ANTIBIOTICS FROM BASIDIOMYCETES. IX[1]

OUDEMANSIN, AN ANTIFUNGAL ANTIBIOTIC FROM *OUDEMANSIELLA MUCIDA* (SCHRADER ex FR.) HOEHNEL (AGARICALES)

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From mycelial cultures of *Oudemansiella mucida* a crystalline optically active antibiotic, oudemansin (**2**), has been isolated; its structure is closely related to strobilurin A (**1**). The relative configuration of oudemansin have been determined by X-ray analysis. The antibiotic exhibits strong antifungal properties and inhibits respiration in fungi, cells of the ascitic form of EHRLICH carcinoma, and rat liver mitochondria.

Recently we elucidated the structures of two antifungal antibiotics from cultures of *Strobilurus tenacellus*[2,3]. Strobilurin A (**1**) has the same molecular formula $C_{16}H_{18}O_3$ as mucidin, an antifungal compound isolated from *Oudemansiella mucida* by VONDRÁCEK *et al.*[4,5] However, in contrast to oily, achiral **1**, mucidin is crystalline (m.p. 51~53°C) and optically active ($[\alpha]_{546}$ +33°)[4]. In this publication we describe the isolation and structural elucidation of oudemansin (**2**), a new antibiotic from *Oudemansiella mucida* showing similar biological activity to **1**.

Results and Discussion

1. Fermentation, Isolation, and Physico-Chemical Properties

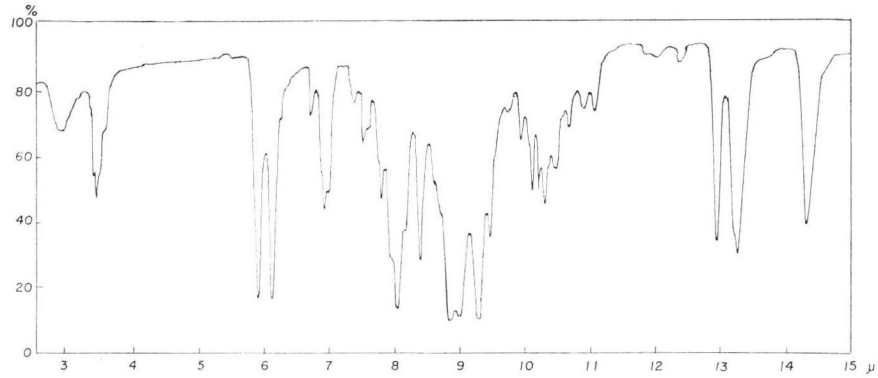
Mycelial cultures of *Oudemansiella mucida* obtained from spore prints were grown on yeast extract-malt extract-glucose (YMG) medium as described in the experimental section. Working up gave a crude extract, which was purified by chromatography on silica gel and Sephadex LH-20. On addition of petroleum ether 50 mg of crystalline oudemansin were obtained from 20 liters of culture. Oudemansin, m.p. 44°C, is soluble in methanol, acetone, chloroform, less soluble in petroleum ether and only sparingly soluble in water, and shows Rf 0.75 on silica plates (Merck 5554; cyclohexane - EtOAc - HCOOH = 120: 40: 1). Its optical rotation $[\alpha]_D^{22}$ −17° (*c* 1, EtOH) and molecular formula prove that it is different from mucidin. In the UV spectrum a maximum at 245 ($\log \varepsilon$ 4.48) is observed, the IR spectrum in KBr disc is shown in Fig. 1.

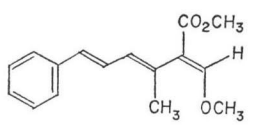
2. Structure Elucidation

The molecular formula $C_{17}H_{22}O_4$ points out a close relationship of oudemansin to strobilurin A (**1**). Like the latter oudemansin contains a methyl β-methoxyacrylate system, which gives rise to signals in the ^{13}C nmr spectrum at δ 51.6, 62.1, 113.1, 161.5, and 170.2 ppm[3]. The ^1H nmr indicates,

Dedication: This paper is dedicated to Dr. FRITZ LIPMANN on occasion of his 80th birthday.

