Noroudemansin A, a New Antifungal Antibiotic from *Pterula* Species 82168 and Three Semisynthetic Derivatives

Michaela Engler-Lohr[a], Timm Anke[a], Veronika Hellwig[b] and Wolfgang Steglich[b]

[a] Lehrbereich Biotechnologie der Universität, Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany
[b] Institut für Organische Chemie der Universität München, Karlstraße 23, D-80333 München, Germany

Z. Naturforsch. **54c,** 163–168 (1999); received November 12/December 18, 1998

Basidiomycetes, Antibiotics, Antifungal, Noroudemansin A

A new antifungal (*E*)-β-methoxyacrylate, noroudemansin A (**1**), was isolated from cultures of *Pterula* sp. 82168. Its biological activities were investigated and compared with oudemansin A. The structure was elucidated by spectroscopic methods. Three semisynthetic noroudemansin A derivatives were synthesized from the natural product.

Introduction

The strobilurins and oudemansins constitute a new class of fungicides (Anke, 1997). To date three antifungal antibiotics of the oudemansin type, oudemansin A (**5**) (Anke *et al.*, 1979), oudemansin B (**6**) (Anke *et al.*, 1983) and oudemansin X (**7**) (Anke *et al.*, 1990) are known. Oudemansins and strobilurins have been described from submerged cultures of a number of basidiomycetes occurring in different habitats and climates (Anke, 1995). They have also been isolated from basidiomycetes cultivated on their natural substrates (Engler *et al.*, 1998a) or growing in the wild (Engler *et al.*, 1998b). A characteristic and essential structure element of the oudemansins and strobilurins is an (*E*)-β-methoxyacrylate moiety which is required for their antifungal activities. Their mode of action is a selective inhibition of mitochondrial electron transport at the hydroquinone oxidation (Q_p) center of the bc_1 complex (Becker *et al.*, 1981). The structural changes conferring resistance to producing fungi have been determined (Kraiczy *et al.*, 1996). In the following we describe the production, isolation, structural elucidation and the biological properties of noroudemansin A (**1**), a new oudemansin derivative from *Pterula* sp. 82168. In addition, three semisynthetic noroudemansin A derivatives **2**, **3** and **4** were synthesized from the natural product by acetylation, oxidation or γ-lactone formation, respectively.

Reprint requests to Prof. Dr. Timm Anke.
Fax: +49–631–205–2999
E-mail: anke@rhrk.uni-kl.de

Materials and Methods

General

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Bruker IFS 48 Fourier Spectrometer or on a Perkin-Elmer 1420 Ratio Recording Infrared Spectrometer (KBr or in $CHCl_3$ between NaCl plates) and UV and CD spectra on an Instruments S. A. Jobin-Yvon CD-6-Dichrograph. NMR spectra were measured with Bruker ARX 300 and AMX 600 spectrometers. Chemical shifts δ in ppm are referenced to the residual solvent signal of $CDCl_3$ at δ_H 7.24 and δ_C 77.0, respectively. MS data were determined using Finnigan MAT 90 and 95 Q instruments (direct inlet, 70 eV). Thin layer chromatography was performed on Silica gel 60 F_{254} (0.25 mm). For analytical HPLC a Hewlett Packard 1090 series II instrument and for preparative HPLC Gilson model 302 or Waters 501 instruments were used.

Producing Organism

The strain of *Pterula* sp. 82168 and its maintenance have been described earlier (Engler *et al.*, 1995).

