9-Hydroxyoudemansin A, a Novel Antifungal (*E*)-β-Methoxyacrylate from a *Mycena* species

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In the course of a screening for new natural antifungal metabolites, the fungus *Mycena* sp. HKI 0153 was found to produce a new member of the (*E*)-β-methoxyacrylate group named 9-hydroxyoudemansin A (**1**). Its structure is closely related to oudemansin A (**2**)[1], which was coisolated from the same strain. The isolation, structure determination and biological evaluation will be described in the following publication.

The producing strain HKI 0153 from the strain collection of the Hans-Knöll-Institut was identified as *Mycena* sp. due to its morphological characteristics.

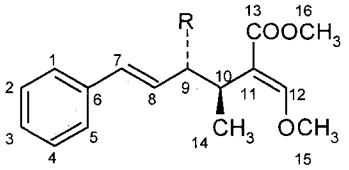
A small piece of a mature slant culture of the strain *Mycena* sp. grown on malt extract agar (malt extract 4%, yeast extract 0.4%, agar 1.5%, deionized water, pH 6.0) was used to inoculate 500 ml-Erlenmeyer flasks containing 100 ml of the producing medium consisting of glycerol 3%, glucose 1%, casein peptone 0.5%, NaCl 0.2%, zeolite 1%, and 0.1% agar, pH 7.0. The surface cultures were incubated for 30 days at 23°C.

The culture broth (5 liters) was extracted with equal volumes of EtOAc for 24 hours. The residue (*ca.* 2 g) was immediately separated and purified by preparative HPLC in 20 mg batches using a binary gradient of 0.1% TFA to CH_3CN (99.5:0.5 to 0.5:99.5, 22 minutes, column 10 × 250 mm, Nucleosil 100-5, C18, flow rate 5 ml/minute, detection at 214 nm). The fractions were concentrated and lyophilized. Retention time (Rt) of the fraction containing **1**: 14.4 minutes (yield 35 mg). Rt of the fraction containing **2**: 16.3 minutes (yield 40 mg). The physico-chemical properties of the new antibiotic **1** are summarized in Table 1. The UV and IR spectra (Table 1) of **1** resemble those of **2**[1]. Structure elucidation was based on mass spectrometric and NMR spectroscopic measurements. The electrospray mass spectrum (ESI-MS, triple quadrupole mass spectrometer Quattro 400, VG Biotech, Altrincham, U.K.) of **1** displayed *m/z* 299 ($[M+Na]^+$). The elemental composition of **1** was suggested by 276.13501 (M^+; calcd. 276.13616 for $C_{16}H_{20}O_4$) in electron impact mass spectrum (EI-MS, double-focusing mass spectrometer AMD 402, Intectra Harpstedt, Germany). Besides the molecular ion strong fragments are visible at *m/z* 258 ($M^+ - H_2O$), 246 ($M^+ - CH_2O$), 143, 133, 115, 111, and 75 ($C_3H_7O_2$), comparable to those reported for **2**[1]. As indicated by its

Table 1. Physico-chemical properties of 9-hydroxyoudemansin A (**1**).

Appearance	Slightly yellow oil
Molecular formula	$C_{16}H_{20}O_4$
ESI-MS (*m/z*)	299 $[M+Na]^+$, 258 $[M-H_2O]^+$
HR-EI-MS (*m/z*)	276.13501 (10%, calcd. 276.13616), 258.12298 (9, $C_{16}H_{18}O_3$), 246.12449 (27, $C_{15}H_{18}O_3$), 147.07069 (89, $C_{10}H_{11}O$), 144.07820 (100, $C_7H_{12}O_3$)
$[\alpha]_D^{20}$	−29.2° (*c* 0.5, $CHCl_3$)
$CD_{extreme}$ nm (θ) (MeOH)	216 (0), 235 (-25.54×10^3), 251 (0), 256 ($+5.83 \times 10^3$), 265 (0), 270 (-3.71×10^3), 299 (0)
UV λ_{max} nm (log ε) (MeOH)	205 (5.36), 248 (5.36), 282 (sh, 4.64), 291 (sh, 4.64)
IR ν_{max} cm^{-1} (KBr)	3430, 2935, 2875, 2845, 1698, 1633, 1489, 1444, 1274, 1249, 1189, 1129, 1020
Solubility	MeOH, DMSO, $CHCl_3$
Rf-value (TLC) (Silica gel sheets, $CHCl_3$/MeOH 9:1)	0.81

Fig. 1. Structure of 9-hydroxyoudemansin A (**1**).



