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Pedein A and B: Production, Isolation, Structure Elucidation and Biological Properties of New Antifungal Cyclopeptides from *Chondromyces pediculatus* (Myxobacteria)

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Abstract Two new secondary metabolites, named pedein A and B, were isolated from the cell mass of the myxobacterium *Chondromyces pediculatus*. Their planar structures were elucidated by spectroscopic methods, in particular 2D NMR as 24-membered cyclic hexapeptides composed of a variable tryptophan residue, glycine, sarcosine and three unusual hydroxy β- and γ-amino acids. The main component, pedein A, strongly inhibited the growth of yeasts and fungi, induced hemolysis of erythrocytes, and caused changes in membrane permeability of *Rhodotorula glutinis*. The structures of the pedeins are closely related to the large family of the microsclerodermins, which have been isolated from lithistid sponges of *Microscleroderma* and *Theonella* species.

Keywords myxobacteria, pedeins, cyclic peptides, antifungal

Introduction

Myxobacteria of the genus *Chondromyces*, suborder *Sorangineae*, have been shown to produce a variety of novel biologically active substances with different mechanisms of action. Thus, chondramides are inhibitors of the actin skeleton [1～3], crocacin and ajudazols are mitochondrial electron transport inhibitors at different sites [4～7], and apicularens are specific inhibitors of V-ATPase [8～10]. Extracts of *Chondromyces pediculatus*, strain Cm p3 were noticed for their marked antifungal activity. HPLC/DAD/MS analysis of the extracts indicated the presence of six major components with molecular weights in the range of 781 to 924 mu of which two were correlated with the antifungal activity. The compounds responsible were isolated by column chromatography of the cell extract and named pedein A (**1**) and B (**2**). A tentative structure of **1** was depicted in the GBF Scientific Annual Report 1993 and at the international conference of microbial secondary metabolism in Interlaken 1994 [11] anticipating the structures of the related microsclerodermins [12～14]. Here we describe in detail production, isolation, physico-chemical properties and structure elucidation of **1** and **2** as well as biological properties of **1**.

Results

Production and Isolation

In order to obtain smooth growth and reasonable cell densities, the producing organism had to be adapted to growth in liquid media by 4 to 8 transfers in shaken cultures. After that, the strain could be cultivated also in media based on technical substrates, *e.g.*, Probion (single cell protein prepared from *Methylomonas clarae*; Hoechst AG, Frankfurt), soy flour, or oatmeal. Table 1 shows the

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effect of various technical substrates on the yields of **1** in 100-ml shake cultures of *Chondromyces pediculatus* strain Cm p3. By increasing the concentration of the technical substrates from 0.4 to 0.9% the amount of **1** usually decreased, with the exception of oatmeal, in which **1** production increased up to 11.9 mg/liter.

The production of pedeins on a larger scale was performed in bioreactors containing media on the basis of different technical substrates. Yet comparable amounts of **1** as produced by cultivating strain Cm p3 in shake cultures, could not be achieved. In a 65-liter fermentation batch described in the experimental section, **1** accumulated after 7 days at the end of the fermentation to about 1.3 mg/liter as determined by HPLC. Isolation of pedeins was best achieved by extraction of the collected cell mass from shake cultures followed by multi-step reversed phase chromatography. Thus, *e.g.* from 36 liters of shake cultures containing 113 mg of **1** and 99 mg of other metabolites 67 mg of pure **1** and 8.0 mg of **2** were isolated. Fig. 1 shows an analytical HPLC/DAD profile of a crude extract of Cm p3 after 7 days of cultivation. In addition to **1** and **2** four other major metabolites **3**∼**6** were observed characterized by specific UV spectra and molecular masses in the range of 700 to 900 mu. For the latter, however, no molecular ions or exact masses were obtained from the on-line ESI-MS measurements.

Table 1 Effect of various technical substrates on the production of pedein A (**1**) in shake cultures

Substrate[a] (%)	Concentration (mg/liter)	Pedein A titer[b]
Probion	0.4	3.7
Skim milk powder	0.4	3
Soy flour	0.4	8.0
Peanut meal	0.4	5
Cornsteep powder	0.4	0.2
Oat meal	0.4	8.2
Oat meal	0.9	11.9

[a] The basal medium was: soluble starch 0.3%, $MgSO_4 \cdot 7H_2O$ 0.1%; $CaCl_2 \cdot 2H_2O$ 0.05%; HEPES buffer 50 mM (pH 7.2); supplemented with standard vitamin- and trace element solutions, 1 ml/liter each. Harvest was at the end of the growth phase after about 5 days.

