Strobilurin M, Tetrachloropyrocatechol and Tetrachloropyrocatechol Methyl Ether: New Antibiotics from a *Mycena* Species

MICHAEL DAFERNER and TIMM ANKE*

LB Biotechnologie der Universität,
Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, FRG

VERONIKA HELLWIG and WOLFGANG STEGLICH*

Institut für Organische Chemie der Universität München,
Karlstr. 23, D-80333 München, FRG

OLOV STERNER

Division of Organic Chemistry 2, Chemical Center, University of Lund,
S-221 00 Lund, Sweden

(Received for publication April 27, 1998)

The antifungal and cytostatic compound strobilurin M (**1**) is a new variant of the strobilurins produced by *Mycena* sp. 96097, a tropical basidiomycete. The same fungus was found to produce tetrachloropyrocatechol (**3a**) and tetrachloropyrocatechol methyl ether (**3b**), new natural products, which exhibit antifungal, antibacterial and cytotoxic activities.

The genus *Mycena* (*Agaricales*, *Tricholomataceae*) has been a good source of new antibiotic metabolites. Cultures of *Mycena leaiana* are known to produce the bioactive sesquiterpenoid leianafulvene[1]. Mycenon, a chlorinated benzoquinone derivative is an inhibitor of isocitrate lyase from *Mycena* sp. 87202[2]. Two tetraacetylenic metabolites, 10-hydroxyundeca-2,4,6,8-tetraynamide and 3,4,13-trihydroxytetradeca-5,7,9,11-tetraynoic acid γ-lactone have been described from *Mycena viridimarginata*[3]. Bioactive derivatives of glutamic acid were isolated from cultures of *Mycena pura*[4]. The same fungus was found to produce α-methylene-γ-aminobutyric acid[5]. Haematopodin, a pyrroloquinoline derivative was isolated from *Mycena haematopus*[6]. Remarkable is the tetrachlorohydroquinone methyl ether (drosophilin A), from *Mycena megaspora*[7], which exhibits several biological activities[8]. Many *Mycena* species have been described to produce strobilurins and the related oudemansins[9], (*E*)-β-methoxyacrylates with high antifungal activities due to an inhibition of the electron transfer within the bc_1 complex (complex III) of the respiratory chain[10,11]. In the following, we wish to describe the production, isolation, biological properties and structure elucidation of strobilurin M (**1**), tetrachloropyrocatechol (**3a**) and tetrachloropyrocatechol methyl ether (**3b**) from *Mycena* sp. 96097.

Materials and Methods

General

For analytical HPLC a Hewlett Packard 1090 series II instrument and for preparative HPLC a Gilson model 302 or Jasco PU-980 instrument were used. TLC experiments were performed on Macherey-Nagel Alugram Sil G/UV_{254} precoated plates. NMR spectra, ^1H NMR at 600 MHz and ^{13}C NMR at 150 MHz, were recorded at room temperature with a Bruker AMX 600 spectrometer, in $CDCl_3$ or CD_3OD with the solvent signals (7.26/77.0 or 3.35/49.0 ppm, respectively) as reference. Mass spectra were recorded with a Finnigan MAT 95 Q (direct inlet, 70 eV) or a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Perkin Elmer λ 16 and a Bruker IFS 48 spectrometer. CD-Spectra were recorded on a Jobin-Yvon Instruments S.A. CD-6 Dichrograph; optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Producing Organism

Fruiting bodies of *Mycena* sp. 96097 were collected in New Caledonia. The specimen showed the characteristics of the genus[1,2]. The species, however, could not be identified. Mycelial cultures were derived from a fruiting body's spore print. The strain is deposited in the culture collection of the LB Biotechnologie, Universität Kaiserslautern.

Fermentation

For the production of strobilurin M (**1**), fermentations were carried out in 20 liters of a cornmeal medium composed of (g/liter): cornmeal 10, glucose 7, KH_2PO_4 1.5, KCl 0.5, $NaNO_3$ 0.5, $MgSO_4 \times 7H_2O$ 0.5, pH 5.8, in a Biolafitte C6 fermenter at 22°C with an aeration rate of 3 liters/minute and agitation (120 rpm). A well grown culture of *Mycena* sp. 96097 (250 ml) in the same medium was used as inoculum.

