New Botrydial Sesquiterpenoids from *Hymenoscyphus epiphyllus*

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Hymenoscyphus epiphyllus, Botrydial Derivatives, Antimicrobial, Cytotoxic, Mutagenic

Four new botrydial derivatives, hymendial (**1**), 7α-hydroxydihydrobotrydial (**2**), 7α-hydroxy-10-*O*-methyldihydrobotrydial (**4**), and 7α-acetoxy-15α-methoxy-10-*O*-methyl-dihydrobotrydial (**5**) were isolated together with dihydrobotrydial (**3**) from the culture fluid of the ascomycete *Hymenoscyphus epiphyllus*. In addition, cytochalasin H (**6a**), 18-deoxycytochalasin H (**6b**) and (+)-mellein (**7**) were produced by this fungus. Hymendial (**1**), possessing an α, β-unsaturated dialdehyde functionality, exhibits antimicrobial and cytotoxic activities and is mutagenic in the Ames *Salmonella* assay.

Introduction

In a screening of higher fungi for antimicrobial secondary metabolites, extracts of the culture filtrate of the ascomycete *Hymenoscyphus epiphyllus*, strain A236–87, were found to be active against the fungi *Nematospora coryli* and *Mucor miehei*. The bioassay-guided fractionation of the extracts yielded several antifungal compounds; a new botrydial sesquiterpene which we have named hymendial (**1**), cytochalasin H (**6a**), 18-deoxycytochalasin H (**6b**) and (+)-mellein (**7**). In addition, several inactive botrydial derivatives were isolated. In this report we describe the fermentation of the producing organism, the isolation and the chemical as well as biological characterization of the new secondary metabolites. Parts of the results have been presented at the 39th Annual Congress on Medicinal Plant Research, Saarbrücken, 1991 (Anke *et al.*, 1991a).

Experimental

General

Materials for preparative HPLC, LiChroSorb CN (7 μm), LiChroGel PS1 (10 μm), LiChroSphere RP18 (7 μm), were obtained from Merck Darmstadt: Preparative HPLC was carried out on JASCO HPLC (PU 980, MD 910), analytical HPLC was carried out on Hewlett-Packard 1090 Type II with LiChroSphere RP 18 (10 μm; 125 x 4 mm) and a water-acetonitrile gradient.

Producing strain

Fruiting bodies of *Hymenoscyphus epiphyllus* growing on oak leaves (collected in Wales) showed characteristics of the genus and species as described by Dennis (1981). A mycelial culture of strain A 236–87 was obtained from ascospores. It is maintained on yeast extract, malt extract and glucose agar. For submerged cultivation, MGP medium (maltose 2%, glucose 1%, soybean peptone 0.1%, yeast extract 0.1%, KH_2PO_4 0.1%, $MgSO_4$ 0.05%, $CaCl_2$ x 2 H_2O 10 mM, $FeCl_3$ 6 mM, $ZnSO_4$ x 7 H_2O 6 μM) was used. A voucher specimen of the fungus is deposited in the herbarium of the Department of Biotechnology, University of Kaiserslautern.

Fermentation and isolation of metabolites

Fermentations were carried out in a 20 l fermenter (Braun Biostat U) at 24 °C with aeration (3.2 liter per minute) and agitation (120 rpm) in MGP medium (*vide supra*). Oxygen saturation was measured using a Braun oxygen electrode. Fermenters were inoculated with 200 ml of a 7 days old culture grown in a 500 ml Erlenmeyer flask with one indentation in the same medium at 22 °C. For the detection of the antimicrobial activity aliquots of the culture fluid (100 ml) were extracted twice with ethyl acetate. The combined ex-

