Oxepinamides A – C and Fumiquinazolines H – I: Bioactive Metabolites from a Marine Isolate of a Fungus of the Genus *Acremonium*

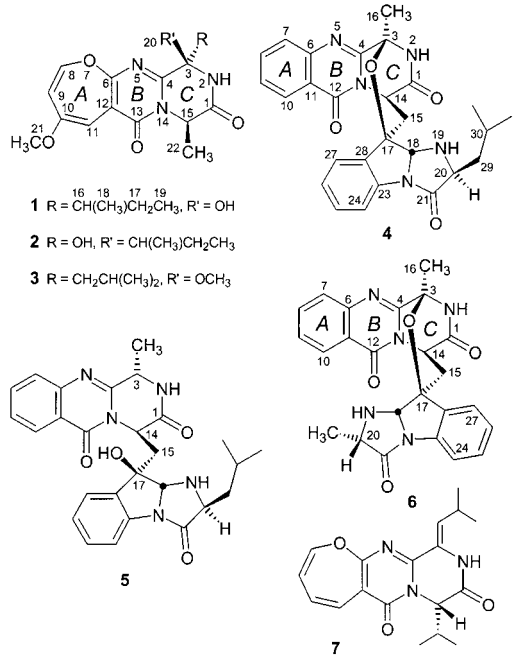
Gilbert N. Belofsky,*[a] Montserrat Anguera,[a] Paul R. Jensen,[a] William Fenical,[a] and Matthias Köck*[b]

Abstract: Three new oxepin-containing natural products (**1** – **3**) and two new fumiquinazoline metabolites (**4** – **5**) have been isolated from organic extracts of the culture broth and mycelia of an *Acremonium* sp., a fungus obtained from the surface of the Caribbean tunicate *Ecteinascidia turbinata*. The structures of the five compounds were determined through extensive analysis of 1D- and 2D-NMR data, and mass spectrometry. Compound **1** exhibited good anti-inflammatory activity in a topical RTX-induced mouse ear edema assay. Compounds **4** and **5** exhibited weak antifungal activity toward *Candida albicans* in a broth microdilution assay.

Keywords: COCON · marine fungi · natural products · NMR spectroscopy · structure elucidation

Introduction

It is well documented that fungi play an important ecological role in the marine environment, for example as primary decomposers,[1] as pathogens of marine invertebrates, and as obligate symbionts.[2, 3] While many of these exact roles have yet to be determined, there is mounting evidence that fungi display highly specific adaptations such as barotolerance[3] in the marine environment, and that these adaptations include the production of unique secondary metabolites. We have previously reported a variety of new bioactive compounds from several different genera of facultative marine fungi isolated from the surfaces of algae,[4] plants,[5] and decaying matter.[6] It is interesting to note, however, that there have been a limited number of chemical studies of fungi associated with marine animals. One prior study described the isolation of fumiquinazolines A – G from *Aspergillus fumigatus*, a fungus separated from the gastrointestinal tract of the marine fish *Pseudolabrus japonicus*.[7, 8] We report here the results of chemical investigations of a fungus of the genus *Acremonium*, isolated from the surface of the Caribbean tunicate *Ecteinascidia turbinata*, collected in the Bahamas. These studies have led to the isolation of three new oxepin-containing metabolites, which we have named oxepinamides A – C (**1** – **3**), as well as two new members of the fumiquinazoline class of compounds, fumiquinazolines H (**4**) and I (**5**).



Results and Discussion

Compounds **1** – **5** were obtained from the organic extract of a 20 L seawater-based fermentation of *Acremonium* sp. using a multi-step fractionation sequence. Steps included chromatography on silica gel, Sephadex LH-20, and C_{18} silica gel, with

[a] Prof. G. N. Belofsky[+], Dr. M. Anguera
P. R. Jensen, Prof. W. Fenical
Center for Marine Biotechnology and Biomedicine
Scripps Institution of Oceanography, University of California
San Diego, La Jolla, CA 92093-0204 (USA)

[b] Dr. M. Köck
Institut für Organische Chemie, J. W. Goethe-Universität
Marie-Curie-Strasse 11, 60439 Frankfurt (Germany)
E-mail: km@org.chemie.uni-frankfurt.de

[+] Current address:
Department of Chemistry and Biochemistry, University of Tulsa
600 South College Ave., Tulsa, OK 74104 (USA)
E-mail: gilbert-belofsky@utulsa.edu

  0947-6539/00/0608-1355 $ 17.50+.50/0

final purification by silica gel HPLC. Compounds **4** and **5** were recognized as belonging to the fumiquinazoline class of compounds based upon literature searches incorporating preliminary mass spectral and NMR spectroscopic evidence.[7, 8] Compounds **1**–**3**, however, exhibited significant differences from the fumiquinazolines and their characterization required more extensive spectroscopic work. Compound **1** was analyzed for the molecular formula $C_{17}H_{21}N_3O_5$ by mass spectral data (FABMS: $m/z = 348$ $[M+H]^+$; HR-FABMS: $m/z = 348.1502$ $[M+H]^+$). This formula required nine degrees of unsaturation, and UV spectral data (i.e., $\lambda =$ 345 nm) indicated an extended π system in the molecule. The ^{1}H-NMR spectrum of **1** contained four methyl signals, one for a methoxy singlet at $\delta = 3.73$, and four signals between $\delta =$ 5.22 and $\delta = 6.18$ due to protons deshielded as a result of unsaturation or attached heteroatoms. Two broad singlets assigned to OH and NH protons were also present in the spectrum at $\delta = 3.19$ and $\delta = 6.79$. The NMR data, overall, suggested that compound **1** was similar to sub-structures within known compounds of the fumiquinazoline type. Detailed comparison of the data for **1** with those of the A–C rings of fumiquinazoline C (**6**)[8] supported this conclusion, but also revealed significant differences from the known members of this class. First, the C-3 position was found to be hydroxylated in **1**, as determined by the carbon signal at $\delta =$ 84.9 and the OH signal at $\delta = 3.19$ in the ^{1}H-NMR spectrum. The substituents at the C-3 position were indicative of a unit derived from isoleucine (Ile), and the ^{1}H- and ^{13}C-NMR data for C-16 through C-19 were characteristic of an Ile side chain. The ^{13}C-NMR assignments for C-1, C-15, and C-22 were appropriate for a unit derived from alanine (Ala). The connection of the C and B rings was supported by key HMBC correlations (see Experimental Section) between H-15 and C-13, and between H-2 and C-4. The ^{13}C-NMR data for the B ring indicated a similar arrangement to that of **6** except for the chemical shifts of C-6 and C-8, which were relatively deshielded in **1** by comparison. At this point, the remaining unassigned portions of the molecule included the methoxy group, another oxygen, and four unsaturated carbons. These data were suggestive of an oxygen-substituted aromatic ring, but the chemical shifts for the remaining unassigned carbons and protons were not consistent with this hypothesis. In particular, the NMR signals for C-6, C-11, H-8, H-9, and H-11 were outside the aromatic region of the spectra.