[b] The concentration of **1** was determined in acetone extracts of the cell mass by HPLC analysis.

Physico-chemical Properties and Structure Elucidation

1 and **2** were obtained as colorless solids, well soluble in dimethyl sulfoxide, DMF and pyridine, less so in methanol and chloroform. The physico-chemical properties of **1** and **2** are summarized in Table 2. The pedeins are stable under normal laboratory conditions, however decomposed in sunlight. This was indicated by the decrease of both antifungal activity and UV absorption at 287 nm. Catalytic hydrogenation of **1** led to a tetrahydro derivative (data not shown) stable in sunlight, however, with 10 times lower activity against *Rhodotorula glutinis*.

For **1** ESI-MS in the positive mode showed a very weak $[M+H]^+$ ion at 925 mu and strong $[(M-H_2O)+H]^+$ and $[M+Na]^+$ ions at 907 and 947 mu, respectively. Similarly FAB-MS gave a strong $[(M-H_2O)+H]^+$ ion which, like in the ESI spectra, showed the 3 : 1 isotope pattern for chlorine. From HRFAB-MS (calc. 907.3393, found 907.3400) the elemental composition $C_{43}H_{53}ClN_8O_{13}$ was calculated, indicative of a peptide structure. Total hydrolysis of **1**, derivatization and GC/MS analysis revealed the presence of glycine, sarcosine (*N*-methylglycine) and of an isomer of threonine. Strong IR

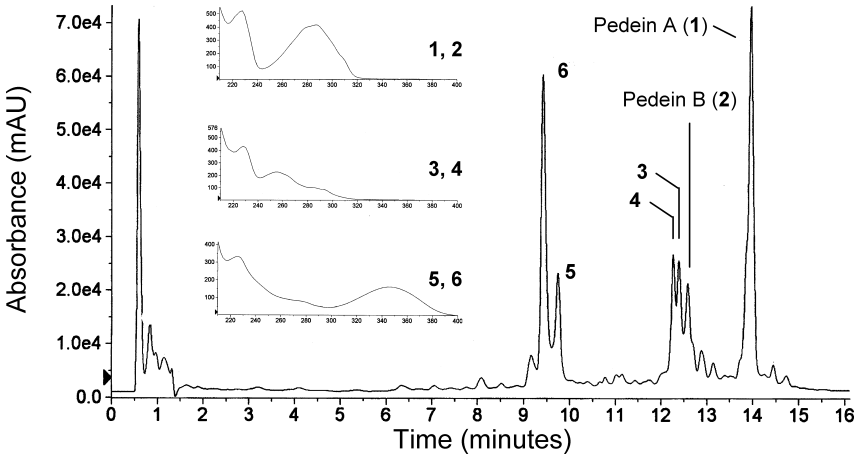

Fig. 1 HPLC/DAD chromatogram of a crude cell extract of *Chondromyces pediculatus*, strain Cm p3

Nucleodur C18 column, CH_3CN/5.0 mM ammonium acetate buffer pH 5.5, flow 0.3 ml/minute, gradient 5 : 95 to 95 : 5 in 30 minutes.

Table 2 Physico-chemical properties of pedein A (**1**) and B (**2**)

	Pedein A (**1**)	Pedein B (**2**)
Appearance	White powder	Colorless solid
Molecular weight	924/926	890
$[\alpha]_D^{20}$	−46.5 (*c* 0.37 in DMSO/MeOH 2 : 8)	—
Formula	$C_{43}H_{53}ClN_8O_{13}$	$C_{43}H_{54}N_8O_{13}$
UV-VIS (λ_{max}, nm) in MeOH	227 (ε=47500), 278sh (29600), 287 (31500), 295sh (ε=26000), 309sh (21500)	227, 278sh, 287, 307sh
IR ν_{max} (KBr) cm^{-1}	3386, 2927, 1732sh, 1674, 1530	—
Rf on TLC[a]	0.44	0.46
Rt (minute) on HPLC[b]	14.0	12.6

[a] Silica gel TLC, solvent: EtOAc/MeOH/H_2O (80 : 15 : 10).
[b] See Fig. 1.

