NOTES

Hydroxystrobilurin A, a New Antifungal *E*-β-Methoxyacrylate from a *Pterula* species

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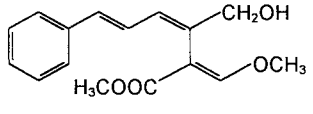
In a search for new antifungal metabolites from basidiomycetes we detected hydroxystrobilurin A (**1**) in the culture broth of *Pterula* spec. 82168. The same fungus was also found to produce strobilurin A and oudemansin A. The isolation, structure determination and biological evaluation of hydroxystrobilurin A will be described in the following. Mycelial cultures of *Pterula* spec. 82168 were derived from tissue plugs of fruiting bodies collected in a forest close to Kaiserslautern, Germany. Voucher specimen and cultures are deposited in the collection of the Lehrbereich Biotechnologie, University of Kaiserslautern.

For maintenance on agar slants and fermentation the fungus was grown in a YMG medium composed of (g/liter): Yeast extract 4, malt extract 10, glucose 4, pH 5.5. For the production of hydroxystrobilurin A a well grown seed culture was used to inoculate 20 liters of YMG in a Biolafitte C6 fermentation apparatus. The fermenter was incubated at 24°C with stirring (120 rpm) and an aeration of 3 liters air/minute. Antifungal activity in fermentations and in fractions during chromatography was measured in the plate diffusion assay using *Mucor miehei* as the test organism. This organism was chosen because with this strain all antifungal metabolites in the culture could be most readily detected. After three weeks of fermentation the active components of the culture filtrate (18 liters) were extracted by adsorption onto Mitsubishi DIAION HP-21 resin. Elution with acetone yielded a crude extract (1.5 g) which was fractionated by chromatography on silica gel (Merck 60, 0.063~0.2 mm, elution with cyclohexane-ethyl acetate, 1:1). 85 mg of a crude product were obtained. Final purification was achieved by preparative HPLC. Column: LiChrosorb-Diol (Merck), 7 μm, 250×25 mm. Mobile phase: Gradient (% cyclohexane in *tert*-butyl methyl ether): 0~10 minutes, 20%; 10~20 minutes, 20%; 20~30 minutes, 20~30%; 30~70 minutes, 30%; 70~85 minutes, 30~40%; 85~125 minutes, 40%; 125~135 minutes, 40~50%; 135~205 minutes, 50%; 205~235 minutes, 50~100%. Flow rate: 5 ml/minute. Detection at 210 nm. Retention time (Rt) of the fraction containing hydroxystrobilurin A: 190 minutes. Yield: 16 mg. Column: LiChrogel PS 1, 250×25 mm, (Merck). Mobile phase: 2-propanol. Flow rate: 3 ml/minutes. Rt of hydroxystrobilurin A: 46 minutes.

For hydroxystrobilurin A the following physicochemical properties were found: brownish yellow oil, Rf 0.16 (toluene-acetone 7:3); UV λ_{max}^{MeOH} nm (log ε) 229 (4.16), 236 (4.13), 260 (4.07), 294 (4.22), 313 (sh, 4.10); EI-MS (direct inlet, 75°C) *m/z* (relative intensity %) 274.1214 (31, M^+, calcd for $C_{16}H_{18}O_4$ 274.1205), 256 (2), 242 (26), 214 (11), 210 (90), 197 (10), 181 (52), 153 (55), 141 (33), 128 (27), 121 (100, C_8H_9O), 115 (48), 104 (17), 91 (57), 75 (46); IR (KBr) cm^{-1} 3630~3100 (m, br), 2946 (m), 2854 (w), 1705 (sst), 1625 (st), 1448 (m), 1436 (m), 1290 (st), 1260 (st), 1242 (sst), 1143 (st), 1120 (sst), 1083 (st), 1029 (m), 988 (m), 968 (m), 927 (w); ^1H NMR (300 MHz, $CDCl_3$) δ 3.75 (s, 16-H), 3.85 (s, 15-H), 4.25 (s, 14-H), 6.51 (d, 9-H), 6.62 (d, 7-H), 6.66 (dd, 8-H), 7.22 (dd, 3-H), 7.30 (dd, 2,4-H), 7.37 (d, 1,5-H), 7.54 (s, 12-H); $J_{1,2}=J_{2,3}=J_{3,4}=J_{4,5}=7.3$ Hz, $J_{7,8}=15.3$ Hz, $J_{8,9}=9.3$ Hz; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 51.8 (C-16), 62.0 (C-15), 66.8 (C-14), 107.8 (C-11), 125.6 (C-8), 126.5 (C-1 and C-5), 127.7 (C-3), 128.5 (C-2 and C-4), 130.9 (C-9), 133.6 (C-6), 134.0 (C-7), 137.4 (C-10), 160.4 (C-12), 168.1 (C-13).