In order to complete the structure determination of **1**, further 2D-NMR experiments, including additional high-field HMBC and ADEQUATE[9] were necessary. Three different delays for the evolution of the long range heteronuclear couplings in ^{1}H,^{13}C-HMBC were used (60, 80, and 120 ms). A portion of the ^{1}H,^{13}C-HMBC spectrum of **1**, obtained with a delay of 80 ms, is shown in Figure 1. A large coupling constant, $^1J_{CH} = 198$ Hz, was observed for C-8 that was typical of an sp^2-carbon atom attached to a heteroatom.[10] Key HMBC correlations included those between H-11 and C-13, and between H-8 and C-6, C-9, C-10, C-11 (four-bond), and C-12 (four-bond). The 1,1-ADEQUATE experiment allowed for discrimination between $^2J_{CH}$ and $^3J_{CH}$ correlations.[11] Additional data obtained from ^{1}H,^{15}N-HMBC experiments provided essential information concerning the connectivity of the

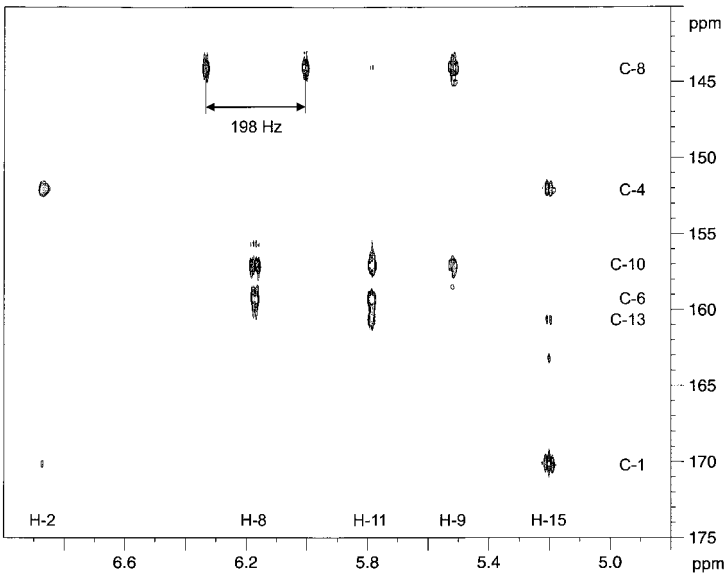


Figure 1. Key ^{1}H,^{13}C-HMBC correlations for Oxepinamide A (**1**).

nitrogen atoms in **1**. The ^{1}H,^{15}N-HMBC experiment was run with two different delays (100 and 150 ms) for the evolution of the heteronuclear couplings. Two of the three nitrogens in the molecule (N-2 and N-14) were easily identified in the ^{1}H,^{15}N-HMBC (Figure 2a and b), but correlations to N-5 were not observed initially. In order to be able to observe correlations

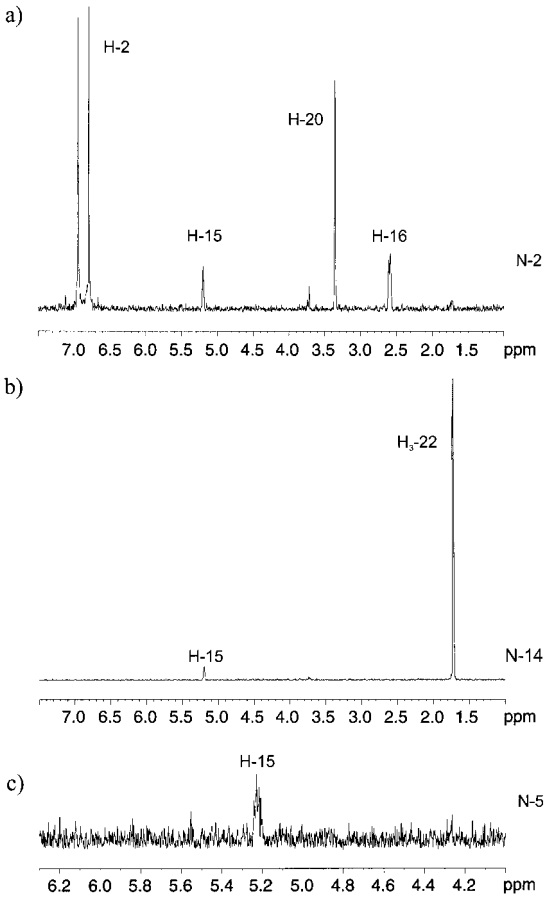


Figure 2. ^{1}H,^{15}N-HMBC correlations for Oxepinamide A (**1**).

0947-6539/00/0608-1356 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 8

due to small coupling constants in the $^{1}H,^{15}N$-HMBC experiment, the delay for the evolution of the heteronuclear long range couplings was set to 200 ms and the number of acquisitions increased from 32 to 64. As a result, it was possible to observe a weak correlation from H-15 to N-5 (Figure 2c), that was concluded to be a $^{4}J_{NH}$ correlation. These data allowed the structure of the compound, which we have named oxepinamide A (**1**), to be proposed as shown. Overall, 38 out of the 40 possible HMBC correlations (^{2}J and ^{3}J) expected for **1** were observed. The oxepin ring, rarely observed in nature, explains the deshielded positions of C-6 and C-8 relative to compound **6**.