Table 3 NMR data of **1** in DMSO-d_6

Residue	Group	δ ^{13}C	δ ^{1}H	Mult.	*J* [Hz]	HMBC
APTD						
1	CO	173.0				2-H and/or Pyrr-4-H, Pyr-4-NH
2	CH	69.4	4.44	m		4-H
3	CH	53.1	4.19	m		5-H[a]
	NH		7.37	br		
4	CH	70.2	3.30	m		3-H, 6-H_2
5	CH	69.1	3.52	m		5-OH, 6-H_2, 7-H
	OH		4.25	br s		
6	CH_2	36.8	2.28	ddd	14; 7; 7	4-H, 5-OH, 7-H, 8-H
			2.32	ddd	14; 7; 7	
7	CH	133.2	5.85	ddd	15; 7.5; 7.5	4-H, 6-H_2, 9-H
8	CH	131.8	6.24	dd	15; 10.5	6-H_2, 9-H, 10-H
9	CH	129.4	6.85	dd	15.5; 10.5	H-7, H-8
10	CH	129.8	6.47	d	15.5	8-H, 12-H, 16-H
11	Cq	137.2				9-H, 10-H, 13-H, 15-H
12, 16	CH	126.0	7.43	d	7.5	10-H, 12-H, 14-H, 16-H
13, 15	CH	128.6	7.31	t	7.5	13-H, 15-H
14	CH	127.1	7.20	t	7.0	12-H, 16-H
Pyrr						
1	CO	170.7				2-H_2, Sark-2-H_2, Sark-3-H_3
2	CH_2	38.9	2.61	d	17	4-H
			2.83	d	17	
3	Cq	82.3				2-H_2, 4-H
4	CH	56.2	4.45	m		3-NH, 5-H, 4-NH[a]
5	CH	79.1	4.06	d	9	3-NH, 4-H, 7-H_3
6	CO	171.5				3-NH[a], 5-H
7	CH_3	56.9	3.40	s		5-H
3	NH		8.18	br s		
4	NH		7.59	d	10	
Sark						
1	CO	170.2				Sark-2-H_2, Cl-Trp-2-H[a], Cl-Trp-2-NH
2	CH_2	49.6	3.69	d	16	3-H_3
			4.31	d	16	

Table 3 Continued

Residue	Group	δ ^{13}C	δ ^{1}H	Mult.	J [Hz]	HMBC
3	CH_3	36.3	2.93	s		2-H_2
Cl-Trp						
1	CO	171.8				2-H, 3-H_2, Gly-2-NH, Gly-2-H_2
2	CH	55.5	4.17	m		2-NH, 3-H_2
	NH		8.68	d	4	
3	CH_2	26.0	2.98	dd	14.5; 8.5	2-H, 2-NH
			3.11	dd	14.5; 6.0	
2′	CH	124.9	7.27	br s		1′-NH, 3-H_2
3′	Cq	111.0				2-H, 3-H_2, 1′-NH, 2′-H, 4′-H
4′	CH	119.6	7.53	d	8.5	
5′	CH	118.6	7.00	d	8.5	7′-H
6′	Cq	125.9				4′-H
7′	CH	110.9	7.37	br s		4′-H, 5′-H
8′	Cq	136.4				2′-H, 1′-NH, 4′-H
9′	Cq	125.7				2′-H, 3-H_2, 5′-H, 1′-NH, 7′-H
1′	NH		11.04	br s		
Gly						
1	CO	168.9				2-H_2, AHB-4-NH, AHB-4-H_2
2	CH_2	42.7	3.37	m		2-NH[a]
			3.72	m		
	NH		8.52	t	4	
AHB						
1	CO	172.3				2-H_2, APTD-3H, APTD-3N-H
2	CH_2	40.9	2.14	dd	14; 11	4-H_2
			2.45	d	14	
3	CH	66.8	3.79	m		2-H_2, 4-H_2
4	CH_2	45.0	2.68	m		4-NH[a]
			3.31	m		
4	NH		7.42	br		
not assign.	OH		6.18	br s		
			6.27	br s		

[a] Weak correlation.