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tracts were dried with Na_2SO_4. After evaporation of the solvent *in vacuo* (45 °C) the oily residue was redissolved in 1 ml methanol. These extracts (10 ml) were used for the determination of the antifungal activity in the agar diffusion assay with *Mucor miehei* and *Nematospora coryli* as test organisms. The fermentation was terminated after 150 hours when glucose was used up and the antifungal activity had reached a peak. The mycelia were separated from the culture fluid by filtration and discarded. The culture fluid was applied onto a column (4 cm x 35 cm) with Mitsubishi HP21 resin. After washing with 2 l H_2O, the bioactive compounds were eluted with 2 l of acetone. The eluate was evaporated *in vacuo* to an aqueous residue which was extracted twice with ethyl acetate. After evaporation of the solvent, 2 g of an oily crude extract were obtained. Upon standing overnight in the cold, a precipitate formed and was separated from the oil (see below). The crude extract was applied onto silica acid (40 g) and the active compounds were eluted using cyclohexane-ethyl acetate. Starting with 10% ethyl acetate, its content in the eluant was rised stepwise by 10%. Fractions containing compound **2** were eluted with 20% ethyl acetate (300 ml). Purification was achieved by preparative HPLC (LiChrosorb CN, cyclohexane-ethyl acetate 7:3) and gel filtration (LiChrogel PS1, 2-propanol) yielding 3 mg of white crystals from 18 liters of culture. Compounds **3**, **4**, and **5** eluted with 30% ethyl acetate (300 ml). Separation was achieved by preparative HPLC (LiChrosphere RP18) elution with a linear gradient of water-methanol. Compound **5** (14.1 mg) eluted with 30% methanol, compound **3** (27.4 mg) with 40% and compound **4** (39.6 mg) with 50%. Compound **1** was eluted with cyclohexane-ethyl acetate 4:6 (300 ml). After evaporation of the solvent, the residue was dissolved in methanol. Chromatography on LH-20 in methanol resulted in 12.3 mg of compound **1**. Figure 1 shows the isolation of compounds **6a, b**. Compound **7** was purified from the precipitate of the HP21 crude product using preparative HPLC (LiChroSphere

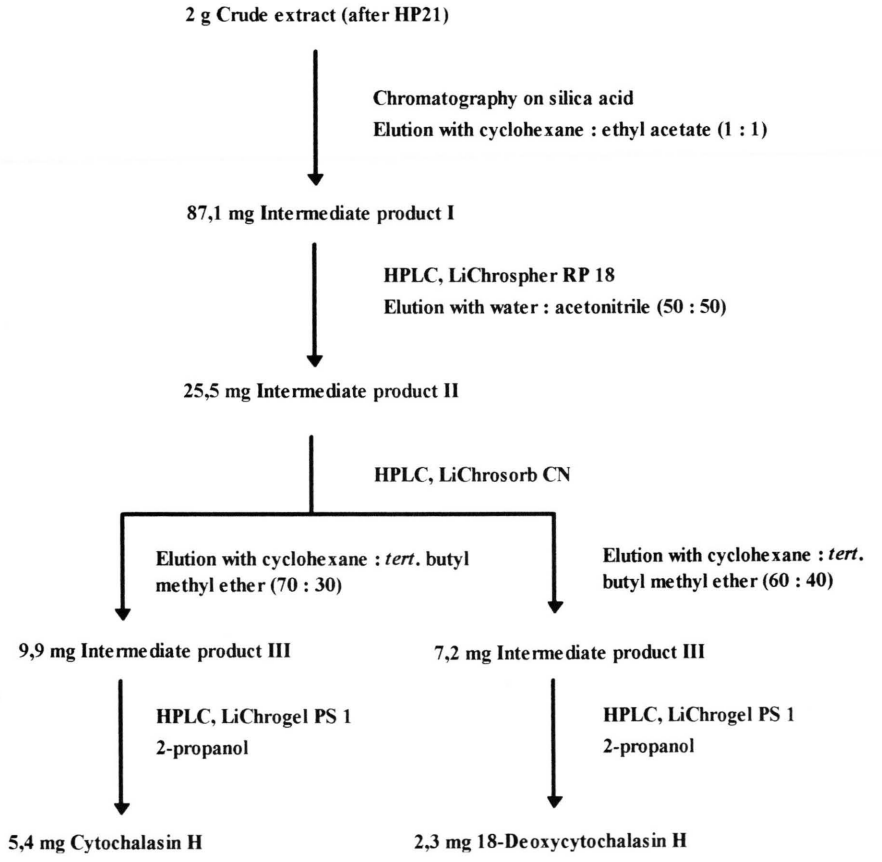


Fig. 1. Isolation of cytochalasin H **(6a)** and deoxycytochalasin H **(6b)**.

RP18, water-methanol, 4:6) and gel filtration (LiChrogel PS1, 2-propanol), yielding 27.4 mg of mellein.