Fermentation

Fermentations were carried out in a Biostat U fermenter (Braun Biotech, Melsungen) containing

0939–5075/99/0300–0163 $ 06.00

100 liters of MGPY medium composed of (g/liter): Yeast extract 1, maltose 20, glucose 10, peptone 2, KH_2PO_4 0.5, $MgSO_4$ x H_2O 1, $FeCl_3$ 0.01, $ZnSO_4$ x H_2O 0.0018, $CaCl_2$ x H_2O 0.074. Prior to sterilization the pH was adjusted to 5.5 with hydrochloric acid, and 2 ml silicone antifoam were added to the medium. For inoculation a well-grown culture of *Pterula* sp. 82168 in the same medium (10 liters) was used. The culture was grown at 22 °C, aerated with 15 liters air per minute, and stirred with 120 rpm. During fermentation 200 ml samples were taken, the mycelia separated by filtration and the culture broth extracted with ethyl acetate. The residue obtained after evaporation of the organic solvent was taken up in 1 ml of methanol. 10 µl of the concentrated solutions were analyzed by analytical HPLC to follow the production of noroudemansin A (Merck LiChrospher 100 RP 18, 5 µm; column 125 x 4 mm; flow 1.5 ml/minute; gradient: H_2O-MeOH, 0–70% in 20 minutes, 70–100% in 10 minutes; Rt [noroudemansin A] 17.9 minutes). The fermentation was terminated when the concentration of noroudemansin A reached the maximal value.

Isolation of noroudemansin A

After 350 hours of fermentation the active components were removed from the culture fluid (60 l) by adsorption onto HP 21 resin (Mitsubishi DIAION). Elution with acetone yielded a crude extract (5.76 g), which was fractionated by chromatography on silica gel (0.063–0.2 mesh, Merck 60, elution with cyclohexane-EtOAc, 8:2 v/v) to yield 0.89 g of a crude product. Further purification was achieved by preparative HPLC in 0.1 g batches (Merck Lichrosorb Diol, 7 µm; column 250 x 25 mm, flow rate 5 ml/minute, detection at 210 nm) with cyclohexane-*tert*butyl methyl ether, 8:2 as eluent. Yield: 106 mg of an enriched product. Pure noroudemansin A was obtained in a final step by preparative HPLC on Merck Lichrogel PS 1 and elution with 2-propanol (10 µm, column 250 x 25 mm; flow rate 5 ml/minutes; Rt [noroudemansin A] 30 minutes). Yield: 57 mg.

Synthesis of noroudemansin A derivatives

Acetylation of noroudemansin A (6.0 mg) was carried out with acetic anhydride (2 ml) in pyridine (1 ml). The reaction mixture was stirred for 2 hours at room temperature. The crude product was purified by thin-layer chromatography on SiO_2 with toluene-acetone-AcOH, 70:30:1 v/v as eluant to give **2** as a colorless solid (5.7 mg, 82%).

Oxidation of noroudemansin A (**1**) (4.5 mg) was performed with MnO_2 (60 mg) in acetonitrile (2 ml). The solution was stirred for 4 hours, then filtered over Celite® and evaporated. Purification by thin-layer chromatography on SiO_2 with petroleum ether$_{40/60}$-EtOAc, 5 : 1 v/v as eluant yielded **3** (4.2 mg, 94%) as a colorless oil.

Formation of the γ-lactone (**4**) was achieved quantitatively by addition of NaH (0.5 mg) to a solution of **1** (1.7 mg) in THF at 0 °C. After stirring for 30 minutes at 0 °C, the mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4 and purified by elution with ethyl acetate over SiO_2 to yield **4** (1.5 mg).

Biological tests

The antimicrobial spectra were tested in an agar diffusion assay and serial dilution assay as described previously (Anke *et al.*, 1989; 1996). The cytotoxicity against L1210 (ATCC CCL 219), HL-60 (ATCC CCL 240), BHK 21 (ATCC CCL 10), and HeLa S3 (ATCC CCL 22) cells was measured as described by Zapf *et al.* (1995). The effect on cell growth of monolayer cell lines was determined using Giemsa stain (Erkel, 1990). The inhibition of respiration of *Penicillium notatum* was measured with a Clark-type oxygen electrode as reported (Weber *et al.*, 1990).