Fig. 1. IR spectrum of oudemansin (**2**) in KBr.





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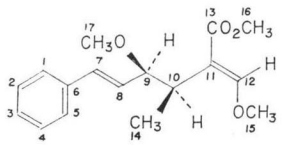


2*

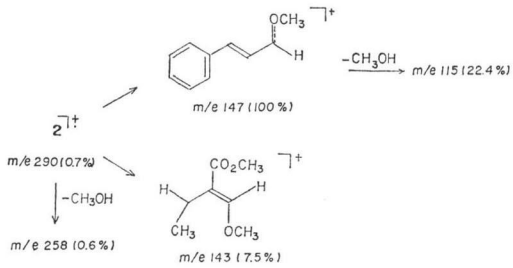
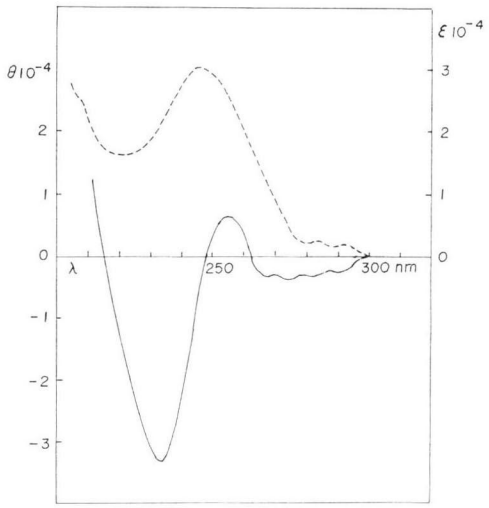


Fig. 2. UV (---) and CD (—) spectra of oudemansin (**2**) in methanol.



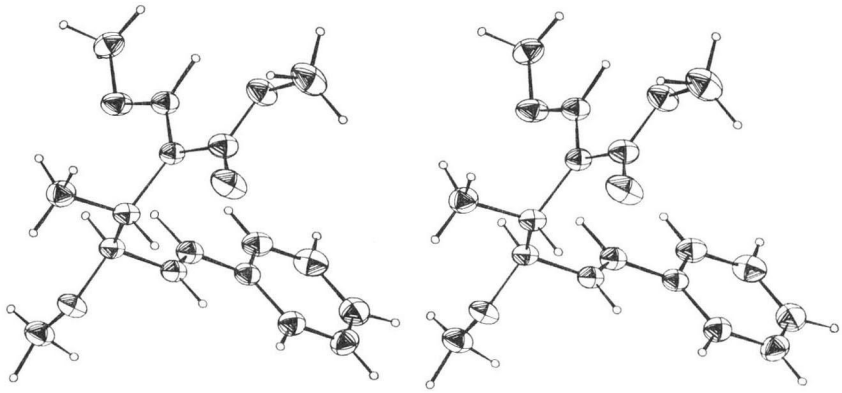
that this moiety is connected with a styryl residue *via* the partial structure –CH(Me)–CH(OMe)–, thus leading to formula **2** for oudemansin. It is in accordance with the ^{13}C nmr spectrum and explains the main fragment ions in the MS.

Besides the molecular ion at *m/e* 290 the MS shows peaks at *m/e* 258 (M – MeOH), 147, 143, and 115, the last ion resulting from α-cleavage next to the ether methoxy group with consecutive loss of MeOH from *m/e* 147. The fragment *m/e* 75 ($C_3H_7O_2$) is typical for the methyl β-methoxyacrylate group[3].