In order to evaluate the correctness of the proposed structure, COCON calculations[12, 13] were carried out based upon the experimental 2D-NMR data obtained for **1**, including correlations observed in COSY, $^{1}H,^{13}C$-HMBC, $^{1}H,^{15}N$-HMBC, 1,1-ADEQUATE, and 1,*n*-ADEQUATE. COCON quantifies the value of connectivity information and generates a series of unbiased alternative structures. The $^{4}J_{NH}$ correlation between N-5 and H-15, and the $^{1}J_{CH}$ coupling constant observed for C-8 were not used as input for the COCON calculations. The COCON analysis generated 42 possible structures, many of which were easily eliminated since they violated Bredt's rule or contained N–O bonds, for which no experimental evidence was obtained. In addition, theoretical ^{13}C-NMR chemical shift calculations (SpecEdit)[14] were carried out for all of the possible structures. Those structures for which the predicted ^{13}C-NMR data (averaged over all carbons in the molecule) deviated significantly from the experimental values were also eliminated. The six best structures generated by the COCON analysis are shown in Figure 3 (structures with N–O bonds were considered). The numbers shown indicate the order in which that particular structure was generated. The chemical shift deviations between the experimental and calculated values are given under each structure. Structures #38 and #40 were eliminated based upon the observed $^{1}H,^{15}N$-HMBC experimental data since they require five- and six-bond correlations, respectively, from H-15 to one of the nitrogens. Furthermore, additional $^{1}H,^{15}N$-HMBC correlations would be expected for structures #38 and #40 that were not observed. In structures #35 and #41 C-6 has no attached heteroatom and therefore would not be expected to have $^{1}J_{CH} = 198$ Hz. The two remaining structures (#36 and #42) may be distinguished based upon the $^{1}H,^{13}C$-HMBC data. In #36 two correlations from olefinic protons to the carbonyl carbon at $\delta = 160.9$ are expected, whereas only one is expected for #42, as was experimentally observed. Thus, the structure proposed for oxepinamide A (**1**) based upon spectroscopic data, is also the preferred structure following COCON analysis.

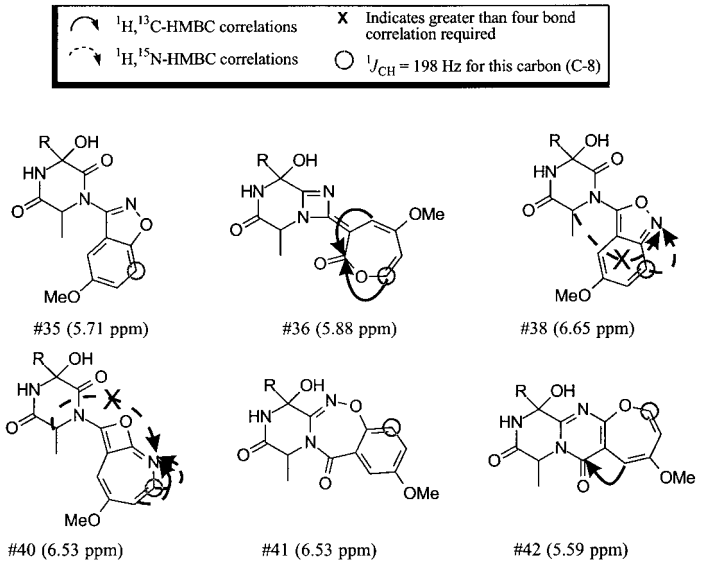


Figure 3. Results of the COCON analysis of the 2D-NMR data obtained for **1**. The final six structural proposals are shown. Numbers in parentheses indicate the averaged ^{13}C-NMR chemical shift deviation calculated by SpecEdit. Criteria used to distinguish between the structural proposals are discussed in the text.

A literature search revealed one compound, cinereain (**7**), with a carbon skeleton close to that of **1**.[15] Although the structure of **7** was determined by single crystal X-ray diffraction, relevant ^{1}H- and ^{13}C-NMR chemical shifts reported for **7** were consistent with those of oxepinamide A. Another oxepin-containing natural product asperloxin was isolated from a terrestrial fungus by Zeeck and co-workers in 1996.[16] Key NOESY correlations (see Experimental Section) between H_3-22 and H-20, and between H-15 and both H_3-18 and H_3-19, helped to assign the relative stereochemistry for oxepinamide A (**1**) as shown.

Compound **2** was analyzed for the same molecular formula as **1**, $C_{17}H_{21}N_3O_5$, and showed similar spectral features to those of **1** (Tables 1 and 2). Analysis of the HMQC- and HMBC-spectral data indicated that the gross structure of **2** was identical to that of **1**. Significant differences in chemical shift between **1** and **2** were observed, however, particularly for H-15, H-16, H-17, and H-20, while the values observed for H_3-22, C-1, C-15, and C-13 remained virtually the same. These differences are consistent with **2** being the C-3 epimer of **1**.

Oxepinamide C (**3**) was shown to have the formula $C_{18}H_{23}N_3O_5$ as determined by ^{13}C-NMR and HR-FABMS methods. The ^{1}H-NMR spectrum of **3** was nearly identical to that of **1** except for the presence of an additional three-hydrogen singlet at $\delta = 3.22$, and the absence of the H-20 signal ($\delta = 3.19$ in **1**) in **3**. These changes indicated that the OH group at C-3 in **1** is replaced by a methoxy in **3**. This was further confirmed by features of the ^{13}C-NMR and DEPT spectra which revealed an additional methyl peak at $\delta = 51.6$.

Table 1. ^{1}H-NMR data and coupling constants *J* (in parentheses [Hz]) for compounds **1–3** in $CDCl_3$.

Position	Compound **1**	Compound **2**	Compound **3**
2	6.79 (brs)	7.00 (brs)	6.30 (brs)
8	6.18 (d, 5.9)	6.15 (d, 5.9)	6.22 (d, 5.9)
9	5.53 (dd, 5.9, 2.0)	5.50 (brd, 5.9)	5.55 (dd; 5.9, 2.0)
11	5.80 (s)	5.74 (s)	5.83 (d, 1.5)
15	5.22 (q, 6.8)	5.04 (q, 6.8)	5.19 (q, 7.0)
16	2.60 (m)	2.11 (m)	2.69 (m)
17/17′	1.96 (m)/1.14 (m)	1.30 (m)/1.13 (m)	1.83 (m)/1.02 (m)
18	0.89 (d, 6.8)	1.04 (d, 6.4)	0.87 (d, 6.8)
19	1.08 (dd, 13.2, 6.8)	0.91 (d, 6.8)	1.05 (d, 6.8)
20	3.19 (s)	4.12 (s)	3.22 (s)
21	3.73 (s)	3.70 (s)	3.75 (s)
22	1.73 (d, 6.8)	1.67 (d, 6.8)	1.72 (d, 7.3)

Table 2. ^{13}C-NMR data for compounds **1**–**3** in $CDCl_3$.