Results and Discussion

Production and isolation of noroudemansin A

From the beginning of the fermentation the crude extracts of the mycelia and the culture fluid exhibited high antifungal activities because of the additional production of strobilurin A, oudemansin A and pterulone and pterulinic acid (Engler *et al.*, 1997a, b). These antibiotics were previously described from fermentations of the same strain in YMG (0.4% yeast extract, 1% malt extract, 0.4% glucose) medium. The production of noroudemansin A (formula **1,** Fig. 1) starting 4 days after inoculation was detected only in MGPY medium with a higher C/N ratio. After 14 days 57 mg noroude-

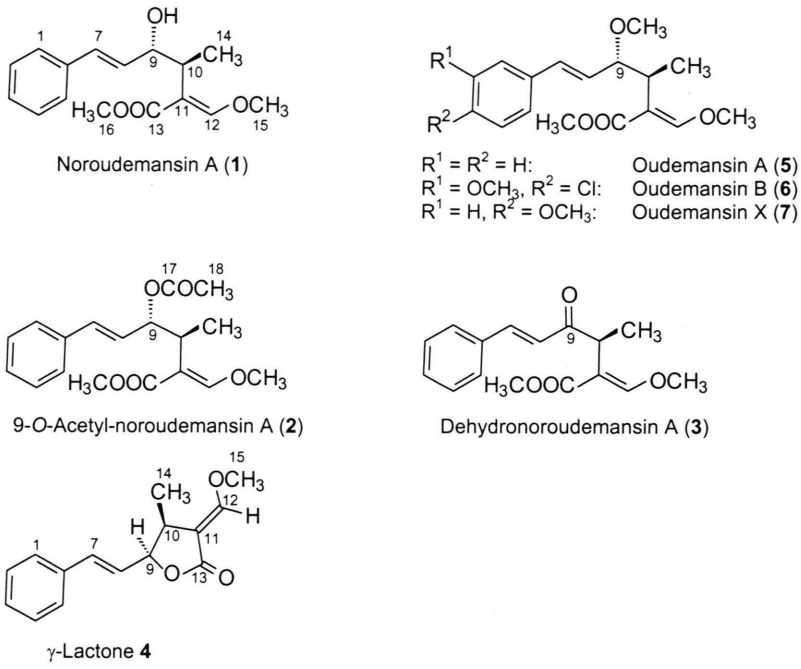

Fig. 1. Chemical structures of noroudemansins and oudemansins.

mansin A were isolated from 60 l culture fluid as described in material and methods.

Structural elucidation of noroudemansin A

The spectral data of noroudemansin A (**1**) are in close agreement with those of oudemansin A (**5**). The IR spectra contains a strong hydroxy absorption at 3430 cm^{-1} in addition to those for a carbonyl at 1700 and a double bond at 1637 cm^{-1}, respectively. The HR-MS showed the molecular ion peak at *m/z* 276, which corresponded to the molecular formula $C_{16}H_{20}O_4$. A comparison with the spectral data of oudemansin A (**5**) indicated that **1** contains a hydroxy group instead of the methoxy group at C-9. In comparison to oudemansin A (**5**), the ^1H and ^{13}C NMR spectra exhibit a downfield shift of the 9-H resonance to δ_H 4.44 as well as an upfield shift of the C-9 resonance to δ_C 75.05. The signal for the hydroxyl proton is detected at δ_H 3.27 in $CDCl_3$. The main fragments in the EI-MS resulting from α-cleavage are shown in Fig. 2.

Due to the similarity of the CD spectra (Fig. 3) of noroudemansin A (**1**) and its *O*-acetyl derivative **2** to that of oudemansin A (**5**) (Anke *et al.*, 1979; Akita *et al.*, 1983) the 9*S*,10*S*-configuration can be proposed for **1**. Moreover, the coupling constants $^3J_{8H,9H}$ = 8.4 and $^3J_{9H,10H}$ = 9.4 Hz of 9-*O*-acetylnoroudemansin A (**2**) are in good agreement with those of the oudemansins A (**5**), B (**6**) (Anke *et al.*, 1983) and X (**7**) (Anke *et al.*, 1990), while the values for the corresponding coupling constants of noroudemansin A (**1**) are smaller. From a calculation of the preferred conformation of **1** (Mohamadi *et al.*, 1990) torsion angles for 8-H/9-H and 9-H/10-H of 45° and 56°,

