ANTIBIOTICS FROM BASIDIOMYCETES

XXXIII†. OUDEMANSIN X, A NEW ANTIFUNGAL *E*-β-METHOXYACRYLATE FROM *OUDEMANSIELLA RADICATA* (RELHAN EX FR.) SING

TIMM ANKE and ANDREAS WERLE

LB Biotechnologie der Universität,
Paul-Ehrlich-Str. 23, D-6750 Kaiserslautern, FRG

MONIKA BROSS and WOLFGANG STEGLICH

Institut für Organische Chemie und
Biochemie der Universität,
Gerhard-Domagk-Str. 1, D-5300 Bonn 1, FRG

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In a search for new natural variants of strobilurins and oudemansins we detected oudemansin X (**1**) in the culture broth of *Oudemansiella radicata*. The same fungus was also found to produce strobilurin A. The isolation, structure determination, and biological evaluation of oudemansin X will be described in the following.

Mycelial cultures of *O. radicata* were obtained from tissue plugs of fruiting bodies collected in Highlands, N.C., U.S.A. Herbarium specimen and the oudemansin X producing strain (No. 86125) are deposited in the collection of the LB 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. Fermentations were carried out in a 150-liter stainless steel vessel (Deutsche Metrohm) containing 100 liters of YMG medium with aeration (15 liters air/minute) and stirring (100 rpm) at 22°C. Eight liters of well grown seed culture were used as inoculum. Antifungal activity in fermentations and in fractions during chromatography was measured in the agar plate-paper disc diffusion assay using *Mucor miehei* as the test organism. After 10 days of fermentation the culture filtrate (90 liters) was extracted with 30 liters of EtOAc yielding after evaporation *in vacuo* 2.7 g of the crude concentrate. The freeze dried mycelia (309 g) were extracted with two 5-liter portions of MeOH and the solvent evaporated. The residue was suspended in 1 liter of water and extracted with two 1-liter portions of EtOAc. Evaporation of the extracts yielded 2.7 g of an oily residue. Oudemansin X was prepared from the combined crude extracts of both culture filtrate and mycelia by silica gel chromatography (Merck 60 PF_{254}) on a Chromatotron (Harrison Research) with cyclohexane-EtOAc (3:1) as eluant. The fractions exhibiting antifungal activity were pooled yielding 840 mg. Final purification was achieved by preparative HPLC in 100 mg batches. Column: LiChrosorb Si60, 7 μm, 50×250 mm (Merck). Mobile phase: Gradient. (% 2-propanol in cyclohexane): 0~10 minutes, 10%; 10~30 minutes, 10%→20%; 30~40 minutes, 20%; 40~60 minutes, 20%→30%. Flow rate: 5 ml/minutes. Detection at 210 nm. Rt of oudemansin X: 31.5 minutes. Overall yield: 200 mg.

Oudemansin X showed the following physicochemical properties: Colorless oil, Rf 0.52 (cyclohexane-EtOAc-HCO_2H, 24:8:1); UV λ_{max}^{MeOH} nm (log ε) 206 (4.38), 260 (4.42), 292 (sh, 3.85); $[\alpha]_{589}$ −20.35° (*c* 0.14, EtOH); CD $\lambda_{extreme}^{MeOH}$ nm (θ) 217.5 (0), 236 (-22.99×10^3), 253 (0), 260 ($+4.74\times10^3$), 267 (0), 292 (-4.49×10^3); EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 320.1635 (0.14, M^+, Calcd for $C_{18}H_{24}O_5$: 320.1624), 288 (1), 177 (100, $C_{11}H_{13}O_2$), 145 (18), 121 (6), 75 (5); IR (KBr) cm^{-1} 2930 (st), 2840 (w), 1705 (sst), 1640 (st), 1600 (sst), 1510 (sst), 1460 (st), 1240 (sst), 1170 (st), 1110 (sst), 1080 (sst), 1035 (st), 970 (w), 850 (w), 820 (w), 720 (w); ^1H NMR (400 MHz, CD_3OD) δ 1.26 (d, 14-H), 2.99 (m, 10-H), 3.32 (s, 17-H), 3.65 (s, 16-H), 3.80, 3.85 (both s, 15- or 18-H), 3.97 (dd, 9-H), 5.74 (dd, 8-H), 6.39 (d, 7-H), 6.86, 7.27 (AA'BB'-system, 2, 4-H and 1, 5-H, respectively), and 7.32 (s, 12-H); $J_{1,2}=J_{4,5}=9$ Hz, $J_{7,8}=15.5$ Hz, $J_{8,9}=9$ Hz, $J_{9,10}=9.5$ Hz, and $J_{10,14}=7$ Hz; ^{13}C NMR (100.6 MHz, CD_3OD) δ 15.1 (C-14), 34.5 (C-10), 50.1 (C-16), 54.4, 55.1 (C-17 or C-18), 60.8 (C-15), 83.8 (C-9), 110.3 (C-11), 113.3 (C-2 and C-4), 125.9 (C-8), 126.8 (C-1 and C-5), 128.3 (C-6), 131.1 (C-7), 158.6 (C-3), 159.2 (C-12), 166.7 (C-13).

