# Hortein, a New Natural Product from the Fungus *Hortaea werneckii* Associated with the Sponge *Aplysina aerophoba*

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The fungus *Hortaea werneckii* isolated from the Mediterranean sponge *Aplysina aerophoba*, collected at Banyuls-sur-Mer in southern France, yielded a new compound named hortein (**1**), which possesses a unique ring system hitherto unknown for natural products. The structure of **1** was established on the basis of 1D and 2D NMR spectroscopic and mass spectrometric (ESI, CI, FAB, EI) data.

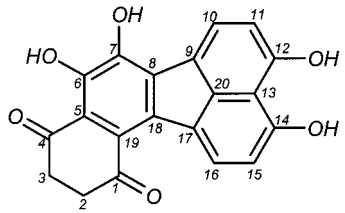
In the search for novel pharmaceutical or agrochemical lead structures increasing attention is being given to secondary metabolites from marine microorganisms, including marine fungi.[1,2] Marine microorganisms play an important ecological role, for example, as primary decomposers[3] and as symbionts,[4] in particular with soft-bodied filter feeding invertebrates such as sponges. To survive in the marine environment, fungi display highly specific adaptations including the production of unique secondary metabolites. In this context sponge- or algae-associated fungi have been previously reported to yield a diverse set of compounds such as chlorinated sesquiterpenes,[5] polyketides,[6] fatty acids,[7] and anthraquinones.[8]

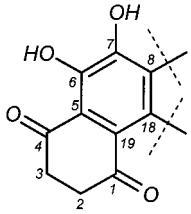
Fungal strains of *Hortaea werneckii* (syn. *Cladosporium werneckii*) are known to be causative agents of the human disease tinea nigra.[9] However, this fungus also exists as nonpathogenic strains,[9] with some being able to grow even at high salt concentrations.[10] To date, the only chemical investigation of the fungus has yielded peptidogalactomannans and peptidosphosphogalactomannans.[11]

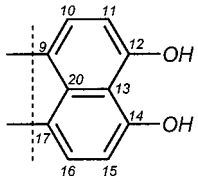
*H. werneckii* analyzed in this study was isolated from the sponge *Aplysina aerophoba*, which occurs frequently in the Mediterranean Sea and is chemically characterized by the accumulation of brominated isoxazoline alkaloids and related compounds.[12] In this paper we describe the isolation and structure elucidation of hortein (**1**), a new phenolic natural product characerized by a unique ring system, hitherto not described in nature.

*H. werneckii* was isolated from the Mediterranean sponge *A. aerophoba* and grown in liquid malt extract medium. Secondary metabolites were extracted with ethyl acetate (EtOAc) from the mycelia and the culture filtrate. The isolation of hortein (**1**) was achieved by chromatography on Sephadex LH-20 using methanol as eluent. Six fractions were collected. Fraction 5 yielded compound **1** (42 mg) and was further purified by semipreparative HPLC. The molecular formula was determined to be $C_{20}H_{12}O_6$ by HRESIMS, thus indicating 15 degrees of unsaturation. The structure of compound **1** was determined from 1D $^1$H and $^{13}$C NMR and 2D COSY and HMBC NMR experiments.

The $^1$H NMR spectrum of hortein (**1**) shows four doublets in the aromatic region, two triplets at 3.08 and 3.18 ppm,




and the signal of a hydrogen-bound OH group at 13.50 ppm. Two pairs of ortho-coupled hydrogens (H-10, H-11, H-15, and H-16, respectively) were evident from the COSY and HMBC correlations. Evaluation of the latter revealed that they belonged to a 12,14-dihydroxynaphthalene system that was further substituted in positions 9 and 17, respectively (partial structure II). The two triplets at 3.08 and 3.18 ppm could be assigned to a 2,3-dihydro-1,4-naphthoquinone system by HMBC correlations from C-19 to H-2 and C-5 to H-3, respectively. This was also corroborated by correlations from the hydrogen of the hydrogen-bonded hydroxyl group at C-6 to C-4, C-5, C-6, and C-7 (partial structure I). Connection of the two fragments was established through $^3J_{C,H}$ correlations from C-8 to H-10 and from C-18 to H-16, respectively. The downfield shift of H-16 (9.02 ppm) as compared to H-10 (8.25 ppm) is due to the spatial proximity of the carbonyl group at C-1.

Hortein features an acenaphtho[1′,2′:7,8]naphthalene ring system, which is most unusual and has to our knowledge previously not been encountered in natural products. Until now, only one synthetic compound featuring this ring system, namely, acenaphtho[1′,2′:7,8]naphth[1,2-*b*]oxirene, has been reported in the literature.[13] Furthermore, to the best of our knowledge, a 2,3-dihydro-1,4-naphthoquinone has not been found as a natural product, despite its apparent structural simplicity. Thus, hortein adds a further example of the remarkable chemical diver-