Biological assays

The Ames test (Ames *et al.,* 1978) was carried out as a pour-plate assay (diameter of the plates 5 cm, 1 ml top layer and 5 ml bottom layer) using *Salmonella typhimurium* strains TA 98 and TA 100. Revertants were counted after 48–60 hours of incubation. Ethyl methanesulfonate *(MES)* and daunomycin (Sigma, St. Louis) were used as control mutagens. Tests were carried out in triplicates and the mean values are given. The tests for the evaluation of the antimicrobial and phytotoxic activities (Anke *et al.,* 1989a) and cytotoxic activities (Erkel *et al.,* 1992) have been previously described. The following concentrations were tested in the antimicrobial assays for the determination of MICs: 1, 2, 5, 10, 20, 30, 50, 100 µg/ml. Cyctotoxicity was tested at concentrations of 0.5, 1, 2.5, 5, 10, 20, 50 and 100 µg/ml. Experiments were repeated twice. For comparison isovelleral, a well known unsaturated dialdehyde sesquiterpene from *Lactarius vellereus,* was used (Anke and Sterner; 1991b, Anke *et al.,* 1989; Jonassohn *et al.,* 1997). Incorporation of ^{14}C-labelled thymidine, uridine, and leucine into DNA, RNA and protein of L1210 cells was tested as described previously (Weber *et al.,* 1990). The cells were grown in roller bottles for 72 hours and suspended in phosphate buffered saline to give $2x10^7$ cells /ml.

Spectroscopy

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$=145 Hz and $^nJ_{CH}$= 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Mass spectra were recorded with a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Perkin Elmer λ16 and a Bruker IFS48 spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 °C.

Physico-chemical properties of the compounds

Hymendial (**1**) was obtained as white crystals, m.p. 144–147 °C. $[\alpha]_D^{22}$+157° (*c* 1.1 in $CHCl_3$). UV (methanol) λ_{max} (ε): 249 nm (7,500). IR (KBr): 2950, 1730, 1680, 1380, 1230 and 1040 cm^{-1}. See Tables I and II for NMR data. MS (EI), *m/z*: 291.1605 (M^+ - OAc, 49%, $C_{17}H_{23}O_4$ requires 291.1596), 230 (24%), 202 (100%), 187 (85%), 173 (59%), 159 (73%).

7α-Hydroxydihydrobotrydial (**2**) was obtained as a colourless oil. $[\alpha]_D^{22}$+50° (*c* 0.6 in $CHCl_3$). UV (methanol) λ_{max} (ε): No maximum above 210 nm. IR (KBr): 3350, 2950, 1710, 1700, 1470, 1420, 1380, 1360, 1270, 1250, 1120, 1070, 1020, 990, 950 and 900 cm^{-1}. See Tables I and II for NMR data. MS (EI), *m/z*: 311.1841 (M^+ - OH, 38%, $C_{17}H_{27}O_5$ requires 311.1858), 250 (45%), 232 (40%), 220 (48%), 202 (51%), 191 (85%), 189 (71%), 43 (100%).

7α-Hydroxy-10-*O*-methyldihydrobotrydial (**4**) was obtained as white crystals, m.p. 178–180 °C. $[\alpha]_D^{22}$+90° (*c* 0.5 in $CHCl_3$). UV (methanol) λ_{max} (ε): No maximum above 210 nm. IR (KBr): 3545, 3505, 2970, 2945, 2920, 1715, 1360, 1265, 1255, 1120, 1080, 1070, 1055, 1005 and 950 cm^{-1}. See Tables I and II for NMR data. MS (EI), *m/z*: 311 (M^+ - OMe, 15%,), 250 (27%), 232 (45%), 204 (44%), 191 (86%), 189 (67%), 180 (73%), 151 (49%), 135 (51%), 109 (54%), 85 (53%), 43 (100%). MS (CI, CH_4), *m/z*: 311.1872 (M^+ -OMe, 17%, $C_{17}H_{27}O_5$ requires 311.1858), 250 (27%), 232 (43%), 204 (42%), 191 (85%), 189 (64%), 180 (69%), 43 (100%).

7α-Acetoxy-15α-methoxy-10-*O*-methyldihydrobotrydial (**5**) was obtained as a colourless oil. $[\alpha]_D^{22}$ +66° (*c* 2.2 in $CHCl_3$). UV (methanol) λ_{max} (ε): No maximum above 210 nm. IR (KBr): 3350, 2950, 1710, 1700, 1470, 1420, 1380, 1360, 1270, 1250, 1120, 1070, 1020, 990, 950 and 900 cm^{-1}. See Tables I and II for NMR data. MS (EI), *m/z*: 414 (M^+, 0.5%), 383 (1%), 354 (1%), 322 (2%), 294 (8%), 262 (11%), 234 (22%), 174 (64%), 159 (37%), 143 (85%), 101 (100%). MS (CI, NH_3), *m/z*: 400 (M^+ – CH_3OH + NH_4^+, 7%), 383 (M^+ – CH_3OH + H^+, 100%), 305 (8%).