For the production of **3a** and **3b** the fermentation was carried out in a Biostat U fermentation apparatus (Braun) with 100 liters of a YMG medium containing (g/liters): yeast extract 4, malt extract 10, glucose 4, pH 5.5. Ten liters of a well grown seed culture in the same medium served as inoculum.

During fermentation 100 ml samples were taken and the mycelia and culture fluid were separated by filtration. The culture fluid was extracted with 100 ml of ethyl acetate and the residue obtained after evaporation of the organic solvent was taken up in 1 ml of methanol. The mycelia were extracted with 100 ml of methanol - acetone 1 : 1 and the crude extract taken up in 1 ml of methanol. 25 μl of the concentrated solutions were assayed for antifungal activity in the agar plate diffusion assay with *Mucor miehei* as test organism. 10 μl were analyzed by analytical HPLC [Merck LiChrospher 100 RP-18, 5 μm; column 125 × 4 mm; flow: 1.5 ml/minute; gradient: H_2O-acetonitrile 0 ~ 60% in 5 minutes, 60% for 10 minutes, 60 ~ 100% in 5 minutes; Rt (strobilurin M) = 19.5 minutes; Rt (**3a**) = 19.1 minutes; Rt (**3b**) = 19.8 minutes].

Isolation of Strobilurin M (**1**)

After 28 days , when the content of strobilurin M in the mycelia was highest, the mycelia (wet weight: 430 g) were separated from the culture fluid (15 liters) and extracted with a total of 4 liters of methanol - acetone 1 : 1. The aqueous remains (112 ml) were extracted twice with 100 ml of ethyl acetate yielding 620 mg of crude extract. This was applied onto a silica gel column (Merck 60, 0.063 ~ 0.2 mm; 14 × 2.5 cm). Upon elution with cyclohexane - ethyl acetate 9 : 1, 210 mg of an enriched extract were obtained. Final purification was achieved by preparative HPLC on LiChrosorb Diol [7 μm; column 250 × 25 mm; flow rate 5 ml/minute; detection at λ = 210 nm) using a cyclohexane - *tert.* butyl methyl ether (*t*BME) gradient: 0 ~ 20 minutes, 100% cyclohexane; 20 ~ 30 minutes, 0 ~ 10% *t*BME; 30 ~ 60 minutes, 10% *t*BME; 60 ~ 70 minutes, 10 ~ 20% *t*BME; 70 ~ 85 minutes, 20% *t*BME; 85 ~ 105 minutes, 20 ~ 100% *t*BME]. 14 mg of strobilurin M were eluted after 60 minutes. Strobilurin D (7 mg) was eluted after 80 minutes, strobilurin E (4 mg) after 55 minutes.

The culture fluid was extracted twice each with 10 liters of ethyl acetate, and the crude extract (1.2 g) was subjected to silica gel chromatography as described above. 370 mg of an enriched product were further purified by preparative HPLC (see above) yielding 7 mg of strobilurin M.

Isolation of Tetrachloropyrocatechol (**3a**) and Tetrachloropyrocatechol Methyl Ether (**3b**)