Oudemansin is optically active, and its CD and UV spectra are shown in Fig. 2. In order to determine the relative configuration of this antibiotic an X-ray analysis was performed. Crystals of **2** are monoclinic, space group $P2_1$, with cell dimensions $a=10.101$ (4), $b=9.596$ (4), $c=8.789(4)$ Å, $\beta=105.40$ (2). Because of the small linear absorption factor [$\mu=6.81$ cm^{-1} (Cu k_α)] and the small

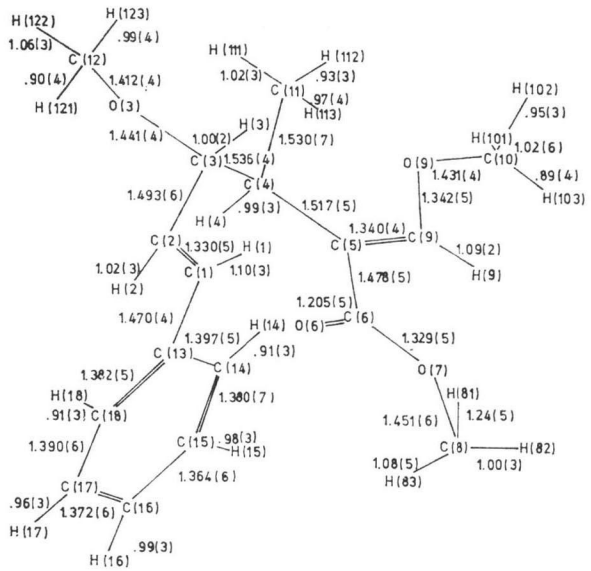
* Absolute configuration unknown.

Fig. 3. ORTEP stereoplot of oudemansin (**2**)[8].



irregular shaped crystal no absorption correction was applied. The structure was solved by direct methods using the MULTAN[6] program. For refinement by least squares techniques the XRAY76 system[7] was used. After anisotropic refinement of carbon and oxygen atoms, hydrogen atoms were determined from difference FOURIER syntheses with isotropic temperature factors included into the refinement. After convergence the final R-value was $R=3.2\%$ for 1,454 observed reflexions. The relative configuration of oudemansin is shown in the stereoplot Fig. 3[8] and in formula **2**. The bond lengths and valence angles are given in Figs. 4 and 5, confirming the structure proposed from spectroscopic arguments. The molecule is arranged in two planar systems forming an angle of 77.9°. Model calculations show this to be a stereochemically favourable conformation, as all rotations around the bonds C(4)–C(5), C(3)–C(4), and C(2)–C(3)* lead to a decrease of contact distances to values less than the sum of VAN DER WAALS radii.

Fig. 4. Bond length of **2** (esd in parantheses).