Known compounds **3, 6a,b** and **7** were identified by their UV-, IR-, ^{1}H-, and ^{13}C NMR-spectra which were in full agreement with the literature (mellein $[\alpha]_D^{22}$-106°: Hoker and Simpson (1981); 18-deoxycytochalasin H: Dombrowski *et al.* (1992);

cytochalasin H: Patwardhan *et al.* (1974); dihydrobotridial: Fehlhaber and Geipel (1974)).

Results and Discussion

Fermentation of Hymenoscyphus epiphyllus and identification of the metabolites

Figure 2 shows a typical fermentation diagram of *Hymenoscyphus epiphyllus* A236–87. Antifungal activity against *Mucor miehei* and *Nematospora coryli* located in the culture fluid, reached its peak after 6 days, at this time, the glucose was almost consumed. For the isolation of secondary metabolites, fermentations were harvested after 7 days and processed as described in the experimental section.

The structures of all secondary metabolites isolated from *Hymenoscyphus epiphyllus* in this investigation are shown in Fig. 3. Several botrydials, among them dihydrobotrydial (**3**) have previously been isolated from *Botrytis* species, e.g. *B. cinerea* (Fehlhaber and Geipel, 1974), but this is the first time such sesquiterpenes are reported from a *Hymenoscyphus* species. Hymendial (**1**) differs from botrydials by the C-1/C-9 double bond, making hymendial (**1**) a member of the widespread group of bioactive terpenoids containing an unsaturated dialdehyde functionality. Interestingly, the two methylated derivatives **4** and **5** are true natural products and not artifacts formed during extraction and work-up. This was established by demonstrating their presence directly in the culture fluids and in ethyl acetate extracts. The elucidation of the structures of the compounds is based on the spectroscopic data presented in the Experimental section and in Tables I and II. The structures and the relative configurations were confirmed by COSY, NOESY, HMQC and HMBC 2D NMR experiments, and pertinent correlations observed in the HMBC and NOESY spectra of hymendial (**1**) are shown in Fig. 4. Cytochalasin H (**6a**), 18-deoxycytochalasin H (**6b**) and mellein (**7**) are known fungal metabolites, and their chemical and some of their biological properties have been reported (Patwardhan *et al.*, 1974, for a review on mellein and other isocoumarins see Hill, 1986) Compounds **6a** and **b,** for example, are inhibitors of HIV-1 protease (Dombrowski *et al.*, 1992) and mellein has insecticidal activities (Grove and Pople, 1981).

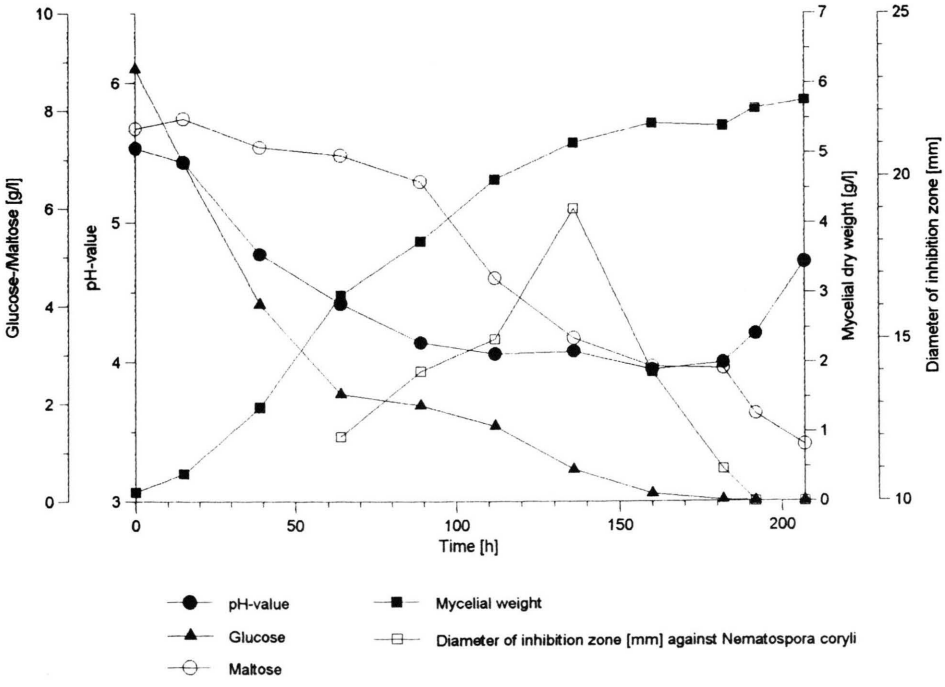
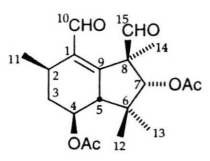


Fig. 2. Fermentation of *Hymenoscyphus epiphyllus* A236–87 in YMG-medium.