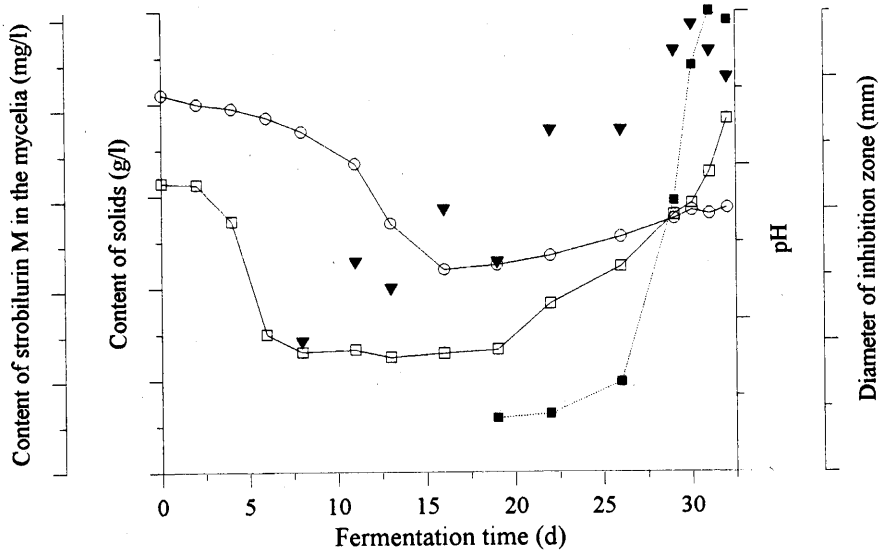
After 25 days of fermentation, the culture fluid (80 liters) was separated from the mycelia and passed through a column (18 × 11 cm) containing Mitsubishi Diaion HP 21 adsorber resin. The column was washed with water and the compounds were eluted with 5 liters of acetone. The acetone eluate was concentrated and the crude product (1 g) was applied onto a silica gel column (Merck 60, 0.063 ~ 0.2 mm; 12 × 6.5 cm). The mycelia were freeze dried (weight: 77 g) and extracted with a total of 8 liters methanol - acetone 1 : 1. The aqueous remains (20 ml) were extracted with twice 20 ml of ethyl acetate yielding 1 g of crude extract. This was applied onto a silica gel column (Merck 60, 0.063 ~ 0.2 mm; 14 × 2.5 cm).

Elution of the silica gel columns with cyclohexane - ethyl acetate 1 : 3 yielded enriched products containing **3a** (48 mg from the culture fluid, 82 mg from the mycelia). These were combined and purified by preparative HPLC on LiChrospher RP-18 [7 μm, 250 × 25 mm; flow rate 5 ml/minute; water - methanol gradient: 0 ~ 40 minutes, 0 ~ 70% methanol; 40 ~ 50 minutes, 70%; 50 ~ 90 minutes, 70 ~ 100%]. **3a** eluted after 70 minutes. Yield: 12 mg.

Elution of the silica gel columns with cyclohexane - ethyl acetate 7 : 3 yielded enriched products containing **3b** (47 mg from the culture fluid, 37 mg from the mycelia). Purification by preparative HPLC on LiChrosorb Diol [7 μm; column 250 × 25 mm; flow rate 5 ml/minute; detection at λ = 210 nm; cyclohexane - *t*BME gradient: 0 ~ 10 minutes, 0 ~ 10% *t*BME; 10 ~ 20 minutes, 10%; 20 ~ 30 minutes, 10 ~ 20%; 30 ~ 40 minutes, 20%; 40 ~

Fig. 1. Fermentation of *Mycena* sp. 96097 in 20 liters cornmeal medium.

□ pH; ○ content of solids (g/liter); ▼ inhibition zone (mm) caused by 25 μl of mycelial extract; ■ content of strobilurin M in the mycelia.



50 minutes, 20~30%; 50~60 minutes, 30%; 60~80 minutes, 30~100%]. Yield: 16 mg of **3b** (elution after 57 minutes).

Tests for Biological Activities

The assays for antimicrobial[13] and cytotoxic[14] activities were carried out as described previously. The inhibition of respiration of *Penicillium notatum* was measured as described by WEBER *et al.*[15]

Results and Discussion

Production of Strobilurin M (1) and Other Strobilurins

Figure 1 shows a graphical representation of several parameters monitored throughout the course of a typical fermentation of *Mycena* sp. 96097 in cornmeal medium. The production of strobilurin M in the mycelia and in the culture fluid, as detected by HPLC, started after 18 days and peaked after 30 days. Activity in the agar diffusion assay was already detected after seven days and was due to the presence of strobilurin A, which disappeared after 20 days. At the same time, strobilurins D, E and M were detected. D and E peaked after 25 days at 0.5 mg/liter and 0.35 mg/liter respectively. Maximum activity was reached after 28 days, which was due to the simultaneous presence of the strobilurins M, D and E. On day 32, when the content of **1** in the culture fluid as well as in the mycelia was highest, the fermentation was terminated. The halogenated phenols could not be detected in fermentations in cornmeal medium.