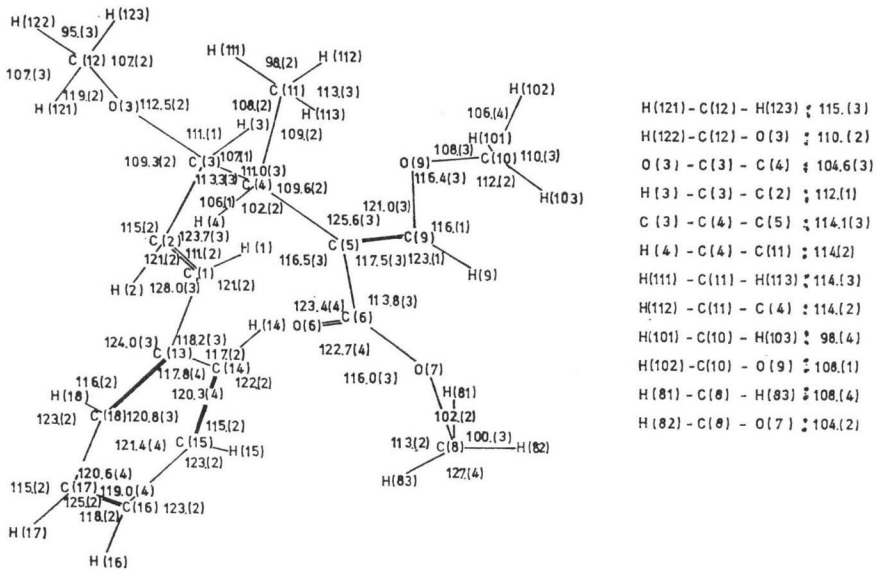


3. Biological Properties

As shown in Table 1 the antimicrobial spectrum of oudemansin closely resembles that of the strobilurins A and B[2], whereas the inhibitory effect of oudemansin on macromolecular syntheses of EHRLICH carcinoma ascitic (ECA) cells is somewhat less pronounced (Table 2). Oudemansin completely inhibits respiration of fungi and ECA cells at the same concentrations which are required to stop protein, RNA, and DNA syntheses. The inhibitory effect on macromolecular syntheses in ECA cells can be

* The atom numbering refers to Figs. 3 and 4.

Fig. 5. Valence angles of **2** (esd in parantheses).



reversed by the addition of glucose (Table 2) under conditions where respiration remains completely blocked (oxygen measured polarographically in the same vessel). The same effects were found for the strobilurins. In rat liver mitochondria prepared and tested according to[9] respiration and ATP synthesis with both α-ketoglutaric acid and succinate as substrates were completely inhibited by concentrations of 10^{-6} M strobilurin A. The inhibitory action of these antibiotics in the absence of glucose is thought to be due to inhibition of respiration and therefore depletion of the cells' ATP pool which can be restored by glycolysis in the presence of glucose. Mucidin

Table 1. Antimicrobial spectrum of oudemansin.

Serial dilution test

Difco antibiotic medium 3	MIC (μg/ml)
Aerobacter aerogenes	>20
Arthrobacter citreus	>20
Bacillus brevis	>20
Bacillus subtilis	>20
Escherichia coli K12	>20
Micrococcus luteus	>20
Micrococcus roseus	>20
Staphylococcus aureus	>20

Plate diffusion assay

YM-medium	Diameter inhibition zone (mm)	
	1 μg/disc	10 μg/disc
Aspergillus panamensis	25	40
Candida albicans	25	30
Fusarium cubense	—	—*
Neurospora crassa	—	—
Paecilomyces varioti	34	43
Rhodotorula glutinis	31	40
Saccharomyces cerevisiae FL200	25	35
Saccharomyces cerevisiae is 1	40	50

* no inhibition.

Table 2. Effect of oudemansin on protein, RNA, and DNA syntheses of EHRLICH carcinoma ascitic cells.

Oudemansin (μg/ml)	Glucose (μg/ml)	Incorporation (cpm), precursor		
		Leucine	Uridine	Thymidine
0	0	31,350	13,806	3,505
1	0	25,118	11,975	3,185
5	0	302	150	1,030
0	300	40,046	21,168	5,119
5	300	39,200	24,000	4,700

has been reported to be a specific inhibitor of the mitochondrial ubiquinol-cytochrome c reductase by ŠUBÍK *et al.*[10] Unfortunately the structure of mucidin has not yet been published.

Experimental

Fermentation and Isolation Procedures

Mycelial cultures of *Oudemansiella mucida* were obtained from spore prints. For maintenance on agar slants or submerged cultivation a yeast extract-malt extract-glucose (YMG) medium (4 g yeast extract, 10 g malt extract, and 4 g glucose per liter) was used. Prior to sterilization the pH was adjusted to 4.7. For the production of oudemansin 20 liters of YMG medium in a Biolafitte fermentation apparatus were inoculated with a 150-ml culture. Two ml of antifoam were added initially and the mycelia were grown at 21°C with stirring (200 rpm) and an aeration rate of 2 liters air/minute. After 6 days the cells were collected, washed with water, and extracted with 1 liter of methanol and subsequently 1 liter of acetone. The combined extracts were evaporated to dryness and the antibiotic extracted from the residue with chloroform yielding 0.63 g of crude product. The culture fluid was extracted with 5 liters of ethyl acetate, the organic phase evaporated, and the crude product (2.47 g) combined with the mycelial extract. The combined extracts were fractionated by chromatography on silica gel (Mallinckrodt, elution with chloroform) yielding 176 mg of a oudemansin-containing oil. After chromatography on Sephadex LH-20 in methanol 110 mg of a colorless oil were obtained from which oudemansin was crystallized by the addition of petroleum ether. Yield: 50 mg. In another batch, besides oudemansin a small amount of strobilurin A was obtained. The possibility that the latter compound was formed by elimination of methanol from oudemansin during the isolation procedure can not be excluded. However, rechromatography of oudemansin on Mallinckrodt silica gel with chloroform did not lead to strobilurin A formation.