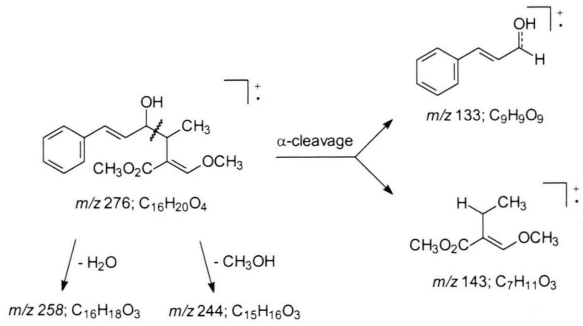

Fig. 2. Important fragmentations in the EI-MS of noroudemansin A (**1**).

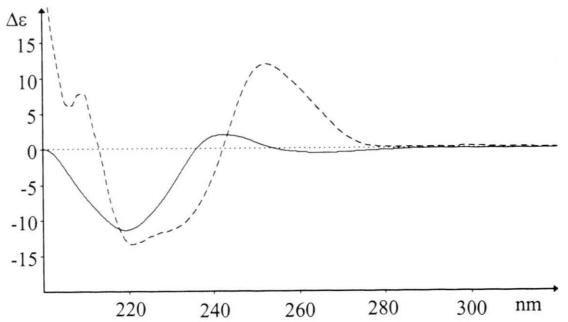

Fig. 3. CD spectra of noroudemansin A (**1**) (———) and 9-*O*-acetylnoroudemansin A (**2**) (---) in MeOH.

respectively, were obtained which agree well with the observed coupling constants ($^3J_{8H,9H}$ = 5.9 and $^3J_{9H,10H}$ = 5.4 Hz).

Noroudemansin A (**1**): Colorless oil; Rf = 0.60 (SiO_2, toluene-acetone-AcOH, 70:30:1); $[\alpha]_D^{25}$ −64.3° (*c* 0.24, MeOH); UV λ_{max}^{MeOH} nm (lg ε) 208 (3.00), 231 (3.42), 270 (2.06), 279 (1.80); CD λ_{max}^{MeOH} nm (Δε) 220 (-11.42), 236 (0), 243 (+2.03), 255 (0), 265 (-0.59); IR (KBr) ν_{max} cm^{-1} 3430 (br, st), 3026 (w), 2938 (m), 2851 (w), 1700 (st), 1637 (st), 1442 (m), 1279 (sh, st), 1248 (st), 1134 (st), 1003 (w), 968 (w), 752 (w), 696 (w); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33–7.13 (5H, m, Ph), 7.31 (1H, s, 12-H), 6.55 (1H, d, 7-H), 6.16 (1H, dd, 8-H), 4.44 (1H, dd, 9-H), 3.77 (3H, s, 16-H), 3.66 (3H, s, 15-H), 3.27 (1H, br s, 9-OH), 3.00 (1H, qd, 10-H), 1.16 (3H, d, 14-H): $J_{7,8}$ = 15.9, $J_{8,9}$ = 5.9, $J_{9,10}$ = 5.4, $J_{7,8}$ = 7.2 Hz; ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.32 (C-13), 159.70 (C-12), 137.19 (C-6), 131.60 (C-8), 129.93 (C-7), 128.42 (C-2/4), 127.22 (C-3), 126.39 (C-1/5), 113.17 (C-11), 75.05 (C-9), 61.56 (C-15), 51.42 (C-16), 36.35 (C-10), 13.03 (C-14), the assignments were confirmed by a HETCOR experiment; MS *m/z* (%) 276 (2) (M^+), 244 (6), 144 (100), 143 (42), 133 (52), 129 (36), 112 (15), 111 (22), 97 (10), 75 (23); HR-MS *m/z* 276 (M, $C_{16}H_{20}O_4$): calcd. 276.1362, found. 276.1377.