Position	Compound **1** (DEPT)	Compound **2** (DEPT)	Compound **3** (DEPT)
1	70.2 (C)	167.4 (C)	170.5 (C)
2	–	–	–
3	84.9 (C)	85.6 (C)	89.1 (C)
4	152.3 (C)	154.7 (C)	149.8 (C)
6	159.5 (C)	159.6 (C)	159.0 (C)
8	144.3 (CH)	144.7 (CH)	144.7 (CH)
9	115.7 (CH)	115.9 (CH)	115.9 (CH)
10	157.4 (C)	157.6 (C)	157.8 (C)
11	94.6 (CH)	94.7 (CH)	94.8 (CH)
12	110.7 (C)	110.8 (C)	111.2 (C)
13	160.9 (C)	160.9 (C)	161.2 (C)
15	52.9 (CH)	53.3 (CH)	53.3 (CH)
16	41.7 (CH)	46.1 (CH)	37.1 (CH)
17	21.1 (CH_2)	24.5 (CH_2)	21.3 (CH_2)
18	14.9 (CH_3)	11.6 (CH_3)	14.6 (CH_3)
19	12.2 (CH_3)	12.0 (CH_3)	12.2 (CH_3)
20	–	–	51.6 (CH_3)
21	55.2 (CH_3)	55.5 (CH_3)	55.5 (CH_3)
22	18.9 (CH_3)	19.4 (CH_3)	18.9 (CH_3)

The remaining chemical shift values in **3** were much more similar to compound **1** than **2**. Analysis of the extensive NMR data for both **1** and **3** provided mutual support for the complete ^{1}H- and ^{13}C-NMR assignments for these compounds, as well as for the proposed relative stereochemistry as shown.

Fumiquinazoline H (**4**) has the molecular formula $C_{27}H_{27}N_5O_4$ as deduced from mass spectral data (FABMS: $m/z = 485$ $[M]^+$; HR-FABMS: $m/z = 485.2060$ $[M]^+$). The ^{1}H-NMR spectrum of **4** contained two methyl doublets, one methyl singlet at $\delta = 2.02$, a broad amide NH signal at $\delta = 8.76$, and eight clearly resolved aromatic proton signals between $\delta = 7.04$ and $\delta = 8.22$. DEPT, COSY, HMQC, and HMBC experiments permitted the corresponding proton and carbon signals to be assigned (see Experimental Section). The NMR data overall was similar to that of fumiquinazoline C (**6**)[7, 8] but with several significant differences. First, it was found that C-20 was substituted with a four carbon unit, with NMR signals typical of a leucine side chain, as evidenced by the two methyl doublets at $\delta = 0.98$ and $\delta = 1.02$, and by the additional ^{13}C-NMR signals at $\delta = 22.4$, 23.2, 26.3, and 42.7. These signals accounted for the difference of C_3H_6 between the molecular formulae of **4** and **6**. Second, there were significant chemical shift differences observed in the ^{1}H-NMR spectra of the two compounds, particularly for those atoms, for example H-14, H_2-15, H-18, H-19, and H-27, that are positioned close to the C-3/C-17 ether bridge that connects the two main ring systems. The presence of this ether bridge allowed for effective analysis by NOESY, since it locks the two main ring systems together. Key NOESY correlations are shown in Figure 4. NOESY correlations that revealed the relative orientation of the upper and lower hemispheres of **4** were observed between H-2 and both H-18 and H-19, and between H-15 and H-27. These data suggested that the absolute stereochemistry at C-17 in **4** is opposite that of **6**. Additional NOESY correlations between H_2-29 and both H-18 and H-19, indicated that these atoms are on the β face of the molecule as shown. Furthermore, a sample of **4** was

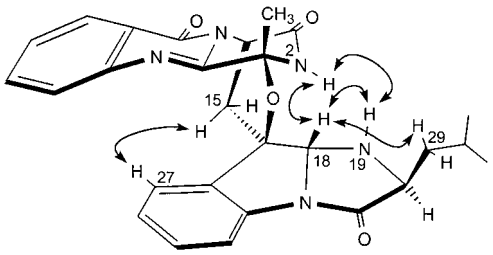


Figure 4. Key NOESY correlations for fumiquinazoline H (**4**).

hydrolyzed in 6 N HCl (110 °C, 24 h) and the pentafluoropropyl isopropyl ester derivatives[17] of the amino acids present in the mixture were prepared. Chiral capillary GC analysis of this mixture revealed the leucine residue of **4** to be in the L (or *S*) configuration at C-20. This information, together with the NOESY data, implied that C-18, C-17, C-14, and C-3 were in the (*R*), (*S*), (*R*), and (*S*) configurations, respectively, as shown.

Fumiquinazoline I (**5**) was shown to have the molecular formula $C_{27}H_{29}N_5O_4$ as determined by ^{13}C-NMR and HR-FABMS methods. The ^{1}H-NMR spectrum of **5** was very similar to that of **4** except for two additional signals: a singlet at $\delta = 5.69$, and a quartet at $\delta = 5.12$. These changes indicated the presence of an OH group at C-17, and a proton at C-3, both of which are present in **5** as a result of the reductive opening of the ether bridge of **4**. This was further confirmed by ^{1}H- and ^{13}C-NMR chemical shift values, particularly for C-3 and C-17, which were significantly shielded in **5** as compared with **4** (see Experimental Section). The conversion of fumiquinazoline H (**4**) to fumiquinazoline I (**5**) was attempted in order to relate the stereochemistry of the two compounds. Reaction of **4** with $NaBH_4$[8] resulted in an approximately 50% conversion to **5** indicating that these two compounds exist in the same configuration. This was further confirmed by comparison of the chemical shift values of C-3, H-3, C-16, and H_3-16, of **5**, to those reported for fumiquinazolines A and B, which have both possible configurations at C-3.[8] This comparison clearly showed **5** to be more similar to fumiquinazoline A, where the methyl group is in the α orientation. Finally, chiral GC analysis revealed the leucine moiety in **5** is also in the (*S*) configuration.