bands at 1674 and 1530 cm^{-1} and 7 carbonyl signals in the ^{13}C-NMR spectrum in the range of 168.0 to 173.0 ppm confirmed a peptide structure incorporating the remaining 7 nitrogen atoms. The strong UV band at 288 nm (ε=31500) was in good agreement with a 1-phenylbutadien chromophor (286 nm, ε=28200 [15]) derived from NMR data. A detailed analysis of COSY, TOCSY and HMBC data obtained from a DMSO-d_6 solution of **1** (Table 3) confirmed the presence of glycine, sarcosine and 6′-chlorotryptophan, and identified three further unusual amino-acid residues abbreviated as APTD, AHB and Pyrr. APTD, carrying the UV chromophor, is a new amino acid, (7*E*,9*E*)-3-amino-10-phenyl-2,4,5-trihydroxy-deca-7,9-dienoic acid. AHB is 4-amino-3-hydroxy-butyric acid. From its carbon backbone, Pyrr is 3-amino-4-keto-5-methoxy-hexadioic acid-6-amide, which in solution exists exclusively in its cyclic hemiacetal (pyrrolidinone) form. However, a shoulder at 1732 cm^{-1} in the IR spectrum indicates the presence of some keto form in KBr. At this point 20 of 21 expected double bond equivalents were identified. The remaining double bond equivalent proved that **1** is a cyclic peptide, which is in good agreement with a negative ninhydrin test and the failure of the Edman degradation. A rigorous proof of the amino acid sequence came from a complete set of C, H correlations across the six amide bonds forming the macrocyle of **1** (Figs. 2 and 3). The core structure of **1** is thus identical with that of the microsclerodermins A∼I. With respect to the side chains, the closest relative of **1** is microsclerodermin D, which carries also a 6′-chloro-tryptophan, but lacks the 5-

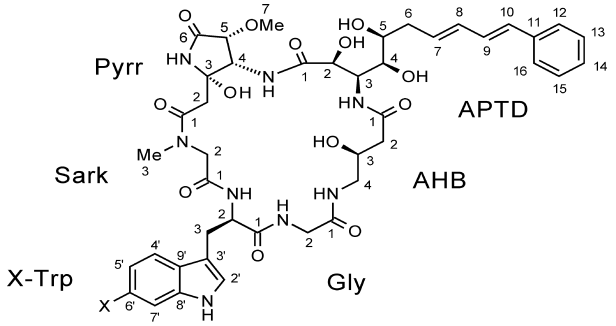

Fig. 2 Structures of pedein A (**1**) and B (**2**).

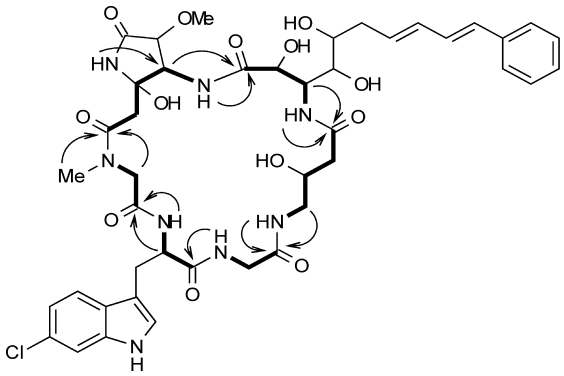

Fig. 3 Amino acid sequence specific correlations from the HMBC NMR spectrum of pedein A (**1**).

methoxy group of Pyrr, and one of the two C,C-double-bonds of APTD. The optical rotation of **1** (α_D −46.5) and microsclerodermin D (α_D −56) are similar, proton and carbon chemical shifts and coupling constant of comparable partial structures nearly identical. Thus, it may be safely assumed that the absolute configuration as well as the conformation of **1** core-structure is identical to microsclerodermin D.

2 according to ESI-MS lacks the chlorine substituent and shows NMR spectral data identical to **1** with the exception of an unsubstituted indole ring.