R = OH (1)
R = OCH_3 (2)

Table 2. ^1H and ^{13}C NMR shifts of 9-hydroxyoudemansin A (**1**) (in $CDCl_3$, δ in ppm).

Carbon No.	δ_C[a] (DEPT)	δ_H[b] (mult, *J* Hz)
1	126.43 (CH)	7.36 (dt, 7.2, 1.1)
2	128.44 (CH)	7.29 (t, 7.4)
3	127.24 (CH)	7.20 (tt, 7.2, 1.3)
4	128.44 (CH)	7.29 (t, 7.4)
5	126.43 (CH)	7.36 (dt, 7.2, 1.1)
6	137.26 (C)	
7	130.02 (CH)	6.60 (dd, 15.8, 1.3)
8	131.67 (CH)	6.21 (dd, 15.8, 6.3)
9	75.11 (CH)	4.49 (ddd, 6.3, 5.4, 1.3)
10	36.48 (CH)	3.04 (qd, 7.2, 5.4)
11	113.27 (C)	
12	159.67 (CH)	7.28 (s)
13	169.29 (C)	
14	13.14 (CH_3)	1.22 (d, 7.2)
15	61.54 (CH_3)	3.70 (s)
16	51.39 (CH_3)	3.82 (s)

[a] 125 MHz, [b] 500 MHz.

Table 3. Antifungal activities of **1** and **2** in a standardized agar diffusion assay[a].

Microorganisms	MIC (μg/ml)	
	1	**2**
1. *Candida albicans* BMSY 212	>50	1.6
2. *Sporobolomyces salmonicolor* SBUG 549	12.5	1.6
3. *Rhodotorula rubra* IMET 25030	>50	50
4. *Penicillium notatum* JP 36	>50	1.6
5. *Fusarium culmorum* JP 15	>50	3.2

1~3 Sabouraud-2%-glucose-agar (Difco).
4~5 Malt-agar.
[a] Description see Deutsches Arzneibuch, 9. Auflage, pp. 47~48 and 424~430, Deutscher Apotheker Verlag, Stuttgart, 1986.

molecular formula **1** contains two less hydrogen and one less carbon atoms than **2**.

The relationship of **1** to **2** was confirmed by the NMR data[1~3]. However, the ^1H NMR spectra of **1** and **2** (Bruker Avance DRX 500, Rheinstetten, Germany) showed a clear difference in that one methoxy signal was observed at 3.32 ppm in **2** but not in **1**. Based on the results of the 2D NMR spectra including COSY, HSQC and HMBC, the structure of **1** was confirmed as hydroxyl derivative of **2** at position 9 (Fig. 1). Correlations among ^1H and ^{13}C NMR signals of **1** in one-bond relationships were determined by DEPT 135 and HSQC (Table 2).

The IR spectrum of **1** showed in addition to signals typical for **2** a band at 3430 cm^{-1} indicating a hydroxy group. **1** gave an acetate on acetylation with acetic anhydride and pyridine, which showed no hydroxyl IR band. It gave a new ^{13}C NMR signal of an additional carbonyl at δ 170.42 ppm along with a strong IR carbonyl band at 1754 cm^{-1}. In the HMBC experiment of the acetate a strong cross peak was observed between 9-H (δ 5.70 ppm) and the acetalic carbon at δ 170.42 ppm.

The CD spectrum of **1** exhibited a positive Cotton effect at 256 nm and corresponds to those of **2**. This establishes the 9*S*,10*S* configuration for **1**, as depicted in Fig. 1. The absolute configuration of **2** was proven by synthesis[4]. **1** could be a connection link between the oudemansins and the strobilurins[5]. Water elimination in **1** gives rise to strobilurin A. The antimicrobial activity of **1** resembles that of other oudemansins and strobilurins[6]. The antimicrobial spectra of **1** and **2** are shown in Table 3. As in the case with activities of hydroxystrobilurin A or D and strobilurin A and D[7,8] **1** exhibited weaker antifungal activities as compared to **2**. Apparently, the substitution at C-9 with a hydroxy group is responsible for the decrease of antifungal activity. **1** does not inhibit antibacterial activities.

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