Production of Tetrachloropyrocatechol (3a) and Tetrachloropyrocatechol Methyl Ether (3b)

For the production of tetrachloropyrocatechol (**3a**) and tetrachloropyrocatechol methyl ether (**3b**) YMG medium was used for fermentation. Antifungal activity appeared after four days in the culture fluid and after nine days in the mycelia. Maximum activity was observed after 24 days due to the simultaneous presence of **3a**, **3b** and traces of the strobilurins M (**1**), D and E (**2**).

Structural Elucidation of Strobilurin M (1)

The UV and IR data of **1** resemble those of strobilurin E (**2**)[15,16]. As indicated by its molecular formula, $C_{26}H_{34}O_6$ (HR-MS), **1** contains two more hydrogen and one less oxygen atoms than **2**. The ^1H and ^{13}C NMR spectra (Table 1) show the characteristic signals of the (*E*)-β-methoxyacrylate unit and signals indicating a 1,4,5-trisubstituted benzene ring fused to a dioxane ring containing a CH_2-group and a quarternary acetalic carbon. Additional signals can be assigned to an isopropyl group and a 3,3-dimethylallyloxy residue. The HMBC-correlation between the CH of the isopropyl group (δ_H 2.41) and the acetalic carbon (δ_C 99.01) confirm the connection between these two fragments. The 3,3-dimethylallyloxy residue is connected to the acetalic carbon across an ether linkage, which completes structure

Table 1. ^{13}C and ^{1}H NMR spectral data for strobilurin M (**1**) (150.92 and 600.15 MHz).

	δ_C [ppm] in CD_3OD	δ_C [ppm] in $CDCl_3$	$^nJ_{CH}$ [Hz][a] in $CDCl_3$		δ_H [ppm] in CD_3OD[b]	δ_H [ppm] in $CDCl_3$[c]
C-1	115.65	114.58	Dt (157.5, 6.1)	1-H	6.89 (s, br)	6.90 (d)
C-2	143.06	141.68	dd (6.5, 4.4)			
C-3	144.15	142.72	tdd (7.4, 5.5, 2.7)			
C-4	117.64	116.78	D (160.2)	4-H	6.79 (d)	6.78 (d)
C-5	121.05	120.29	Ddd (159.7, 7.6, 4.9)	5-H	6.88 (dd)	6.83 (dd)
C-6	133.17	131.72	tm (6.8)			
C-7	131.41	130.81	Dm (148.4)	7-H	6.39 (d)	6.36 (d)
C-8	126.38	124.92	D (153.1)	8-H	6.49 (dd)	6.46 (dd)
C-9	131.02	129.85	Dm (155.80)	9-H	6.19 (d)	6.21 (d)
C-10	131.44	130.30	m			
C-11	111.73	110.92	m			
C-12	160.88	158.79	Dq (182.6, 5.3)	12-H	7.55 (s)	7.40 (s)
C-13	169.70	167.85	quint (3.5)			
C-14	23.74	23.61	Qd (127.2, 6.5)	14-H	1.94 (s)	1.94 (s)
C-15	62.31	61.86	Qd (145.5, 6.1)	15-H	3.89 (s)	3.82 (s)
C-16	51.96	51.56	Q (146.8)	16-H	3.75 (s)	3.71 (s)
C-1′	66.19	65.00	DDd (149.9, 146.1, 4.9)	1′$_a$-H	4.18 (d)	4.13 (d)
				1′$_b$-H	3.99 (d)	3.91 (d)
C-2′	100.38	99.01	m			
C-3′	32.70	31.46	Dhept (127.0, 4.1)	3′-H	2.47 (hept)	2.41 (hept)
C-4′	17.36	17.15	Qm (126.4)	4′-H	1.10 (d)	1.03 (d)
C-5′	16.67	16.43	Qm (127.9)	5′-H	1.10 (d)	1.03 (d)
C-1″	58.73	57.68	T (142.2)	1″$_a$-H	4.11 (dd)	4.05 (dd)
				1″$_b$-H	4.06 (dd)	3.98 (dd)
C-2″	121.89	120.62	Dm (155.3, 5.3)	2″-H	5.14 (tm)	5.13 (tm)
C-3″	137.57	136.75	m			
C-4″	17.77	17.69	Qm (126.3)	4″-H	1.54 (s)	1.47 (s)
C-5″	25.80	25.70	Qm (124.8)	5″-H	1.65 (s)	1.60 (s)