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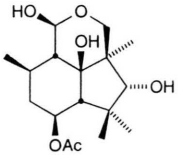

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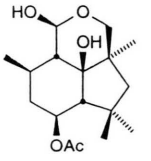

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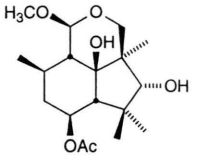

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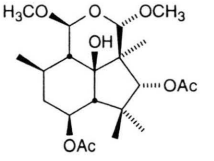

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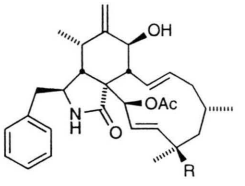

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a: R = OH; b: R = H.

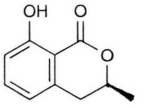

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Fig. 3. Structures of the metabolites isolated from *Hymenoscyphus epiphyllus.*

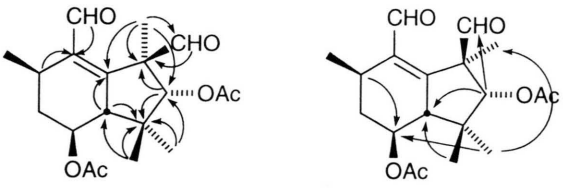

Fig. 4. Significant HMBC (left) and NOESY (right) correlations observed with hymendial **(1)**.

Biological properties

The biological activities of compounds detected in this study are summarized in Tables III–VI. The antibacterial effects were rather low. Weak antibacterial activities of mellein have been reported before (Berdy, 1997) and therefore were not recorded. Compounds **1, 6a, b**, and **7** exhibited antifungal activities. The antifungal activity of mellein was low, only *Paecilomyces varioti* was sensitive. Hymendial and both cytochalasins were active towards bacteria and fungi, with minimal inhibitory concentrations for *Nematospora coryli* and *Mucor miehei,* the most sensitive organisms, ranging from 5 to 20 μg/ml (as shown in Table III). The other compounds were not active. The cytotoxic activities listed in Table IV, show that cell lines growing as monolayer were more sensitive towards hymendial than suspension cell cultures. Both cytochalasins were highly cytotoxic, the IC_{50}

Table I. ^1H (500 MHz) data (δ, mult., *J* in Hz) for compounds **1, 2, 4** and **5** in $CDCl_3$.

H	**1**	**2**	**4**	**5**
1	-	1.56; d; 12.6	1.52; d; 12.5	1.50; dd; 12.2, 1.9
2	2.94; m	1.83; m	1.84; m	1.81; m
3α	2.17; ddd; 4.1, 6.6, 13.0	2.05; m	2.04; ddd; 4.0, 4.5, 14.5	2.05; m
3β	1.40; ddd; 10, 10, 13.0	1.05; m	1.03; ddd; 11, 12, 13	1.06; ddd; 11, 12, 13
4	4.96; ddd; 4.1, 9.0, 10.5	5.00; ddd; 4.7, 10, 11	4.99; ddd; 4.5, 11, 11	5.09; ddd; 4.7, 11, 11
5	2.59; dd; 3.6, 9.0	1.94; d; 10.7	1.97; d; 10.7	1.97; d; 10.8
7	4.75; s	4.02; s	3.98; s	5.37; s
10	9.71; s	5.36; s	4.82; s	4.93; d; 1.9
11	1.05; d; 6.8	0.98; d; 6.2	0.95; d; 6.3	0.96; d; 6.5
12	1.19; s	1.26; s	1.24; s	1.28; s
13	0.92; s	0.93; s	0.93; s	0.94; s
14	1.32; s	1.15; s	1.16; s	1.03; s
15α	9.59; s	3.32; d; 10.6	3.28; d; 10.6	5.00; s
15β	-	4.24; d; 10.6	4.00; d; 10.6	-
4-OAc	2.06; s	2.03; s	2.02; s	2.03; s
7-OAc	2.13; s	-	-	2.07
9-OH	-	-	4.08; s	3.76; s
10-OCH_3	-	-	3.37; s	3.47; s
15-OCH_3	-	-	-	3.44; s

Table II. ^{13}C (125 MHz) data (δ, mult.) for compounds **1, 2, 4** and **5** in $CDCl_3$.