Semisynthetic derivatives of noroudemansin A

9-O-Acetyl-noroudemansin A (**2**): Colourless solid; Rf = 0.79 (SiO_2, toluene-acetone-AcOH, 70:30:1); $[\alpha]_D^{33}$ +58.3° (*c* 0.02, MeOH); UV λ_{max}^{MeOH} nm (lg ε) 208 (3.70), 245 (3.73), 281 (2.58), 291 (2.38); CD λ_{max}^{MeOH} nm (Δε) 209 (+7.82), 213 (0), 219 (-13.04), 242 (0), 253 (+11.88); IR (film) ν_{max} cm^{-1} 2940 (m), 2850 (w), 1735 (st), 1705 (st), 1640 (st), 1450 (sh, m), 1435 (m), 1375 (m), 1240 (st), 1120 (st), 1025 (w), 965 (m), 775 (w), 750 (w), 695 (w); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.20 (5H, m, Ph), 7.28 (1H, s, 12-H), 6.53 (1H, d, 7-H), 5.99 (1H, dd, 8-H), 5.67 (1H, dd, 9-H), 3.79 (3H, s, 16-H), 3.65 (3H, s, 15-H), 3.16 (1H, dq, 10-H), 2.05 (3H, s, 18-H), 1.17 (3H, d, 14-H): $J_{7,8}$ = 15.9, $J_{8,9}$ = 8.4, $J_{9,10}$ = 9.4, $J_{10,14}$ = 7.1 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.47 (C-13 or C-17), 168.18 (C-13 or C-17), 160.10 (C-12), 136.65 (C-4), 133.20, 128.44 (C-2/4 or C-1/5), 127.67, 127.03, 126.56 (C-2/4 or C-1/5), 111.02 (C-11), 77.20 (C-9), 61.61 (C-15), 51.24 (C-16), 34.83 (C-10), 21.37 (C-18), 15.39 (C-14); MS *m/z* (%) 318 (1) (M^+), 275 (4), 143 (100), 133 (54), 111 (21), 75 (25). HR-MS *m/z* 276 (M, $C_{18}H_{22}O_5$): calcd. 318.1467, found. 318.1456.

Dehydronoroudemansin A (**3**): Colourless oil; Rf = 0.53 (SiO_2, toluene-acetone-AcOH, 70:30:1); $[\alpha]_D^{30}$ +266.3° (*c* 0.20, MeOH); UV λ_{max}^{MeOH} nm (lg ε) 225 (3.51), 242 (3.45), 286 (3.53); CD λ_{max}^{MeOH} nm (Δε) 204 (+7.43), 216 (-0.57), 221 (+1.43), 224 (+0.85), 229 (+2.00), 248 (-16.29), 285 (+19.57); IR ν_{max} (film) cm^{-1} 2940 (m), 2850 (w), 1705 (vst), 1640 (st), 1615 (st), 1450 (m), 1440 (m, sh), 1315 (m), 1260 (st, sh), 1240 (st), 1195 (m), 1130 (st), 1075 (m), 1045 (m), 990 (w), 930 (w), 775 (m); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (1H, d, 7-H), 7.49 (2H, m, Ph), 7.38 (1H, s, 12-H), 7.34 (3H, m, Ph), 6.75 (1H, d, 8-H), 3.82 (3H, s, 16-H), 3.80 (1H, q, 10-H), 3.70 (3H, s, 15-H), 1.29 (3H, d, 14-H): $J_{7,8}$ = 15.8, $J_{10,14}$ = 6.9 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.87 (C-9), 172.44 (C-13), 160.22 (C-12), 141.32 (C-7), 134.91 (C-6), 130.01 (C-3 or C-8), 128.78 (C-2/4 or C-1/5), 128.23 (C-2/4 or C-1/5), 123.83 (C-3 or C-8), 110.91 (C-11), 61.82 (C-15), 51.49 (C-16), 42.42 (C-10), 13.77 (C-14); MS *m/z* (%) 274 $(M)^+$ (2), 246 (5), 143 (24), 131 (100), 111 (10), 103 (14), 75 (15); HR-MS *m/z* 274 (M, $C_{16}H_{18}O_4$): calcd. 274.1205, found. 274.1198.