[a] Capital letters denote $^1J_{CH}$-couplings, whereas small letters are used for $^nJ_{CH}$-couplings (n = 2, 3).
[b] J_{HH} [Hz] in CD_3OD: 1,5 = 1.5; 4,5 = 8.2; 7,8 = 15.6; 8,9 = 10.4; 1′$_a$,1′$_b$ = 11.2; 3′, 4′/5′ = 7.1; 1″$_a$,1″$_b$ = 11.4; 1″$_a$,2″ = 6.7; 1″$_b$,2″ = 7.1.
[c] J_{HH} [Hz] in $CDCl_3$: 1,5 = 1.8; 4,5 = 8.3; 7,8 = 15.5; 8,9 = 10.3; 1′$_a$,1′$_b$ = 11.0; 3′, 4′/5′ = 7.0; 1″$_a$,1″$_b$ = 11.5; 1″$_a$,2″ = 7.1; 1″$_b$,2″ = 6.8.

1 for strobilurin M.

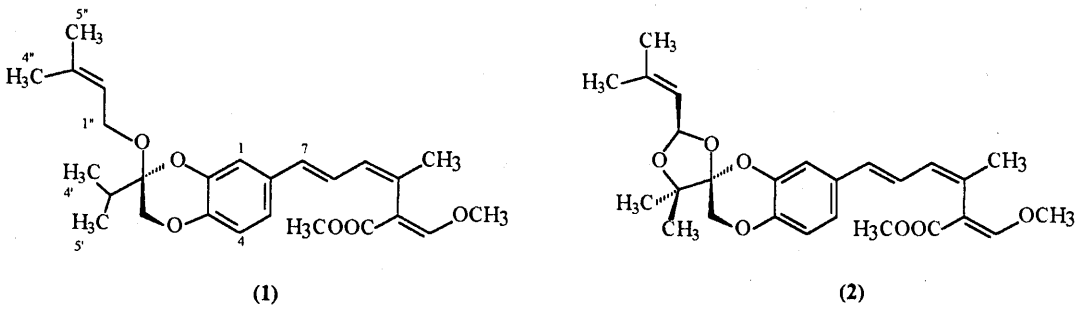
The attachment of the dioxane ring to the benzene nucleus was established by selective decouplings of the ^1H-coupled ^{13}C NMR spectrum (Table 1). The pair of doublets at δ_C 141.68 ($^3J_{C\text{-}2,H\text{-}4}$ = 6.5 Hz, $^2J_{C\text{-}2,H\text{-}1}$ = 4.4 Hz) becomes a singlet after simultaneous irradiation at the resonances of 1-H, 4-H and 5-H and has therefore to be assigned to C-2. The complex multiplet for C-3 at δ_C 142.72 can be simplified to a triplet of doublets ($^3J_{C\text{-}3,H\text{-}1} = {}^3J_{C\text{-}3,H\text{-}5}$ = 7.4 Hz, $^2J_{C\text{-}3,H\text{-}4}$ = 2.7 Hz) by selective irradiation at the frequency of the CH_2-group in the dioxane ring. Simultaneous irradiation at all aromatic resonances changes the signal to a doublet ($^3J_{C\text{-}3,H\text{-}1'}$ = 5.5 Hz) due to the remaining coupling to one of the methylene protons (Figure 3).

The substitution of **1** at the dioxane ring given in Figure 2 corresponds to that of strobilurin E (**2**) whose structure has been proven by total synthesis[16]. From the close correspondence of their CD spectra the identical, but unknown absolute configuration can be assigned to **1** and **2**.