Biological Properties

1 inhibited the growth of a broad spectrum of yeasts and fungi, while bacteria were not sensitive to the antibiotic (Table 4). MIC for *Rhodotorula glutinis* was 0.6 μg/ml, for both *Saccharomyces cerevisiae* and *Candida albicans* 1.6 μg/ml, and for *Ustilago maydis* 3.1 μg/ml. For cultivated L929 mouse fibroblasts **1** showed only weak cytotoxicity with an IC_{50} of 1.1 μg/ml. **1** proved to be highly hemolytic. In a two step dilution assay with 10% sheep blood in 0.9% NaCl solution, the addition of 100 μg/ml down to 1.8 μg/ml led to the immediately lysis of the erythrocytes. The IC_{50} was 0.8 μg/ml. Because of this hemolytic activity, the effect of **1** on membrane integrity of the yeast *R. glutinis* was investigated. As Fig. 4 shows, UV absorption of the supernatant at 260 nm increased under these conditions. The degree and rate of these changes depended on **1** concentration applied and showed that **1** indeed causes changes of membrane permeability of sensitive cells.

Table 4 **1**: Biological Activity

Test organism[a]	Diameter of inhibition zone[b] (mm)
Gram-negative bacteria	
Escherichia coli DSM[c] 423	0
Enterobacter aerogenes	0
Pseudomonas aeruginosa DSM 1117	0
Salmonella typhimurium DSM 5091	0
Gram-positive bacteria	
Bacillus subtilis DSM 10	0
Brevibacterium ammoniagenes DSM 20306	0
Corynebacterium fascians DSM 20131	0
Micrococcus luteus GBF[d] 26	0
Staphylococcus aureus GBF 16	0
Yeasts	
Candida albicans GBF 129	32
Candida utilis	27
Rhodotorula glutinis	28
Saccharomyces cerevisiae GBF 36	20
Schizosaccharomyces pombe Tü[e] 501	24
Torulopsis glabrata DSM 70398	23
Filamentous fungi	
Botrytis cinerea DSM 877	22
Gibberella fujikuroi DSM 893	26
Pythium debaryanum DSM 62948	27
Rhizopus arrhizus DSM 905	35
Trichoderma koningii DSM 63060	23
Ustilago maydis	27

[a] Bacteria were tested on nutrient agar, fungi on malt extract peptone agar.
[b] Determined by the agar diffusion test with 20 μg pedein per 6-mm paper disc.
[c] Deutsche Sammlung von Mikroorganismen.
[d] Strain of the GBF, Dept. of Natural Biology.
[e] Collection University Tübingen.

Discussion

The pedeins are further new basic structures isolated from

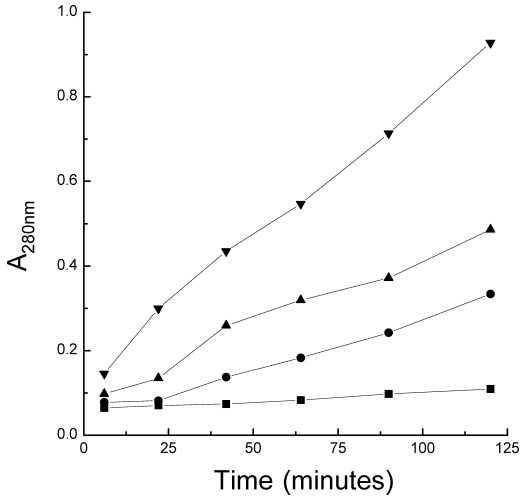

Fig. 4 Effect of pedein A on membrane permeability of *Rhodotorula glutinis* cells.

■ Without pedein A, ● 1.5 μg/ml pedein A, ▲ 3.1 μg/ml pedein A, ▼ 6.2 μg/ml pedein A.