Determination of the isolated yields of compounds **1**–**5** from the *Acremonium* sp. culture broth and mycelia was limited by the complexity of the mixtures. Based upon the actual isolated amounts compounds **1**–**5** were produced at concentrations ranging from 0.2 mg L^{-1} to 0.8 mg L^{-1}.

Fumiquinazolines H and I were found to have weak antifungal activity toward *Candida albicans* in a broth microdilution assay.[18] Both compounds exhibited activity to a dilution of 0.5 mg mL^{-1} (1 mM). By comparison, the antifungal antibiotic amphotericin B exhibited activity at a dilution of 1 μg mL^{-1} (1 μM) in this assay. Compounds **1**–**3** showed no activity in this assay. Compounds **1**–**5** were tested in-house for antimicrobial activity (e.g. *Bacillus subtilus*) and for cancer cell cytotoxicity in the National Cancer Institute's 60 cell-line panel,[19] but showed no significant activity against any cell-line. More notably, oxepinamide A showed good topical anti-inflammatory activity using the resiniferatoxin (RTX)-in-

0947-6539/00/0608-1358 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 8

duced mouse ear edema assay,[20] a test for neurogenic inflammation. Compound **1** exhibited 82% inhibition of edema (induced by RTX at 0.1 μg per ear) at the standard testing dose of 50 μg per ear. Further biological testing of these compounds is in progress.

Experimental Section

General: Preparative HPLC separations were accomplished using a Rainin Dynamax 60-A silica column (8 μm particles, 10 mm × 25 cm) at 3.0 mL mL^{-1} with UV detection at 254 nm. A two-chamber linear gradient apparatus coupled to a column packed with silica gel (60–200 mesh) was used for the gradient silica gel chromatography. For the thin-layer chromatography (TLC) silica gel (0.25 mm) coated glass plates were employed with CH_2Cl_2/MeOH 9:1. TLC spots were visualized by exposure to UV light at 254 nm or to a vanillin/H_2SO_4 (1% *w*/*v*) spray reagent. ^{1}H-NMR, COSY, and NOESY data were obtained at 300 MHz and ^{13}C-NMR data were obtained at 100 MHz. All carbon multiplicities were verified by DEPT and NMR spectra. Initial HMBC and HMQC data were obtained at 300 MHz (^{1}H dimension) and experiments were optimized for $^nJ_{CH} = 10$, 8, or 4 Hz, and $^1J_{CH} = 150$ Hz, respectively. Higher field 2D-NMR data for compound **1** including, HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{15}N-HMBC, 1,1-ADEQUATE, and 1,*n*-ADEQUATE were obtained on Bruker DMX500 (Bruker, Karlsruhe, Germany) and Bruker DRX600 (Institut für Organische Chemie, Universität Frankfurt) instruments. Spectra were recorded in $CDCl_3$ or [D$_6$]acetone and ^{1}H- and ^{13}C-NMR chemical shifts were referenced using the corresponding solvent signals (e.g. $\delta = 2.05$ and $\delta = 29.8$ for [D$_6$]acetone). Chiral GC analyses were obtained on an HP5890A GC using a Heliflex Chirasil-Val capillary column (Alltech, 0.32 mm × 25 m, He carrier gas). Optical rotations were recorded at 21 °C in the solvents indicated. IR spectra were recorded as thin films on NaCl. Fast atom bombardment mass spectral data (FABMS) were obtained using an NBA matrix. The antifungal microdilution broth assay for *C. albicans* (ATCC 32354) was performed in-house with alamar blue as indicator. Compounds were tested at 10 mg mL^{-1} using methods according to previously published procedures.[18]

Fungal isolation, fermentation, and extraction: The producing strain (CNC 890), identified as an *Acremonium* sp. by fatty acid methyl ester (FAME) analysis,[21] was isolated from the surface of the Caribbean tunicate *Ecteinascidia turbinata* collected in the center of a mangrove channel at a depth of −2 m at Sweetings Cay, Grand Bahama Island, Bahamas, in 1996. The fungus was grown in static liquid culture in 20 replicate 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium comprised of 0.5% yeast extract, 0.5% peptone, 1.0% glucose, and 0.2% crab meal. Following a 23 day fermentation period, the mycelium and broth were separated by filtration and the broth extracted twice with equal volumes of EtOAc. The combined mycelial mats were freeze-dried and extracted twice with 4 L of 1:1 CH_2Cl_2/MeOH.

Isolation of compounds 1–5: The broth and mycelial extracts were combined (10.7 g) and subjected to silica gel vacuum liquid chromatography (VLC)[22] over a pre-packed column bed (6 cm (i.d.) × 5 cm (h)). The column was eluted with a stepwise gradient of EtOAc (0–100%) in hexane, followed by MeOH (1–50%) in CH_2Cl_2, and a total of twelve 200 mL fractions were collected. The seven fractions eluting with 60% EtOAc to 10% MeOH were combined (1.3 g) and fractionated on Sephadex LH-20 (2.5 × 28 cm) with 3:1:1 hexane/toluene/MeOH. Fractions of similar composition as determined by TLC were pooled. The fourth and fifth fractions contained mixtures of compounds with TLC R_f values between 0.4 and 0.7 that exhibited a distinctive olive-green color with spraying. These two fractions were combined (225 mg) and further purified by silica gel chromatography (2.5 × 15 cm) using a linear gradient of MeOH (0–7%) in CH_2Cl_2. The first and third fractions (both 31 mg), eluted with ≈4% and ≈5% MeOH, respectively, were each subjected to preparative silica gel HPLC (isocratic elution: EtOAc/hexane 7:3) to afford compounds **1** (16 mg) and **2** (13 mg). The second (427 mg) fraction from the LH-20 column (above) was purified in three successive steps by silica gel chromatography (linear gradient of 0–5% MeOH in CH_2Cl_2), reversed-phase C_{18} VLC (step gradient of 35–100% CH_3CN in H_2O), and another silica gel column (linear gradient of 0–50% EtOAc in hexane) to afford a fraction (38 mg) containing a mixture of compounds with TLC R_f values between 0.8 and 0.9. This material was subjected to preparative silica gel HPLC (EtOAc/hexane) to yield compounds **3** (5.3 mg) and **4** (13 mg). A second batch (20 L) of the fungus was produced and the resulting mycelial extract (10.9 g) was subjected to fractionation procedures similar to those described above (i.e., VLC, LH-20). Final purification by preparative reversed-phase HPLC (CH_3CN/H_2O) afforded compound **5** (8.0 mg).