Strobilurin M (**1**) was obtained as a yellow oil. Rf 0.73 (toluene - acetone 70 : 30); $[\alpha]_D^{30}$ +65.5 (*c* 0.17, CH_3OH); UV λ_{max} (MeOH) nm (log ε) 229 (4.48), 301 (4.44), 321 (4.43); CD (CH_3OH) $\lambda_{extreme}$ nm (Δε) 208 (+3.39), 221 (+3.09), 240 (−1.43), 319 (+4.26); EI-MS (direct inlet, 80°C) *m/z* (relative intensity %) 442.2291 (100, M^+, calcd for $C_{26}H_{34}O_6$ 442.2355), 374 (39), 342 (17), 237 (65); IR_{max} (KBr) cm^{-1} 3439 (w, br), 2937 (m), 1710 (st), 1628 (m), 1507 (st), 1433 (m), 1288 (st), 1274 (st), 1237 (m), 1120 (st); ^1H and ^{13}C NMR data see Table 1.

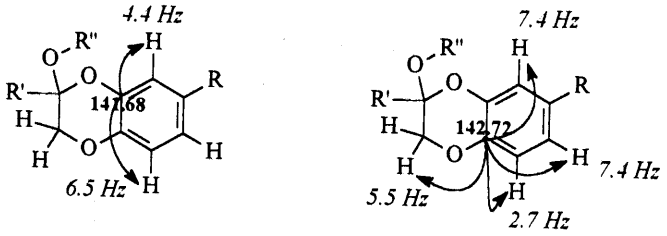
Strobilurin E (**2**)[15,16]: $[\alpha]_D^{30}$ +103.59 (*c* 0.19, CH_3OH); UV λ_{max} (MeOH) nm (log ε) 226 (3.49), 299 (3.44), 318 (3.41); CD (CH_3OH) $\lambda_{extreme}$ nm (Δε) 207

Fig. 2. Structures of strobilurin M (**1**) and strobilurin E (**2**).



(1) (2)

Fig. 3. $^{n}J_{CH}$-coupling-partners of C-2 (δ_C 141.68) and C-3 (δ_C 142.72) in **1**.



(+6.31), 219 (+3.72), 237 (−4.81), 319 (+8.28).

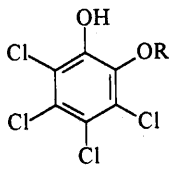
Identification of Tetrachloropyrocatechol (**3a**) and Tetrachloropyrocatechol Methyl Ether (**3b**)

The two phenols **3a** and **3b** were identified by NMR spectroscopy and mass spectrometry. The EI mass spectra showed the typical isotope pattern of four chlorine atoms, and high resolution measurements indicated an elemental composition of $C_6H_2Cl_4O_2$ and $C_7H_4Cl_4O_2$ for **3a** and **3b**, respectively. From the ^{13}C NMR spectrum and comparison with an authentic sample **3a** was identified as tetrachloropyrocatechol (**3a**). In **3b**, the presence of an additional *O*-methyl group was supported by the ^1H and the ^{13}C NMR spectra, and due to the loss of symmetry all carbons are nonequivalent. Since the ^{13}C chemical shifts of **3b** are close to those of **3a** the compound was identified as 3,4,5,6-tetrachloro-2-methoxy-phenol[17].

Tetrachloropyrocatechol (**3a**) is a metabolite of the biocide pentachlorophenol *in vitro* and *in vivo*[18], but to our knowledge **3a** and its monomethyl ether **3b** are not known yet as natural products.

3a was obtained as a colourless solid. ^1H NMR (δ, mult.): 3.77, br s, 1-OH and 2-OH. ^{13}C NMR (δ): 140.1 C-1 and C-2; 123.8 C-4 and C-5; 118.9 C-3 and C-6. EI-MS, *m/z* (% rel. int.): 252 (12%); 250 (50%); 248 (100%); 245.8823 (M^+, 81%, $C_6H_2Cl_4O_2$ requires 245.8809), 212 (8%), 182 (8%), 147 (22%), 84 (35%).

Fig. 4.



3a: R = H: tetrachloropyrocatechol;
3b: R = CH_3: tetrachloropyrocatechol methyl ether.