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**Table 1.** NMR Data of Hortein (**1**)[a]

| position | $\delta_C$ | $\delta_H$ ($J$ Hz) | HMBC correlations |
|---|---|---|---|
| 1 | 196.6 (s) | | |
| 2 | 36.8 (t) | 3.08 (t, 7.0) | C1, C2,[b] C3, C4, C19 |
| 3 | 38.3 (t) | 3.18 (t, 7.0) | C1, C2, C3,[b] C4, C5 |
| 4 | 204.9 (s) | | |
| 5 | 116.0 (s) | | |
| 6 | 148.9 (s) | 13.50 (s) | C4, C5, C6,[b] C7 |
| 7 | 145.4 (s) | | |
| 8 | 131.1 (s) | | |
| 9 | 123.8 (s) | | |
| 10 | 127.4 (d) | 8.25 (d, 7.82) | C8, C10,[b] C11, C12, C20 |
| 11 | 110.1 (d) | 6.99 (d, 7.82) | C9, C11,[b] C12, C13 |
| 12 | 156.6 (s) | | |
| 13 | 111.0 (s) | | |
| 14 | 156.1 (s) | | |
| 15 | 110.2 (d) | 6.96 (d, 8.2) | C13, C14, C15,[b] C17 |
| 16 | 130.5 (d) | 9.02 (d, 8.2) | C14, C16,[b] C17, C18, C20 |
| 17 | 125.2 (s) | | |
| 18 | 129.9 (s) | | |
| 19 | 122.1 (s) | | |
| 20 | 136.6 (s) | | |

[a] Spectra were recordet in DMSO-$d_6$. [b] C−H direct correlations.

sity of sponge-derived fungi. It is also interesting to note that this compound does not exhibit any structural similarity to brominated isoxazoline alkaloids or related secondary metabolites of the sponge *A. aerophoba*,[12] from which the fungus was isolated.

When tested for antibiotic or insecticidal activity, hortein (**1**) proved to be inactive against *Escherichia coli* HB101, *Staphylococcus aureus*, *Bacillus subtilis*, the fungus *Candida albicans*, and the pest insect *Spodoptera littoralis*, respectively.

## Experimental Section

**General Experimental Procedures.** $^1$H NMR and $^{13}$C NMR (including DEPT) spectra (chemical shifts in ppm) were recorded in DMSO-$d_6$ on a Bruker ARX 300 NMR spectrometer. Mass spectra (ESI, CI, FAB) were recorded on a Finnigan MAT 8430 and on an Intectra AMD 402 spectrometer. HRESIMS data were determined on a Intectra AMD 402 spectrometer. Solvents were distilled before use, and spectral grade solvents were used for spectroscopic measurements. HPLC analysis was performed using a HPLC (Gynkotek) system coupled to a photodiode array detector. Routine detection was at 254, 280, and 340 nm.

The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher $C_{18}$ (4 $\mu$m) (Knauer, Germany) using the following gradient (MeOH, 0.02% $H_3PO_4$ in $H_2O$): 0 min, 10% MeOH; 5 min, 10% MeOH; 35 min, 100% MeOH; 45 min, 100% MeOH.

**Fungal Material.** The fungus *H. werneckii* was isolated from fresh samples of the marine sponge *A. aerophoba*. The sponge was collected by scuba diving in June 1996 from the Mediterranean along the shores of Banyuls-sur-Mer (France). Tissue samples from inside the sponge were taken under sterile conditions, inoculated on malt agar slants (containing 15 g/L malt extract, 15 g/L agar in a mixture of seawater and distilled water adjusted to 74% of natural salinity), and incubated at 27 °C. From the growing cultures pure strains of *H. werneckii* were isolated by repeated reinoculation on malt agar plates. The fungus was identified by the Centraalbureau voor Schimmelcultures (CBS), Baarn, Netherlands.

**Extraction and Isolation.** For the extraction of natural products liquid cultures (20 L) were grown in malt-broth medium, containing 25 g/L malt extract. Mass growth of the fungus was performed at the Hans-Knöll-Institut für Naturstofforschung, Jena (Germany). After 62 days of incubation without shaking at 27 °C the mycelia and culture filtrate were collected and extracted with EtOAc. The crude extract (2.66 g) was taken to dryness and then chromatographed over Sephadex LH-20 using methanol as solvent system. TLC plates with SiO gel F254 (Merck, Darmstadt, Germany) were used for monitoring the fractions. Detection was done by UV at 254, 340, and 366 nm or by spraying the plates with anisaldehyde reagent. Further purification was achieved by semipreparative HPLC (Merck, Darmstadt, Germany) on a Eurospher $C_{18}$ (300 × 8 mm, i.d.) column using the following gradient: 0 min, 60% MeOH; 15 min, 80% MeOH; 20 min, 80% MeOH; 25 min, 100% MeOH; 30 min, 100% MeOH; 35 min, 60% MeOH.

**Bioassay.** Bioassays for antibacterial and antifungal activity were carried out as described previously,[14] using *B. subtilis* 168, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. coli* HB 101, and *C. albicans*. The bacterial strains were from the laboratory cultures of the Institute for Hygiene, University of Würzburg, Germany. The chronic feeding assays were carried out with larvae of the polyphagous pest insect *S. littoralis* (Noctuidae, Lepidoptera). The larvae were from a laboratory colony reared on an artificial diet under controlled conditions at 26 °C as described previously.[15]

**Hortein** (**1**): brown powdery solid; UV (MeOH) $\lambda_{max}$ 204, 257, 358, 444 nm; NMR data, see Table 1; EIMS (70 eV) *m/z* 348 $[M]^+$ (100), 319 (14), 292 (10), 235 (8), 208 (10); CIMS *m/z* $[M + H]^+$ 349 (100), $[M + NH_4]^+$ 366 (32); (−)FABMS *m/z* $[M - H]^-$ 347 (52), 346 (38), 307 (26), 305 (100); (−)ESIMS *m/z* 347 $[M - H]^-$ (100), 283 (17), 277 (30), 235 (11); HRESIMS *m/z* 349.0719 $[M + H]^+$ (calcd for $C_{20}H_{13}O_6$, 349.0634).

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