Oxepinamide A (1): yellow oil; $[\alpha]_D = +43$ ($c = 0.0012$ g mL^{-1}, $CHCl_3$); UV (MeOH): λ_{max} (ε) = 250 (6100), 345 nm (4900 mol^{-1} dm^3 cm^{-1}); IR (film on NaCl): $\tilde{\nu} = 3248$ (br) 2966, 1691, 1658, 1583, 1541, 1412, 1193, 753 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; COSY correlations (*H* →*H*) H-8 →H-9; H-9 →H-11; H-15 →H$_3$-22; H-16 →H-17′; H-16 →H$_3$-18; HMBC correlations (*H* →*C*): H-2 →C-1, C-3, C-4, C-15, C-16; H-8 →C-6, C-9, C-10, C-11 (four-bond), C-12 (four-bond); H-9 →C-8, C-10, C-11; H-11 →C-6, C-9, C-10, C-13; H-15 →C-1, C-4, C-13, C-22; H-16 →C-3, C-4, C-17, C-18, C-19; H-17 →C-3, C-16, C-18, C-19; H-17′ → C-3, C-18, C-19; H$_3$-18 →C-16, C-17; H$_3$-19 →C-16, C-17; H-20 →C-3, C-4; H$_3$-21 →C-10, C-11 (four-bond); H$_3$-22 →C-1, C-15; ^{1}H,^{15}N-HMBC correlations (*H* →*N*): H-15 →N-2, N-14; H-16 →N-2; H-22 →N-14; H-20 →N-2; 1,1-ADEQUATE correlations (*H* →*C*): H-11 →C-12; H-15 →C-1, C-22; H-16 → C-3, C-17; H-18 →C-16; H-19 →C-17; H-22 →C-15; 1,*n*-ADEQUATE correlations (*H* →*C*): H-21 →C-9, C-11; NOESY correlations (*H* →*H*): H-2 →H-16, H-17, H$_3$-18, H$_3$-19, H$_3$-22; H-8 →H-9; H-9 →H$_3$-21; H-11 →H$_3$-21; H-15 →H$_3$-18, H$_3$-19, H$_3$-22; H-20 →H$_3$-22; LR-FABMS: *m*/*z* (%): 348 (26) $[M+H]^+$, 330 (4.9), 279 (36), 205 (3.4), 165 (6.3); HR-FABMS (NBA): found: 348.1502 $[M+H]^+$, calcd. for $C_{17}H_{21}N_3O_5+H^+$: 348.1559.

Oxepinamide B (2): yellow oil; $[\alpha]_D = +52$ ($c = 0.0012$ g mL^{-1}, $CHCl_3$); UV (MeOH): λ_{max} (ε) = 252 (4900), 347 nm (2600 mol^{-1} dm^3 cm^{-1}); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (*H* →*C*): H-2 →C-4, C-15; H-8 →C-6, C-9, C-10; H-9 →C-8, C-10, C-11; H-11 →C-6, C-9, C-10, C-13; H-15 →C-1, C-4, C22; H$_2$-17 →C-18, C-19; H$_3$-18 →C-3, C-16, C-17; H$_3$-19 →C-17; H$_3$-21 →C-10; H$_3$-22 →C-1, C-15; LR-FABMS: *m*/*z* (%): 348 (9.8) $[M+H]^+$, HR-MALDI-FTMS: found: 348.1548 $[M+H]^+$, calcd. for $C_{17}H_{21}N_3O_5+H^+$: 348.1559.

Oxepinamide C (3): yellow oil; $[\alpha]_D = -35$ ($c = 0.0015$ g mL^{-1}, $CHCl_3$); UV (MeOH): λ_{max} (ε) = 250 (3000), 345 nm (2100 mol^{-1} dm^3 cm^{-1}); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; LR-FABMS: *m*/*z* (%): 362 $[M+H]^+$ (84), 345 (7.3), 279 (5.7), 192 (17), 167 (7.8); HR-FABMS (NBA): found: 362.1748 $[M+H]^+$, calcd. for $C_{18}H_{23}N_3O_5+H^+$: 362.1716.