The IR spectrum of the compound shows in addition to signals typical for strobilurins a broad band from 3630 to 3100 cm^{-1} indicating a hydroxy group. The main fragment ion of the mass spectrum is *m/z* 121 (C_8H_9O) which is also the base peak of the mass spectra of strobilurin A[1] and 9-methoxystrobilurin A[2]. The relationship to strobilurin A is confirmed by the NMR data, but instead of the signals for the methyl group C-14 there are signals (δ 4.25 in the ^1H NMR and δ 66.8 in the ^{13}C NMR) which can be assigned to a hydroxymethylene group. The NMR data are in good agreement with those of hydroxystrobilurin D[3]. This establishes structure **1** for hydroxystrobilurin A. The biological activity of hydroxystrobilurin A resembles that of other strobilurins and oudemansins[4]. As is the case with the activities of hydroxystrobilurin D and strobilurin D[3,5], hydroxystrobilurin A exhibits much weaker antifungal activities as compared to strobilurin A. Apparently, the substitution at C-14 with a hydroxyl group is responsible for the decrease of antifungal activity. In the plate



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diffusion assay (Table 1) most filamentous fungi and yeasts are inhibited by $1 \sim 10\,\mu g$ of hydroxystrobilurin A. Incomplete inhibition zones are observed with *Saccharomyces cerevisiae* is 1 and other fungi because aerobic growth on the surface of the medium is completely inhibited while markedly slower anaerobic growth is still possible. This is due to a strong inhibition of fungal respiration. Oxygen uptake by freshly germinated spores of *Penicillium notatum* (30 mg wet weight/ml in 1% glucose solution) is inhibited 60% at $10\,\mu g$/ml of **1**. This effect, however, is considerably lower than the inhibition of respiration by strobilurin A[6]. Hydroxystrobilurin A does not exhibit antibacterial activities.

Table 1. Antifungal activity of hydroxystrobilurin A in the agar diffusion assay.

Organism	Diameter of inhibition zone		
	1[a]	10[a]	50[a]
Absidia glauca (+)	—	—	7, 5
A. glauca (−)	—	—	8
Alternaria porri	—	13i	N.T.
Ascochyta pisi	—	17i	N.T.
Aspergillus ochraceus	—	20i	N.T.
Botrytis cinerea	—	10	N.T.
Cladosporium cladosporioides	—	—	23
Epicoccum purpurascens	—	15i	N.T.
Fusarium fujikuroi	—	11i	N.T.
F. oxysporum	—	15i	N.T.
Mucor miehei	—	10	N.T.
Nematospora coryli	—	15	N.T.
Neurospora crassa	—	10	N.T.
Paecilomyces variotii	—	11	N.T.
Penicillium notatum	9	20i	N.T.
Phoma clematidina	7	10	N.T.
Pythium irregulare	—	—	9
Rhodotorula glutinis	—	11	N.T.
Saccharomyces cerevisiae is 1[b]	12i	30i	N.T.
Ustilago nuda	—	—	20
Zygorhynchus moelleri	—	14i	N.T.

—: No inhibition zone.
i: Incomplete.
N.T.: Not tested.
[a] μg/disc, [b] gift of Prof. F. LACROUTE, Strasbourg.

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