γ-Lactone **4**: Colourless oil; Rf = 0.77 (SiO_2, toluene-acetone-AcOH 70:30:1); $[\alpha]_D^{25}$-56.5° (*c* 0.09, EtOH); UV λ_{max}^{MeOH} nm (lg ε) 252 (3.00); CD λ_{max}^{MeOH} nm (Δε) 214 (-4.36), 237 (+6.90), 259 (-8.08), 300 (+0.10); IR ν_{max} (film) cm^{-1} 3440 (br, m), 2910 (m), 2830 (w), 1740 (st), 1665 (st), 1440 (w), 1230 (m), 1100 (m), 1075 (st), 1010 (w), 955 (w), 935 (w), 750 (w), 690 (w); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.4–7.2 (6H, m, Ph and 12-H), 6.68 (1H, dm, 7-H), 6.17 (1H, dd, 8-H), 5.05 (1H, ddd, 9-H),

3.86 (3H, s, 15-H), 3.34 (1H, m, 10-H), 1.11 (3H, d, 14-H): $J_{7,8}$ = 15.9, $J_{8,9}$ = 7.3, $J_{9,10}$ = 7.3, $J_{10,14}$ = 6.9 Hz; MS *m/z* (%) 244 $(M)^+$ (82), 216 (12), 185 (2), 167 (8), 153 (11), 140 (25), 131 (14), 115 (11), 112 (100), 103 (10), 84 (84), 69 (50); HR-MS *m/z* 244 (M, $C_{15}H_{16}O_3$): calcd. 244.1099, found. 244.1102.

Biological properties

In the plate diffusion assay (Table I) most filamentous fungi were inhibited by 50 µg/disc of **1** and of **2,** but not of **3** and **4**. The antifungal activities of oudemansin A (**5**), B (**6**) and X (**7**) are considerably higher at concentrations starting at 0.1 or 1 µg/disc (Anke *et al.*, 1979; 1983; 1990). In the same assay 1 µg/disc of oudemansin A resulted in inhibition zones of 34 mm for *P. variotii* and 40 mm for *S. cerevisiae* is1.

As shown in Fig. 4, oudemansin A (**5**), noroudemansin A (**1**) and the *O*-acetyl derivative **2** inhibited the oxygen uptake by freshly germinated spores of *Penicillium notatum* (30 mg wet weight/ml in 1% glucose solution) in a dose-dependent manner. The IC_{50} values of oudemansin A and **1** were determined at 0.89 µM (0.25 µg/ml) and 261 µM (72 µg/ml), respectively. The *O*-acetyl derivative **2** blocked the oxygen uptake of *Penicillium notatum* 50% at 23 µM (7.3 µg/ml). No inhibition of oxygen uptake was observed at 100 µg/ml of the dehydro derivative **3** and at 50 µg/ml of lactone **4**. Like oudemansin A (**5**), B (**6**) and X (**7**), the new compounds **1**, **2** and **3** contain the (*E*)-β-methoxyacrylate system responsible for the antifungal and respiration-inhibiting properties (Anke, 1997). However, the inhibitory effects appear to be strongly dependent on the substituents of the oxygen at C-9 and their influence on the conformation. The relatively weak activity of **1** can be increased by acetylation of the hydroxy group at this position (**2**), whereas the oxidation product **3** and the γ-lacton (**4**) totally lack the respiration-inhibiting properties.