3b was obtained as a colourless solid. ^1H NMR (δ, mult.): 3.95, s, 2-OMe; 3.60, br s, 1-OH. ^{13}C NMR (δ): 146.0 C-1; 143.4 C-2; 128.3 C-4; 126.4 C-5; 123.9 C-3; 119.4 C-6; 61.3 2-OMe. EI-MS, *m/z* (% rel. int.): 266 (10%), 264 (44%), 262 (90%), 259.8978 (M^+, 69%, $C_7H_4Cl_4O_2$ requires 259.8965), 251 (12%), 249 (49%), 247 (100%), 245 (82%), 221 (17%), 219 (38%), 217 (30%), 181 (15%), 129 (20%), 95 (47%).

Biological Properties

Strobilurin M (**1**)

Like the other strobilurins and oudemansins, strobilurin M (**1**) exhibits high antifungal activity in the agar diffusion assay (Table 2). Growth of most test organisms was inhibited at concentrations starting from 1 μg/disc.

Table 2. Antifungal activity of strobilurin M in the agar diffusion assay.

Organism	Diameter of inhibition zone (mm)		
	0.1[a]	1[a]	10[a]
Absidia glauca (+)	—	8i	10i
Absidia glauca (−)	8i	11i	13i
Alternaria porri	11i	15i	19i
Ascochyta pisi	—	—	15i
Aspergillus ochraceus	9i	13i	16i
Botrytis cinerea	—	—	15
Cladosporium cladosporioides	8	12	18
Epicoccum purpurascens	—	8i	12i
Fusarium fujikuroi	—	8i	10i
Fusarium oxysporum	—	—	—
Mucor miehei	9	10	12
Nadsonia fulvescens	—	—	—
Nematospora coryli	—	9i	11i
Paecilomyces variotii	—	—	9
Penicillium islandicum	8i	10i	16i
Penicillium notatum	—	8i	10i
Phoma clematidina	—	—	10
Pythium irregulare	—	—	—
Rhodotorula glutinis	7	11	13
Saccharomyces cerevisiae is 1[b]	—	—	12i
Saccharomyces cerevisiae 288c	—	10i	15i
Zygorhynchus moelleri	7i	9i	11i

[a] μg/disc.
[b] Gift of Prof. LACROUTE, Strasbourg.
i: Incomplete (=with a narrow, hazy zone of growth on the edge of the inhibition zone).

Table 3. Antimicrobial activity of tetrachloropyrocatechol (**3a**) and tetrachloropyrocatechol methyl ether (**3b**) in the serial dilution assay.

Organism	Minimal inhibitory concentration (μg/ml)	
	3a	**3b**
Bacteria		
Arthrobacter citreus	20	50
Bacillus brevis	10	20
Bacillus subtilis	20	50
Corynebacterium insiduosum	>100	>100
Escherichia coli K12	20	50
Micrococcus luteus	>100	>100
Mycobacterium phlei	20	50
Streptomyces sp. ATCC 23836	50	50
Fungi		
Fusarium oxysporum	20	20
Mucor miehei	5	20
Nematospora coryli	20	50
Paecilomyces variotii	10	50
Penicillium notatum	10	20
Rhodotorula glutinis	10	20
Saccharomyces cerevisiae is 1[a]	20	50
Saccharomyces cerevisiae 288c	50	50

[a] Gift of Prof. LACROUTE, Strasbourg.

Antibacterial effects were not observed. Oxygen uptake of *Penicillium notatum* is inhibited starting at 0.2 μM strobilurin M (IC_{50}=2.5 μM). Starting at 5 μg/ml, **1** inhibits the growth HeLa S3 (human epithelial carcinoma) cells. Up to concentrations of 100 μg/ml strobilurin M did not cause cell lysis and a residual growth of the cells was observed. The antifungal and cytostatic properties of strobilurin M are somewhat lower as compared to strobilurin E which is thought to reflect the markedly different substitution patterns.

Tetrachloropyrocatechol (**3a**) and Tetrachloropyrocatechol Methyl Ether (**3b**)

3a and **3b** both exhibit antimicrobial and cytostatic properties. In the serial dilution assay, MIC's of 10~50 μg/ml for **3a** and of 20~50 μg/ml for **3b** were observed with several test organisms (Table 3). Cytotoxic effects on HeLa S3 cells were observed with IC_{50} values of 2~5 μg/ml for **3a** and **3b**. As is to be expected both compounds appear to be biocides with a broad spectrum of activities.