strains of the myxobacterial genus *Chondromyces*. The structurally unrelated crocacins, chondramides, chondrochlorens, and ajudazols are simultaneously produced by all strains of the species *C. crocatus* screened and show different mechanisms of action [1~10]. While the cytotoxic V-ATPase inhibitors apicularen [8~10] were widely distributed in most of the other *Chondromyces* species, the new pedeins were hitherto only found in *C. pediculatus*, strain Cm p3 and Cm T270. Biological studies showed that especially **1** showed good antifungal activity, which corresponds to the structurally related microsclerodermins. Yet we could find neither potent antitumor activity nor microtubule aberrations as has been described for some of the microsclerodermins [14, 16]. First investigations into the mechanism of action showed that **1** seem to interfere with membrane integrity. The pedeins are a further example of myxobacterial compounds that are chemically related to metabolites isolated from marine sponges. After myxobacterial saframycin MX1 [17], which is chemically related to renieramycin from the sponge *Reniera* sp. [18], the chondramides [1~3], which resemble jasplakinolide from the sponge *Jaspis johnstoni* [19], the apicularens [8, 9], which are structurally related to the salicylihalamides from *Haliclona* sp. [20], and rhizopodin [21] which resembles sphinxolid B from the deep sea sponge *Nosiphonia superstes* [22], the pedeins resemble the microsclerodermins, which have been isolated by Faulkner and co-workers from several species of the lithistid sponges *Microscleroderma* sp. and *Theonella* sp. [12~14]. It is remarkable that chemically closely related but otherwise unique compounds occur in phylogenetically so distant organisms as myxobacteria and marine sponges. However, recently it was shown, that one of such peptides, namely theopalauamide from *Theonella swinhoei* is located in and presumably produced by a symbiotic proteobacterium, *Candidatus Entoheonella palauensis* that is by 16S rRNA analysis closely related to terrestrial myxobacteria [23]. Most remarkably, microsclerodermins C~E have also been isolated from *Theonella* species in addition to theopalauamide [13]. Thus theopalauamide related compounds may likewise be discovered in myxobacteria in the future. On the other hand, one could hypothesize that more relatives of myxobacterial metabolites will be discovered in the future in sponges having been produced by their bacterial feed or bacterial symbionts genetically related with myxobacteria.

Experimental

General

Spectral and physico-chemical data were obtained with the following instruments: Optical rotation, Perkin Elmer 241 MC polarimeter; UV, Shimadzu UV/Vis-2102 PC spectrometer. IR, Nicolet 20 DXB FT-IR spectrometer; NMR, Bruker DMX-600 spectrometer (^{1}H: 600 MHz, ^{13}C: 150 MHz). HPLC/DAD/ESI-MS: PE Sciex Api-2000 LC/MS; Nucleodur C18 column, 5.0 μm, 2×125 mm (Machery-Nagel) (0.3 ml/minute CH_3CN/5 mM ammonium acetate buffer pH 5.5 gradient 5 : 95 to 95 : 5 in 30 minutes). ESI-MS: TSQ 700 (Finnigan MAT) (solvent $CH_3OH/CH_3CN/H_2O$/HCOOH 4 : 2 : 1 : 0.1). GC/MS: Carlo Erba Mega series gas chromatograph interfaced with a Kratos MS50 fast-scan mass spectrometer (+EI); column: 30 m DB1; gradient: 5 minutes isotherm at 60°C, then 10°C/minute to 300°C; carrier gas He. FAB-MS: Kratos MS50 RF TC instrument equipped with a high field magnet and using a 8 kV Xenon beam. Approximately 10 μg of sample was dissolved in 3-nitrobenzylalcohol. Spectra were recorded with an acceleration potential of 8 kV and a magnet scan rate of 10 seconds/decade. High-resolution data were acquired by peak matching at a resolution of approximately 8000 using PEG.

Producing Microorganism and Culture Conditions

Chondromyces pediculatus strain Cm p3 was isolated at the Helmholtz Centre for Infection Research (formerly GBF) in 1982 from a soil sample collected in Key Largo, Florida, USA. Later on pedein A was also detected in strain Cm T292, which was isolated from a soil sample from Costa Rica. Initially Strain Cm p3 was grown on VY/2 [24] agar plates and in MD1 [24] liquid medium supplemented with

1 ml/liter of each of a standard vitamin and a trace element solution. Batch cultures of 20, 100 or 400 ml in 100-ml, 250-ml or 1,000-ml Erlenmeyer flasks, respectively, were incubated at 30°C on a rotary shaker at 160 rpm for 4～7 days. Cm p3 grew in small clumps. After the strain had been adapted to growth in liquid media by 4～8 transfers in shaken cultures, it also could be cultivated in media containing various technical substrates *e.g.* Probion (single cell protein prepared from *Methylomonas clarae*; Hoechst AG, Frankfurt), soy flour (Tipo S, gift from Novartis) or oatmeal.