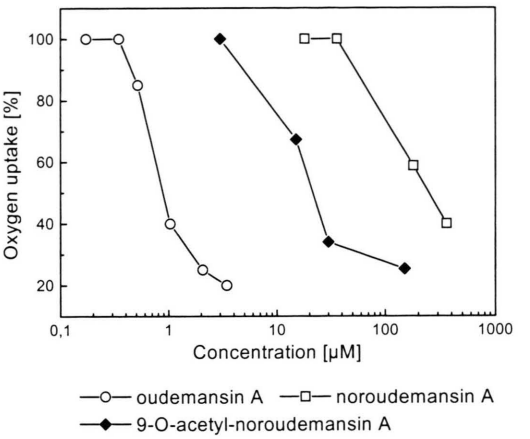

Fig. 4. Effects of **1**, **2** and oudemansin A on the oxygen uptake of germinated *Penicillium notatum* spores.

Table I. Antifungal activities of 50 µg/disc of noroudemansin A (**1**), 9-*O*-acetyl-noroudemansin A (**2**), dehydronoroudemansin A (**3**) and γ-lacton (**4**) in the agar diffusion assay.

Organism	Diameter of inhibition zone [mm]			
	1	**2**	**3**	**4**
Absidia glauca (+)	–	–	–	10
Absidia glauca (–)	–	–	–	–
Alternaria porri	–	12d	–	–
Aspergillus ochraceus	14	20d	–	–
Botrytis cinerea	–	11	n.t.	n.t.
Curvularia lunata	15	15	–	–
Epicoccum purpurascens	15	n.t.	n.t.	n.t.
Fusarium fujikuroi	20d	25d	n.t.	n.t.
Fusarium oxysporum	–	15	–	–
Mucor miehei	20	20	9	–
Nematospora coryli	–	14	–	–
Paecilomyces variotii	15d	22	–	–
Penicillium notatum	15i	18i	11	8
Phoma clematidina	27d	18d	n.t.	n.t.
Pythium irregulare	–	–	n.t.	n.t.
Saccharomyces cerevisiae is1[a]	18	22	–	–
Saccharomyces cerevisiae S288	11	n.t.	–	–
Ustilago nuda	–	10	n.t.	n.t.
Zygorrhynchus moelleri	15	16	10	8

[a]: gift of Prof. F. Lacroute, Strasbourg;
-: no inhibition zone; i: incomplete; d: diffuse inhibition zone; n.t.: not tested.

1 did not exhibit antibacterial activities. In the serial dilution assay 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* and *Streptomyces* sp. ATCC 23836 at 100 µg/ml.

Table II shows that **2** and **3** were moderately cytotoxic, while **1** exhibited only weak cytotoxic activities. **4** showed no cytotoxic effects.

Table II. Cytotoxic activities of **1**, **2**, **3** and **4** towards mammalian cell lines.

Cell line	IC_{50} [μg/ml]			
	1	**2**	**3**	**4**
L1210	50–100	25	10	>50
HL 60	>100	20	10	>50
BHK	50	n.t.	n.t.	n.t.
Hela S3	>100	50	25	>50

n.t.: not tested.

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft is gratefully acknowledged. We thank Dipl. Chem. C. Paulitz for performing the conformational analyses with MacroModel®.

Anke T., Hecht H. J., Schramm G. and Steglich W. (1979), Antibiotics from basidiomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (Schrader ex Fr.) Hoehnel (*Agaricales*). J. Antibiotics **32**, 1112–1117.

Anke T., Besl H., Mocek U. and Steglich W. (1983), Antibiotics from basidiomycetes. XVIII. Strobilurin C and Oudemansin B, two new antifungal metabolites from *Xerula* species (*Agaricales*). J. Antibiotics **36**, 661–666.

Anke H., Bergendorff O. and Sterner O. (1989), Assays of the biological activities of guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. Food. Chem. Toxicol. **27**, 393–397.