C	**1**	**2**	**4**	**5**
1	139.1; s	58.8; d	54.8; d	58.7; d
2	29.8; d	28.4; d	28.3; d	29.0; d
3	36.9; t	39.5; t	39.6; t	39.3; t
4	69.6; d	72.4; d	72.4; d	72.4; d
5	54.3, d	58.9; d	58.6; d	55.2; d
6	42.2; s	41.4; s	41.4; s	41.3; s
7	82.9; d	82.5; d	82.5; d	82.5; d
8	57.5; s	47.8; s	47.9; s	51.2; s
9	155.7; s	79.5; s	79.1; s	82.6; s
10	191.6; d	92.6; d	98.8; d	100.6; d
11	20.4; q	20.0; q	20.1; q	20.8; q
12	27.0; q	34.2; q	34.1; q	34.0; q
13	17.3; q	19.8; q	19.9; q	20.0; q
14	18.5; q	16.5; q	16.5; q	12.3; q
15	198.4; d	66.8; t	66.5; t	102.0; d
4-O_2**C**CH_3	170.0; s	170.5; s	170.5; s	170.4; s
4-O_2C**C**H_3	21.2; q	21.4; q	21.4; q	21.4; q
7-O_2**C**CH_3	170.9; s	-	-	170.6; s
7-O_2C**C**H_3	20.4; q	-	-	21.1; q
10-OCH_3	-	-	55.0; q	56.8; q
15-OCH_3	-	-	-	55.5; q

Table III. Antimicrobial activities of compounds **1, 6a** and **6b**.

Organism	MIC [μg/ml]		
	Compound		
	1	**6a**	**6b**
Bacteria:			
Bacillus brevis ATCC 9999	>100	50	50
Bacillus subtilis ATCC 6633	>100	50	50
Escherichia coli K12	50	>100	>100
Micrococcus luteus ATCC 381	50	50	100
Yeasts:			
Nematospora coryli ATCC 10647	20	10	10
Rhodotorula glutinis ATCC 26086	50	n.t.	n.t.
Fungi:			
Mucor miehei Tü 284	10	5	5
Penicillium notatum	20	50	50
Paecilomyces variotii ETH 114646	20	50	100

n.t.: not tested.

for all mammalian cell lines tested were between 1 and 10 μg/ml. For comparison, the ED_{50} of cytochalasin B towards HeLa S3 cells was reported to be 1–2.5 μg/ml (Cole and Cox, 1981). In addition, as has been described for other cytochalasins, the formation of giant cells was observed (Mac Lean-Fletcher and Pollard, 1980). In L1210 cells the effect of hymendial on the incorporation of uridine, leucine and thymidine into macromolecules was measured. The results, shown in Table V, indicate a non selective inhibition of all macromolecular syntheses, which is in accord with the chemical reactivity of the compound.

Table IV. Cytotoxic activities of compounds **1, 2** and **6a, b** towards different mammalian cell lines in comparison with isovelleral.

Cell line	IC_{50} [μg/ml]				
	Compound				
	1	**2**	**6a**	**6b**	**Isovelleral**
L1210	10–20	>100	1	1	2
RBL-1	10–20	>100	2.5	2.5	5
BHK 21	5–10	>100	2.5	2.5	2
HeLa S3	5–10	>100	2.5	2.5	2

Table V. Inhibition of the incorporation of radioactive labelled precursors into macromolecules in L1210 cells by hymendial.

Hymendial [μg/ml]	Incorporation [cpm]		
	Thymidine	Uridine	Leucine
0	11191	26622	24781
0.1	11098	26544	24763
0.5	9524	21845	23245
1	9613	20883	21123
2	5562	6628	11300

Upon incubation with L-cysteine, hymendial (**1**) lost its biological activities and after 30 minutes ninhydrine-positive adducts were detected by TLC. This suggests that the bioactivities of this compound are due to the reactivity of the electrophilic unsaturated dialdehyde moiety as has been reported for other unsaturated dialdehydes (Anke *et al.*, 1989b). Like some of these compounds (Anke and Sterner, 1991b), hymendial (**1**) is weakly mutagenic in the Ames test (results are shown in Table VI). However, it lacks the cyclopropane ring that previously has been associated with the mutagenicity of the unsaturated dialdehydes (Sterner *et al.*, 1987). Hymendial (**1**) exhibited no phytotoxic activities towards *Lepidium sativum* and *Setaria italica* up to 100 μg. *O*-Methyldihydrobotrydial, a metabolite produced by *Botrytis squamosa*, has been reported to inhibit the hypocotyl elongation of lettuce seedlings (Kimura *et al.*, 1988). Botrydienal and dehydrobotrydienal from *Botryotinia squamosa* (teleomorph of *Botrytis squamosa*) were also phytotoxic whereas deacetyldihydrobotrydienal had no effect on turnip seedlings (Ki-

Table VI. Mutagenic activity of hymendial in the Ames test with *Salmonella typhimurium*.