Acknowledgements

We thank the BMBF (Bundesministerium für Bildung und Forschung) for financial support.

References

1) HARTTIG, U.; T. ANKE, A. SCHERER & W. STEGLICH: Leianafulvene, a sesquiterpenoid fulvene derivative from cultures of *Mycena leaiana*. Phytochem. 29: 3942~3944, 1990
2) HAUTZEL, R.; H. ANKE & W. S. SHELDRICK: Mycenon, a new metabolite from a Mycena species TA 87202 (*Basidiomycetes*) as an inhibitor of isocitrate lyase. J. Antibiotics 43: 1240~1244, 1990
3) JENTE, R.; F. BOSOLD, J. BÄUERLE & T. ANKE: Tetraacetylenic metabolites from *Mycena viridimarginata*. Phytochem. 24: 553~559, 1985
4) HATANAKA, S.-I. & H. KATAYAMA: L-γ-Propylidene glutamic acid and related compounds from *Mycena pura*. Phytochem. 14: 1434~1436, 1975
5) HATANAKA, S.-I. & K. TAKISHIMA: α-Methylene-γ-aminobutyric acid from *Mycena pura*. Phytochem. 16: 1820~1821, 1977
6) BAUMANN, C.; M. BRÖCKELMANN, B. FUGMANN, B. STEFFAN, W. STEGLICH & W. S. SHELDRICK: Haematopo-

din, ein ungewohnliches Pyrrolchinolin-Derivat aus dem Blut-Helmling (*Mycena haematopus*, *Agaricales*). Angew. Chem. 105: 1120~1121, 1993

7) VAN EIJK, G. W.: Drosophilin, a methyl ether from *Mycena megaspora*. Phytochem. 14: 2506, 1975

8) BASTIAN, W.: PhD thesis, University of Kaiserslautern, 1985

9) ANKE, T.: Strobilurins. *In* Fungal Biotechnology. *Ed.* ANKE, T., pp. 206~212, Chapmann & Hall, 1997

10) BECKER, W. F.; G. VON JAGOW, T. ANKE & W. STEGLICH: Oudemansin, strobilurin A, strobilurin B and myxothiazol: New inhibitors of the bc_1 segment of the respiratory chain with a (*E*)-β-methoxyacrylate system as common structural element. FEBS-Letters 132: 329~333, 1981

11) KRAICZY, P.; U. HAASE, S. GENCIC, S. FLINDT, T. ANKE, U. BRANDT & G. VON JAGOW: The molecular basis for the natural resistance of the cytochrome bc_1 complex from strobilurin-producing basidiomycetes to Center Qp inhibitors. Eur. J. Biochem. 235: 54~63, 1996

12) MAAS GEESTERANUS, R. A.: *Mycenas* of the northern hemisphere. North-Holland, Amsterdam, 1992

13) ANKE, H.; O. BERGENDORFF & O. STERNER: Assays of the biological activities of guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. Food Chem. Toxicol. 27: 393~398, 1989

14) ZAPF, S.; M. HOBFELD, H. ANKE, R. VELTEN & W. STEGLICH: Darlucins A and B, new isocyanide antibiotics from *Sphaerellopsis filum* (*Darluca filum*). J. Antibiotics 48: 36~41, 1995

15) WEBER, W.; T. ANKE, B. STEFFAN & W. STEGLICH: Strobilurin E: A new cytostatic and antifungal (*E*)-β-methoxyacrylate antibiotic from *Crepidotus fulvotomentosus* Peck. J. Antibiotics 43: 207~212, 1990

16) BERTRAM, G.; A. SCHERER, W. STEGLICH, W. WEBER & T. ANKE: Total synthesis of (±)-strobilurin E. Tetrahedron 37: 7955~7958, 1996

17) THAKORE, A. N. & A. C. OEHLSCHLAGER: Structures of toxic constituents in kraft mill caustic extraction effluents from ^{13}C and ^{1}H nuclear magnetic resonance. Can. J. Chem. 55: 3298~3303, 1977

18) AHLBORG, U. G.; J. E. LINDGREN & M. MERCIER: Metabolism of pentachlorophenol. Arch. Toxicol. 32: 271~281, 1974