The spectral data of oudemansin X are in close agreement with those of the oudemansins A[2] and B[3]. As indicated by the MS, oudemansin X contains an additional methoxy group in comparison to oudemansin A. The methoxy group forms part of a 4-methoxyphenyl residue which gives rise to an AA'BB'-pattern in the ^1H NMR spectrum. In addition, the presence of an oudemansin side chain with the usual anti arrangement of the two stereocenters[4] can be deduced. The CD spectrum of oudemansin X exhibits a positive Cotton effect at 260 nm and corresponds to those of oudemansins

† See ref 1.

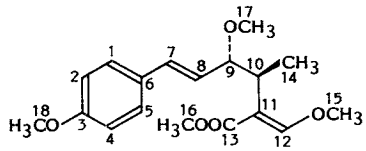

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Table 1. Antifungal activity of oudemansin X in the agar diffusion assay.

Organism	Diameter of inhibition zone (mm)		
	0.1[a]	1[a]	10[a]
Absidia glauca (+)	—	13	24
A. glauca (−)	—	13	23
Alternaria porri	19i[b]	40i	55
Ascochyta pisi	20i	40i	50i
Botrytis cinerea	—	18	25
Curvularia lunata	—	—	40
Fusarium fujikuroi	—	16i	29i
F. oxysporum	15i	20i	21i
Mucor miehei	15	29	37
Nematospora coryli	—	14i	29i
Neurospora crassa	—	—	25i
Paecilomyces varioti	14i	25i	30i
Penicillium islandicum	—	16	16
P. notatum	28i	35i	45i
Pythium debaryanum	—	—	11
Rhodotorula glutinis	20i	30i	35i
Saccharomyces cerevisiae iS 1[c]	19i	29i	40i
Ustilago nuda	16i	32i	37i
Zygorhynchus moelleri	17	37	45

−: No inhibition zone.
[a] μg/disc.
[b] Inhibition incomplete.
[c] Gift of Prof. F. LACROUTE, Strasbourg.

A[2] and B[3]. This establishes the 9*S*,10*S*-configuration[5] for oudemansin X as depicted in formula **1**. A strobilurin analogue of **1** has been isolated by VONDRÁČEK *et al.*[6] from cultures of *Oudemansiella mucida*.

The biological activities of oudemansin X closely resemble those of the other strobilurins and oudemansins[4]. The compound does not exhibit antibacterial activities. In the serial dilution assay[2] at 100 μg/ml no inhibition of growth was observed of *Acinetobacter calcoaceticus*, *Arthrobacter citreus*, *Bacillus brevis*, *Bacillus subtilis*, *Corynebacterium insidiosum*, *Escherichia coli* K-12, *Micrococcus luteus*, *Mycobacterium phlei*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Streptomyces bambergiensis*. As shown in Table 1 oudemansin X exhibits high antifungal activities. This activity is due to a strong inhibition of respiration. Oxygen uptake by freshly germinated spores of *Penicillium notatum* (30 mg wet weight/ml in 1% glucose solution) is inhibited 50% at 0.1 μg/ml of oudemansin X. Like oudemansin A[2] and other *E*-β-methoxyacrylate antibiotics oudemansin X strongly inhibits the incorporation of ^{14}C-thymidine, ^{14}C-uridine, and ^{14}C-leucine into DNA, RNA, and protein of Ehrlich ascitic carcinoma cells at low concentrations (2 μg/ml). This inhibition can be reversed in the presence of glucose in the medium.

Acknowledgments

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References

1) WEBER, W.; T. ANKE, B. STEFFAN & W. STEGLICH: Antibiotics from basidiomycetes. XXXII. Strobilurin E: A new cytostatic and antifungal (*E*)-β-methoxyacrylate antibiotic from *Crepidotus fulvotomentosus* peck. J. Antibiotics 43: 207~212, 1990
2) ANKE, T.; H. J. HECHT, G. SCHRAMM & W. STEGLICH: Antibiotics from basidiomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (SCHRADER ex FR.) HOEHNEL (Agaricales). J. Antibiotics 32: 1112~1117, 1979
3) ANKE, T.; H. BESL, U. MOCEK & W. STEGLICH: Antibiotics from basidiomycetes. XVIII. Strobilurin C and oudemansin B, two new antifungal metabolites from *Xerula* species (Agaricales). J. Antibiotics 36: 661~666, 1983
4) ANKE, T. & W. STEGLICH: β-Methoxyacrylate antibiotics: from biological activity to synthetic analogues. *In* Biologically Active Molecules. *Ed.*, U. P. SCHLUNEGGER, pp. 1~25, Springer-Verlag Berlin, 1989
5) AKITA, H.; H. KOSHIJI, A. FURUICHI, K. HORIKOSHI & T. OISHI: The absolute configuration of oudemansin; total synthesis of (−)-oudemansin. Tetrahedron Lett. 24: 2009~2010, 1983
6) VONDRÁČEK, M.; J. VONDRÁČKOWÁ, P. SEDMERA & V. MUSÍLEK: Another antibiotic from the basidiomycete *Oudemansiella mucida*. Coll. Czech. Chem. Commun. 48: 1508~1512, 1983