Test strain	S9-Mix [µl/ml]	NR	Number of revertants per plate Hymendial 10 µg	MES 2 µl	Daunomycin 2 µl
	0	7 +/- 0	138 +/- 12	-	2370 +/- 113
TA98	10	7 +/- 0	107 +/- 12	-	13 +/- 1
	25	8 +/- 1	99 +/- 9	-	15 +/- 2
	0	93 +/- 11	187 +/- 19	5230 +/-210	-
TA 100	10	96 +/- 5	205 +/- 15	118 +/- 14	-
	25	107 +/- 9	135 +/- 7	109 +/- 12	-

NR = natural number of revertants.

mata *et al.*, 1985). Whether these different phytotoxic effects of botrydial derivatives are due to differences in the chemical structure or due to different sensitivity of the plant species used in the assays is not clear. Botrydial and its derivatives are produced by phytopathogenic fungi. Especially *Botrytis cinerea* turned out to be a very prolific producer of botrydial derivatives (Welmar *et al.*, 1979; Collado et al., 1995 and 1996). None of these compounds, but most of those isolated from *H. epiphyllus* A 236–87, an endophytic fungus, are oxidized at C-7. Pyrichalasin H (4'-methoxycytochalasin H) exhibited strong inhibitory effects towards rice seedlings (Nukina, 1987) and cytochalasin H (**6a**) inhibited the floral development in tobacco (Wells *et al.*, 1976). Mellein was not phytotoxic towards *L. sativum* and *S. italica*. It has been reported to affect corn seedlings (Devys *et al.*, 1974). The phytotoxic activity of compound **4** remains to be investigated. None of the compounds **1–5** was nematicidal towards *Caenorhabditis elegans* at concentrations up to 100 µg/ml.

It is interesting to note that the bioactive secondary metabolites produced by *Hymenoscyphus epiphyllus*, strain A236–87, originate from three different biogenetic pathways. The botrydial derivatives are sesquiterpenes, derived from mevalonic acid (Turner and Aldridge, 1983), mellein is a pentaketide, built from acetyl-CoA and malonyl-CoA (Hoker and Simpson, 1981), while the cytochalasins are synthesized from phenylalanine, a polyketide chain and methionine as donator of the methyl groups (Tamm, 1978). Thus *Hymenoscyphus epiphyllus* may be grouped among the more creative fungi (Dreyfuss and Chapela, 1994).

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Ames B. N., McCann J. and Yamasaki R.(1978), Methods for detecting carcinogens and mutagens in the *Salmonella*/mammalian microsome mutagenicity test. Mut. Res. **31**, 347–364.

Anke H., Bergendorff O. and Sterner O (1989a), Assays of the biological activities of guaiane sesquiterpenoids from the fruiting bodies of edible *Lactarius* species. Food Chem. Toxicol. **27**, 93–397.

Anke H., Helfer A., Arendtholz W. R., Casser I. and Steglich W. (1991a), Two new botrydial derivates from *Hymenoscyphus epiphyllus* and their biological activities. Planta Med. **57**, A34.

Anke H., Sterner O. and Steglich W. (1989b), Structure-activity relationships for unsaturated dialdehydes. 3. Mutagenic, antimicrobial, cytotoxic and phytotoxic activities of merulidial derivates. J. Antibiotics **42**, 738–744.

Anke H. and Sterner O (1991b), Comparison of the antimicrobial and cytotoxic activities of twenty unsaturated sesquiterpene dialdehydes from plants and mushrooms. Planta Med. **57**, 344–346.

Berdy, J. (1997), Bioactive Natural Products Database. Szenor Management Consulting Company, Budapest.

Cole R. J. and Cox R. H. (1981), The cytochalasins. In Handbook of Toxic Fungal Metabolites, Academic Press, New York, pp. 264–343.

Collado I. G., Hernandez-Gala R., Duran-Patron R. and Cantoral J. M. (1995), Metabolites from a shake culture of *Botrytis cinerea*. Phytochemistry **38**, 647–650.

Collado I. G., Hernandez-Galan R., Prieto V., Hanson, J. R. and Rebording L. G. (1996), Biologically active sesquiterpenoid metabolites from the fungus *Botrytis cinerea*. Phytochemistry **41**, 513–517.