Anke T., A. Werle A., Bross M. and Steglich W. (1990), Antibiotics from basidiomycetes. XXXIII. Oudemansin X, a new antifungal *E*-β-methoxyacrylate from *Oudemansiella radicata* (Relhan ex Fr.) Sing. J. Antibiotics **43**, 1010–1011.

Anke T. (1995), The antifungal strobilurins and their possible ecological role. Abstr. IMC 5, Vancouver 1994 and Can. J. Bot. **73** Suppl. 1, 940–945.

Anke H., Morales P. and Sterner O., (1996), Assays of the biological activities of two fatty acids derivatives formed in the fruit bodies of *Cantharellus* species as a reponse to injury. Planta Med. **62**, 181–184.

Anke T. (1997), Agricultural fungicides: The strobilurins. In: Fungal Biotechnology, T. Anke Ed., pp. 206–212, Chapman & Hall.

Akita H., Koshiji H., Furuichi A., Horikoshi K. and Oishi T.(1983), The absolute configuration of oudemansin – Total synthesis of (-)-oudemansin. Tetrahedron Lett. **24**, 2009–2010.

Becker W. F. von Jagow G., Anke T. and Steglich W. (1981), Oudemansin, strobilurin A, strobilurin B and myxothiazol: New inhibitors of the bc_1 segment of the respiratory chain with a *E*-ß-methoxyacrylate system as common structural element. FEBS-Lett. **132**, 329–333.

Engler M., Anke T., Klostermeyer D. and Steglich W. (1995), Hydroxystrobilurin A, a new antifungal *E*-β-methoxyacrylate from a *Pterula* species. J. Antibiotics **48**, 884–885.

Engler,M., Anke T., Sterner O. and Brandt U. (1997a), Pterulinic acid and pterulone, two novel inhibitors of NADH : ubiquinone oxidoreductase (complex I) produced by a *Pterula* species I. Production, isolation and biological activities. J. Antibiotics **50**, 325–329.

Engler M., Anke T. and Sterner O. (1997b), Pterulinic acid and pterulone, two novel inhibitors of NADH : ubiquinone oxidoreductase (complex I) produced by a *Pterula species* II. Physico-chemical properties and structure elucidation. J. Antibiotics **50**, 330–333.

Engler M., Anke T. and Sterner O. (1998a), Production of antibiotics by *Collybia nivalis*, *Omphalotus olearius*, a *Favolaschia* and a *Pterula* species on natural substrates. Z. Naturforsch. **53c**, 318–324.

Engler M., Anke T. and Sterner O. (1998b), Tintinnadiol, a new sphaeroane diterpene from fruiting bodies of *Mycena tintinnabulum*. Phytochemistry, in print.

Erkel G. (1990), Neue Inhibitoren der AMV-Reverse Transkriptase aus Basidiomyceten. Ph. D. Thesis, Univ. Kaiserslautern.

Kraiczy P., Haase U., Gencic S., Flindt S., Anke T., Brandt U. and von Jagow G. (1996), The molecular basis for the natural resistance of the cytochrome bc_1 complex from strobilurin-producing basidiomycetes to Center Q_p inhibitors. Eur. J. Biochem. **235**, 54–63.

Mohamadi F. Richards N. G. J., Guida W. C., Liskamp R., Lipton M., Canfield C., Chang G., Hendrickson T. and Still W. C. (1990), MacroModel – an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. J. Comput. Chem. **11**, 440–467. The conformational analysis was performed with MacroModel®, Version 5.5 with MMFF (Merck Molecular Force Field).

Zapf S., Hossfeld M., Anke H., Velten R. and Steglich W. (1995), Darlucins A and B, new isocyanide antibiotics from *Sphaerellopsis filum* (*Darluca filum*). J. Antibiotics **48**, 36–41.

Weber W., Anke T., Steffan B. and Steglich W. (1990), Antibiotics from basidiomycetes. XXXII. Strobilurin E: a new cytostatic and antifungal (*E*)-β-methoxyacrylate antibiotic from *Crepidotus fulvotomentosus Peck*. J. Antibiotics **43**, 207–212.