Fermentation and Isolation

Batch fermentations of *C. pediculatus* strain Cm p3 were performed in media on the basis of technical substrates. To give an example, eleven 400-ml cultures in 1-liter Erlenmeyer flasks grown for 3 days in soy flour liquid medium (soy flour 0.4%, soluble starch 0.3%, $MgSO_4 \cdot 7H_2O$ 0.15 %, $CaCl_2 \cdot 2H_2O$ 0.05%, vitamin B_{12} 0.25 mg/liter, and 1.0 ml/liter of a standard trace element solution, HEPES 50 mM, pH 7.2) on a gyratory shaker at 160 rpm were inoculated into 65 liters of the same medium without the addition of HEPES in a type b 50 bioreactor (Giovanola Frères, Monthey, Switzerland). In order to reduce foam formation, 0.05% silicone antifoam agent (Tegosipon, Goldschmidt AG, Essen) was added. The bioreactor was kept at 30°C and agitated at 100 rpm with a turbine plate stirrer. The aeration rate was 0.05 volume air per culture volume and minute and the pH value was regulated during fermentation at pH 7.0. The pO_2 in the culture was recorded continuously with a polarographic oxygen electrode and remained during the whole fermentation between 90 and 83%, respectively. On day 7 the fermentation was terminated by centrifugation of the culture broth at a titer of 1.3 mg/liter **1**. For isolation of **1** and **2**, wet cell mass from 36 liters of culture from shaking flasks containing 4.0 mg/liter **1** was extracted twice with acetone. After evaporation of the organic solvent and lyophilisation, the crude extract (6.0 g) was distributed between methanol (0.5 liter) and heptane (5×0.5 liter). Evaporation of the methanol layer yielded a refined extract (370 mg) which was dissolved in a small amount of DMSO and separated in two runs on a HD-Sil-18-20-60 column (6×54 cm) with the solvent system methanol/water 70 : 30 (detection 206 nm). The pedein containing fraction (113 mg) was re-chromatographed on a Nucleosil 100-C18 column (20×250 mm) with the solvent system methanol/water 60 : 40; yield 67 mg of **1** and 8.0 mg of **2**.

Analysis of Secondary Metabolites

Secondary metabolite production by Cm p3 was monitored by analytical HPLC of acetone cell extracts using a Waters 991 instrument. Chromatographic conditions were as follows: column 250×4 mm, 7 μm, Nucleosil C18, solvent: MeOH/H_2O 60 : 40 or 70 : 30, flow 1.5 ml/minute, detection at 280 nm. Rt for **1** was 10.1 and 5 minutes, respectively. Analytical TLC was performed on silica gel Si 60 F254 aluminium sheets (Merck), solvent: EtOAc/MeOH/H_2O 80 : 15 : 10, detection UV absorption at 254 nm, and as blue spots after spraying with vanillin/sulfuric acid/heating to 120°C.

Amino Acid Analysis

1 (1.0 mg) was hydrolyzed [4 N TFA, 100°C, 4 hours] and the resulting amino acid mixture converted to ethyl esters [4 N HCl in EtOH, 100°C, 1 hour]. The solvents evaporated in a stream of nitrogen and the residue trifluoroacetylated [100 μl CH_2Cl_2, 50 μl TFAA, 110°C, 15 minutes]. The solvents were again evaporated in a stream of nitrogen, the residue dissolved in dichloromethane and analyzed by GC/MS: Three major peaks were observed identified by retention time and mass spectrum as glycine and sarcosine and an isomer of threonine which according to NMR data was 4-amino-3-hydroxy-butyric acid (AHB).

Biological Activity

Standard strains for testing the biological activity were obtained from the DSMZ and the stock collection of our laboratory at the HZI. The antimicrobial spectrum of **1** was determined by an agar-plate diffusion assay using paper discs as described previously [25]. The minimal inhibitory concentrations were determined with the conventional serial two-fold dilution method. As inoculum 1×10^6 cells were used, and the antibiotics were dissolved in MeOH, giving MeOH concentrations in the cultures of not more than 3.0%. Cytotoxicity against L929 mouse cells (connective tissue, ATCC CCL 1) was determined as reported [26].

Measurement of Membrane Integrity

To measure the effect of **1** on membrane integrity, washed cells of a culture of the yeast *R. glutinis* were suspended in 0.1 M TRIS-HCl buffer to give an optical density of $E_{546} = 1$. After the addition of different concentrations of **1** to parallel suspensions, the efflux of UV-absorbing material was measured by determination of the extinction at 260 nm of the culture supernatant at different times.

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