Dennis R. W. G. (1981): British Ascomycetes. J. Cramer Verlag, Vaduz, pp. 92–93, 116–117, 124–126, 135–141.

Devys M., Bousquet J. F., Skajennikoff M. and Barbier M. (1974), Ochracin (mellein), a phytotoxin from the culture medium of *Septoria nodorum*. Phytopathol. Z. **81**, 92–96.

Dombrowski A. W., Bills G. F., Sabnis G., Koupal L. R., Meyer R., Ondeyka J. K., Giacobbe R. A., Monaghan R. L. and Lingham R. B. (1992), L-696,474, a novel cytochalasin as an inhibitor of HIV-1 protease. J. Antibiotics **45**, 671–691.

Dreyfuss M. M. and Chapela I. H. (1994), Potential of fungi in the discovery of novel, low molecular weight pharmaceuticals. In: The Discovery of Natural Products with Therapeutic Potential (Gullo V. P. ed.). Butterworth-Heinemann, Boston, London, Oxford, Singapore, Sidney, Toronto, Wellington, pp. 49–80.

Erkel G., Anke T., Gimenez A. and Steglich W. (1992), Clavicoronic acid, a new inhibitor of reverse transcriptases from *Clavicorona pyxidata* (Pers. ex Fr.) Doty. J. Antibiotics **45**, 29–37.

Fehlhaber H. W. and Geipel R. (1974), Botrydial, ein Sesquiterpen-Antibiotikum aus der Nährlösung von *Botrytis cinerea*. Chem. Ber. **1974**, 1720–1730.

Grove J. F. and Pople M. (1981), The insecticidal activity of some fungal dihydroisocoumarins. Mycopathologia **76,** 65–67.

Hill R. A. (1986), Naturally occurring isocoumarins. In Progress in the Chemistry of Organic Natural Products **49,** 1–78.

Hoker J. S. E. and Simpson T. J. (1981), Studies on fungal metabolites. 2. Carbon-13-nuclear magnetic resonance studies on pentaketide metabolites of *Aspergillus melleus*: 3-(1',2'-epoxypropyl)-5,6-dihydro-5-hydroxy-6 methylpyrane-2-one and mellein. J. Chem. Soc. Perkin Trans. I **1981**, 1397–1400.

Jonassohn M., Hjertberg R., Anke H., Dekermendjian K., Szallasi A., Thines E., Witt R. and Sterner O. (1997), The preparation and bioactivities of (-)-isovelleral. Bioorg. Med. Chem. in print.

Kimata T., Natsume M. and Marumo S. (1985), Botrydienal, a new phytotoxin, and ist related metabolites, dehydrobotrydienal and deacetyldihydrobotrydial produced by *Botryotinia squamosa*. Tetrahedron Lett. **26**, 2097–2100.

Kimura Y., Fujioka H., Nakajima H., Hamasaki T. and Isogai A. (1988), Isolation and structures of O-methyldihydrobotrydial and deacetyl-O-methyldihydrobotrydialone produced by *Botrytis squamosa*. Agric. Biol. Chem. **52**: 1845–1847.

MacLean-Fletcher S. and Pollard T. D. (1980), Mechansim of action of cytochalasin B on actin. Cell **20**, 329–341.

Nukina, M. (1987), Pyrichalasin H, a new phytotoxic metabolite belonging to the cytochalasans from *Pyricularia grisea* (Cooke) Saccardo. Agric. Biol. Chem. **51**, 2625–2628.

Patwardhan S. A., Pandey R. C. and Dev S. (1974), Toxic cytochalasins of *Phomopsis paspalli,* a pathogen of kodo millet. Phytochemistry **13,** 1985–1988.

Sterner O., Carter R. and Nilson L. (1987), Structure-activity relationship for unsaturated dialdehydes. 1. The mutagenic activity of 18 compounds in the *Salmonella*/microsome assay. Mutat. Res. **188,** 169–174.

Tamm Ch. (1978), in Cytochalasins – Biochemical and Cell Biological Aspects (J. Tannenbaum, ed.). North-Holland, Amsterdam p.15.

Turner W. B. and Aldridge D. C. (1983), Fungal Metabolites II. Academic Press, London, New York, Paris, San Diego, San Francisco, Sao Paulo, Sydney, Tokyo, Toronto.

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

Welmar K., Tschesche R. and Breitmaier E. (1979), Botrylacton, ein neuer Wirkstoff aus der Nährlösung des Pilzes *Botrytis cinerea*, 2. Chem. Ber. 112, 3598–3602.