Fumiquinazoline H (4): pale yellow solid; m.p.: 144–147 °C; $[\alpha]_D = -59$ ($c = 0.001$ g mL^{-1}, $CHCl_3$); UV (MeOH): λ_{max} (ε) = 224 (27700), 230sh (25800), 255 (11500), 265 (9700), 276 (7600), 303 (3000), 314 nm (2300 mol^{-1} dm^3 cm^{-1}); IR (film on NaCl): $\tilde{\nu} = 3236$ (br), 2954, 1717, 1693, 1608, 1487, 1466, 1386, 1094, 757 cm^{-1}; ^{1}H NMR ([D$_6$]acetone): $\delta = 8.76$ (br s, H-2), 8.22 (d, $J = 7.8$ Hz, H-10), 7.87 (br t, $J = 7.3$ Hz, H-8), 7.75 (d, $J = 7.8$ Hz, H-7), 7.59 (t, $J = 7.6$ Hz, H-9), 7.43 (d, $J = 7.8$ Hz, H-24), 7.31 (t, $J = 7.6$ Hz, H-25), 7.24 (d, $J = 7.8$ Hz, H-27), 7.04 (t, $J = 7.6$ Hz, H-26), 5.92 (d, $J = 5.9$ Hz, H-18), 5.54 (d, $J = 5.9$ Hz, H-14), 3.82 (t, $J = 7.3$ Hz, H-20), 3.16 (dd, $J = 14.9$, 5.6 Hz, H-15′), 2.58 (d, $J = 5.9$ Hz, H-19), 2.06 (dd, buried, H-15), 2.02 (s, H$_3$-16), 1.85 (m, H-30), 1.70 (m, H$_2$-29), 1.02 (d, $J = 6.4$ Hz, H$_3$-31), 0.98 (d, $J = 6.8$ Hz, H$_3$-32); ^{13}C NMR ([D$_6$]acetone): 173.6 (C-21), 169.7 (C-1), 160.1 (C-12), 153.0 (C-4), 148.3 (C-6), 138.4 (C-23), 138.9 (C-28), 135.5 (C-8), 130.8 (C-25), 129.1 (C-7), 128.4 (C-9), 127.6 (C-10), 127.0 (C-27), 126.1 (C-26), 122.1 (C-11), 115.6 (C-24), 88.9 (C-18), 88.0 (C-17), 85.7 (C-3), 63.4 (C-20), 54.1 (C-14), 42.7 (C-29), 35.5 (C-15), 26.3 (C-30), 24.6 (C-16), 23.2 (C-31), 22.4 (C-32); HMBC correlations (*H* →*C*): H-7 →C-11; H-8 →C-6, C-9, C-10; H-9 →C-7, C-8, C-10, C-11; H-10 → C-6, C-8, C-12; H-14 →C-1, C-4, C-12, C-15, C-17; H-15 →C-1, C-14, C-17, C-18, C-28; H-15′ → C-14, C-17, C-18; H$_3$-16 →C-3, C-4; H-18 →C-15, C-17; H-19 →C-21, C-29; H-20 →C-18, C-21, C-29, C-30; H-24 →C-26, C-28; H-25 →C-23, C-26, C-27; H-26 →C-24, C-25, C-28; H-27 →C-17, C-23, C-25; H$_2$-29 →C-20, C-21, C-30, C-31, C-32; H-30 →C-20, C-29, C-31, C-32; H$_3$-31 →C-29, C-30, C-32; H$_3$-32 →C-29, C-30, C-31; NOESY correlations (*H* →*H*): H-2 →H$_3$-16, H-18, H-19; H-14 →H-15; H-15 →H-15′, H-27; H$_3$-16 →H-18; H-18 →H-19, H$_2$-29; H-19 →H-20, H$_2$-29, H-30, H$_3$-31, H$_3$-32; H-20 →H$_2$-29, H$_3$-30, H$_3$-32; H$_2$-29 →H-30, H$_3$-31, H$_3$-32; H-30 →H$_3$-31, H$_3$-32; LR-FABMS: *m*/*z* (%): 485 (84) $[M]^+$, 428 (4.2), 413 (9.9), 391 (22), 371 (4.0), 341 (4.2), 279 (40), 241 (11), 228 (13), 192 (15); HR-FABMS (NBA): found: 485.2060 $[M]^+$, calcd. for $C_{27}H_{27}N_5O_4$ 485.2063.

Fumiquinazoline I (5): white solid; m.p.: 116–120 °C; $[\alpha]_D = -138$ ($c = 0.001\ g\,mL^{-1}$, $CHCl_3$); UV (MeOH): λ_{max} (ε) = 224 (63300), 230sh (57200), 255 (26000), 266sh (21700), 277 (17000), 304 (5100), 317 nm ($2800\ mol^{-1}\,dm^3\,cm^{-1}$); ^1H NMR ([D_6]acetone): $\delta = 8.24$ (dd, $J = 8.3$, 1.5 Hz, H-10), 7.88 (ddd, $J = 7.6$, 7.6, 1.5 Hz, H-8), 7.74 (s, H-2), 7.73 (d, $J = 7.8$ Hz, H-7), 7.57 (t, $J = 8.3$ Hz, H-9), 7.56 (d, $J = 8.3$ Hz, H-27), 7.36 (d, $J = 7.3$ Hz, H-24), 7.29 (t, $J = 7.6$ Hz, H-25), 7.15 (t, $J = 7.3$ Hz, H-26), 5.75 (dd, $J = 9.8$, 4.4 Hz, H-14), 5.69 (s, H-17), 5.40 (d, $J = 7.3$ Hz, H-18), 5.12 (q, $J = 6.5$ Hz, H-3), 3.61 (br s, H-20), 2.74 (dd, $J = 14.7$, 9.8 Hz, H-15′), 2.64 (br d, $J = 3.9$ Hz, H-19), 2.14 (dd, $J = 14.8$, 4.6 Hz, H-15), 1.75 (d, $J = 6.4$ Hz, H_3-16), 1.43 (m, H_2-29), 1.22 (m, H-30), 0.79 (d, $J = 6.4$ Hz, H_3-31), 0.79 (d, $J = 6.4$ Hz, H_3-32); ^{13}C NMR ([D_6]acetone): 174.1 (C-21), 169.9 (C-1), 163.0 (C-12), 153.6 (C-4), 148.2 (C-6), 140.4 (C-28), 138.5 (C-23), 135.5 (C-8), 129.9 (C-25), 128.3 (C-7), 127.9 (C-9), 127.5 (C-10), 125.9 (C-26), 125.5 (C-27), 121.2 (C-11), 115.9 (C-24), 63.0 (C-20), 88.8 (C-18), 81.8 (C-17), 54.2 (C-14), 49.7 (C-3), 42.3 (C-29), 38.5 (C-15), 25.6 (C-30), 23.5 (C-31), 21.6 (C-32), 16.9 (C-16); HMBC correlations ($H \rightarrow C$: H-2 →C-4, C-14; H-3 →C-4, C-16; H-7 →C-9, C-11; H-8 →C6, C-10; H-9 →C-7, C-8, C-11; H-10 →C-6, C-8, C-12; H-14 →C-1, C-4, C-12, C-15, C-17; H-15 →C-1, C-14, C-17, C-18, C-28; H-15′ → C-1, C-14, C-17, C-18, C-28; H_3-16 →C-3, C-4; H-17 →C-15, C-18, C-28; H-24 →C-23, C-26, C-28; H-25 →C-23, C-24, C-26, C-27, C-28; H-26 →C-24, C-28; H-27 →C-17, C-23, C-25; H_2-29 → C-20, C-21, C-30, C-31, C-32; H-30 →C-29, C-31, C-32; H_3-31 →C-29, C-30, C-32; H_3-32 →C-29, C-30, C-31; LR-FABMS: m/z (%): 488 (81) $[M+H]^+$, 470 (40), 449 (4.9), 391 (9.4), 369 (9.8), 338 (8.7), 313 (6.0), 273 (8.1), 241 (22), 229 (17), 213 (11); HR-FABMS (NBA): found: 488.2294 $[M+H]^+$, calcd. for $C_{27}H_{29}N_5O_4+H^+$: 488.2297.