Oudemansin (**2**)

m.p. 44°C. $[\alpha]_{578}^{22}$ −20°, $[\alpha]_{546}^{22}$ −27°, $[\alpha]_{436}^{22}$ −57° (*c* 0.03, EtOH).

UV (MeOH): λ_{max}(log ε) = 292 (3.23), 283 (3.36), 254 (4.44, sh), 245 (4.48), 216 (4.23), 208 nm (4.39).

CD (MeOH): $[\theta]_{290.5} = -2.61 \times 10^3$, $[\theta]_{280} = -3.04 \times 10^3$, $[\theta]_{274.5} = -3.89 \times 10^3$, $[\theta]_{262.5} = 0$, $[\theta]_{255} = +6.93 \times 10^3$, $[\theta]_{248} = 0$, $[\theta]_{234} = -33.43 \times 10^3$, $[\theta]_{225} = 0$.

IR ($CHCl_3$): 3035(m), 2960(m), 2870(w), 2845(w), 1708(ss), 1646(s), 1636(sh, s), 1452(m), 1285 (sh, s), 1275(sh, s), 1254(s), 1158(ss), 1142(ss), 1100(ss), 1010(w), 980(m), 925(w), 699(w), 550 cm^{-1}(w).

^1H-NMR ($CDCl_3$, TMS internal standard): δ 1.26 (d, 14-H), 2.99 (dq, 10-H), 3.32 (s, 17-H), 3.64 (s, 15-H), 3.78 (s, 16-H), 3.96 (dd, 9-H), 5.92 (dd, 8-H), 6.46 (d, 7-H), 7.20 (s, 12-H), ~7.2 (br, C_6H_5); $J_{7,8} = 15.9$ Hz, $J_{8,9} = 8.3$, $J_{9,10} = 9.8$, $J_{10,14} = 6.8$.

^{13}C-NMR (CD_3OD): δ 16.2 (C-14), 37.0 (C-10), 51.6 (C-16), 56.9 (C-17), 62.1 (C-15), 86.7 (C-9), 113.1 (C-11), 127.5 (C-1,5), 128.7, 130.2 (C-3 or 8), 129.7 (C-2,4), 134.4 (C-7), 138.1 (C-6), 161.5 (C-12), 170.2 (C-13).

MS (AEI MS 50): *m/e* 290.1512 (0.7%, calc. $C_{17}H_{22}O_4$ 290.1518), 258 (0.6, $C_{16}H_{18}O_3$), 147 (100, $C_{10}H_{11}O$), 143 (7.5, $C_7H_{11}O_3$), 115 (22.4, C_9H_7), 75 (13.5, $C_3H_7O_2$).

Anal. calcd. for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64
found: C, 70.18; H, 7.69

X-Ray Analysis

Space group and preliminary lattice constants were obtained from oscillation and Weissenberg photographs. For determination of accurate unit cell parameters and intensity data collection a STOE diffractometer with Cu k_α radiation and $\theta-2\theta$ scan was used. Listings of the atom parameters may be obtained from H. J. HECHT.

Biological Assays

The antimicrobial spectrum was evaluated and macromolecular syntheses in cells of the ascitic form of EHRLICH carcinoma tested as described earlier[2]. Oxygen-uptake from oxygen-saturated cell suspensions was measured polarographically with a CLARK electrode.

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