Conversion of fumiquinazoline H (4) to fumiquinazoline I (5): A sample of fumiquinazoline H (**4**, 2.6 mg) was dissolved in 1,4-dioxane (0.26 mL). $NaBH_4$ (1 mg) and MeOH (0.75 mL) (to aid solubility) was added to this solution. The reaction mixture was allowed to stand 45 min, then evaporated under dry N_2. The residue was dissolved/suspended in H_2O (1 mL) and extracted twice with EtOAc (1 mL each). The EtOAc extract was evaporated to afford the product (3.1 mg). The product consisted of a 1:1 mixture of compounds **4** and **5**, by comparison with authentic samples, by TLC, HPLC (silica column, 7:3 EtOAc/hexane), and ^1H-NMR spectroscopic analyses.

Acknowledgement

This research was supported by the National Institutes of Health and the National Cancer Institute, under grant CA44848 and the Deutsche Forschungsgemeinschaft, under grant Ko1314/3-1 and 3-2. The University of California, Riverside, and The Scripps Research Institute Mass Spectrometry facilities are gratefully acknowledged. The authors would especially like to thank Mr. Chris Kauffman, Mr. Akkharawit Kanjana-Opas, and Ms. Chelsea Clough for their technical assistance. We thank Dr. Rainer Kerssebaum at Bruker, Karlsruhe for valuable discussions and measurement time. We thank Prof. Robert S. Jacobs (UC Santa Barbara) for anti-inflammatory testing of compound **1**.

[1] G. Rheinheimer, *Aquatic Microbiology*, Wiley, New York, **1992**, pp. 243–253.
[2] A. K. Sparks, *Synopsis of Invertebrate Pathology*, *Exclusive of Insects*, Elsevier, New York, N.Y., **1985**.
[3] R. Lorenz, H. P. Molitoris, *Mycol. Res.* **1997**, *101*, 1355–1365.
[4] G. N. Belofsky, P. R. Jensen, M. K. Renner, W. Fenical, *Tetrahedron* **1998**, *54* 1715–1724.
[5] G. N. Belofsky, P. R. Jensen, W. Fenical, *Tetrahedron Lett.* **1999**, *40*, 2913–2916.
[6] K. M. Jenkins, M. K. Renner, P. R. Jensen, W. Fenical, *Tetrahedron Lett.* **1998**, *39*, 2463–2466.
[7] A. Numata, C. Takahashi, T. Matsushita, T. Miyamoto, K. Kawai, Y. Usami, E. Matsumura, M. Inoue, H. Ohishi, T. Shingu, *Tetrahedron Lett.* **1992**, *33*, 1621–1624.
[8] C. Takahashi, T. Matsushita, M. Doi, K. Minoura, T. Shingu, T. Kumeda, A. Numata, *J. Chem. Soc. Perkin Trans. 1* **1995**, 2345–2353.
[9] B. Reif, M. Köck, R. Kerssebaum, H. Kang, W. Fenical, C. Griesinger, *J. Magn. Reson.* **1996**, *A118*, 282–285.
[10] P. Crews, J. Rodriguez, M. Jaspars, *Organic Structure Analysis*, Oxford University Press, New York, **1998**, p. 136.
[11] M. Köck, B. Reif, W. Fenical, C. Griesinger, *Tetrahedron Lett.* **1996**, *37*, 363–366.
[12] a) T. Lindel, J. Junker, M. Köck, *J. Mol. Model.* **1997**, *3*, 364–368; b) T. Lindel, J. Junker, M. Köck, *Eur. J. Org. Chem.* **1999**, 573–577; c) M. Köck, J. Junker, W. Maier, M. Will, T. Lindel, *Eur. J. Org. Chem.* **1999**, 579–586; d) J. Junker, W. Maier, T. Lindel, M. Köck, *Org. Lett.* **1999**, *1*, 737–740; e) M. Köck, J. Junker, T. Lindel, *Org. Lett.* **1999**, *1*, 2041–2044.
[13] A COCON calculation can be carried out in the internet under http://cocon.org.chemie.uni-frankfurt.de.
[14] W. Maier in *Computer Enhanced Analytical Spectroscopy* (Ed.: C. L. Wilkins), Plenum Press, New York, **1993**, pp. 37–55.
[15] H. G. Cutler, J. P. Springer, R. F. Arrendale, B. H. Arison, P. D. Cole, R. G. Roberts, *Agric. Biol. Chem.* **1988**, *52*, 1725–1733.
[16] a) A. Zeeck, J. Fuchser, personal communication; b) J. Fuchser, Ph. D. thesis, Universität Göttingen, **1996**.
[17] Alltech kit catalogue No. 18093; see: K. E. Ibrahim, M. W. Couch, C. M. William, M. Budd, R. A. Yost, J. M. Midgley, *Anal. Chem.* **1984**, *56*, 1695.
[18] M. A. Pfaller, Q. Vu, M. Lancaster, A. Espinel-Ingroff, A. Fothergill, C. Grant, M. R. McGinnis, L. Pasavell, M. G. Rinaldi, L. Steele-Moore, *J. Clin. Microbiol.* **1994**, *32*, 1625–1628.
[19] M. R. Boyd, K. D. Paull, *Drug Dev. Res.* **1995**, *34*, 91–109.
[20] A. M. S. Mayer, P. B. Jacobsen, W. Fenical, R. S. Jacobs, K. B. Glaser, *Life Sci.* **1998**, *62*, 401–407.
[21] FAME (fatty acid methyl ester) analysis (Microbial ID Inc., Newark, DE).
[22] J. C. Coll, B. F. Bowden, *J. Nat. Prod.* **1986**, *49*, 934–936.

Received: July 14, 1999 [F1912]

  0947-6539/00/0608-1360 $ 17.50+.50/0