Ascosalipyrrolidinone A, an Antimicrobial Alkaloid, from the Obligate Marine Fungus *Ascochyta salicorniae*

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From the green alga *Ulva* sp., the endophytic and obligate marine fungus *Ascochyta salicorniae* was isolated. *A. salicorniae* was mass cultivated and found to produce the unprecedented and structurally unusual tetramic acid containing metabolites ascosalipyrrolidinones A (**1**) and B (**2**). Additionally, the new natural product ascosalipyrone (**3**) and the known metabolites **4** and **5** were obtained. Ascosalipyrrolidinone A (**1**) has antiplasmodial activity toward *Plasmodium falciparum* strains K1 and NF 54, as well as showing antimicrobial activity and inhibiting tyrosine kinase $p56^{lck}$.

Terrestrial fungi are known as sources of secondary metabolites with significant therapeutic potential. The biosynthetic capabilities of marine fungi, however, are less well studied and interest in this group of organisms is increasing.[1,2] It was estimated by Kohlmeyer that one-third of all known higher marine fungi are associated with algae.[3] For this reason, and on the basis of the idea that algicolous fungi may produce metabolites as a means of dealing with their host plant, algae were regarded as a valuable source for the isolation of unusual and obligate marine fungal strains. In the last 5 years compounds with novel chemical structures and biological activities have been isolated from algicolous fungi, e.g., communesins,[4] leptosins,[5−7] penochalasins,[8] penostatins,[9,10] and pyrenocine E.[11] All of these compounds are cytotoxic toward cultured P-388 lymphocytic leukemia cells. Further examples of biologically active compounds isolated from fungi associated with algae include the halymecins with antimicroalgal activity,[12] exumolides A and B,[13] also with antimicroalgal activity, and finally some sesquiterpenoid nitrobenzoyl esters, which were shown to be cytotoxic toward HCT-116 human colon carcinoma cells.[14] Terrestrial species of the genus *Ascochyta* have been studied for their natural product content, with pinolidoxin being isolated from *A. pinodes*,[15] chrysanthones B and C from *A. chrysanthemi*,[16] and ascochalasin from *A. heteromorpha*,[17] indicating the genus *Ascochyta* to have a highly developed and diverse secondary metabolism.

During our investigations dealing with the isolation, cultivation and screening of fungal strains associated with marine algae, the obligate marine fungus *Ascochyta salicorniae* was obtained from the marine green alga *Ulva* sp., collected from the North Sea, Tönning, Germany. Algal material was surface sterilized with EtOH 70% to ensure fungi were only isolated from the inner algal tissue. Thus, *A. salicorniae* is assumed to be an algal endophyte. The EtOAc extract of this fungal isolate was found to have antimicrobial activity. On the basis of this activity the fungus was further investigated in order to identify the biologically active natural products.

Results and Discussion

In the present study the isolation, structure elucidation and biological activities of five secondary metabolites obtained from *Ascochyta salicorniae* are described. The fungus was cultivated on a solid biomalt medium with added artificial sea salt. Successive fractionation of the EtOAc extract by vacuum-liquid chromatography (VLC) and normal (Si-60) and reversed (RP-18) phase HPLC

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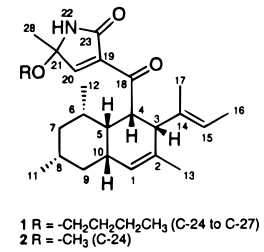
Table 1. ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR Data for Ascosalipyrrolidinone A (1) and ^{1}H NMR Data (200 MHz, $CDCl_3$) for Ascosalipyrrolidinone B (2)

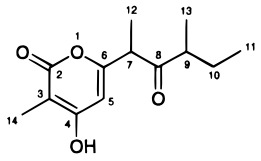
carbon	δ ^{13}C (**1**)	δ ^{1}H (**1**)	HMBC[a] (**1**)	NOE[b] (**1**)	δ ^{1}H (**2**)
1	128.6 d[c]	5.58 (d, J = 6.1 Hz)	3, 5, 10, 13	9, 10	5.57 (d, J = 6.1 Hz)
2	132.4 s				
3	57.5 d	2.87 (brd, J = 7.6 Hz)	1, 2, 14, 15	15	2.86 (brd, J = 7.6 Hz)
4	43.2 d	4.07 (brm)		7, 20	4.08 (brm)
5	41.6 d	2.32 (m)		3, 6, 10	2.31 (brm)
6	37.0 d	1.73 (m)			1.69
7	38.5 t	1.17 (m), 1.29 (m)			1.19 (m), 1.28 (m)
8	32.9 d	1.44 (m)			1.44 (m)
9	39.0 t	1.65 (brd, J = 13.0 Hz)			1.55 (brd, J = 13.0 Hz)
		0.95 (ddd, J = 13.0 Hz)			0.91 (ddd, J = 13.0 Hz)
10	39.4 d	2.07 (brm)	1, 2, 4, 9	4, 5, 6, 9	2.08 (m)
11	22.4 q	0.91 (d, J = 6.6 Hz)	7, 8, 9		0.92 (d, J = 6.6 Hz)
12	23.5 q	0.67 (d, J = 7.4 Hz)	5, 6, 7		0.68 (d, J = 7.4 Hz)
13	21.0 q	1.42 (brs)	1, 2, 3		1.41 (brs)
14	135.2 s				
15	122.7 d	5.12 (m)	3, 16	3, 20	5.11 (m)
16	13.2 q	1.42 (brs)			1.43 (brs)
17	11.9 q	1.47 (brs)	3, 14, 15		1.46 (brs)
18	201.9 s				
19	137.0 s				
20	155.8 d	7.38 (m)	18, 19, 21, 23, 28	4, 24	7.32 (m)
21	87.1 s				
23	167.5 s				
24	63.2 t	3.33 (m), 3.07 (m)	21, 25, 26		3.09 (s)
25	32.0 t	1.46 (m)			
26	19.2 t	1.32 (m)			
27	13.8 q	0.89 (t, J = 7.3 Hz)	25, 26		
28	25.1 q	1.57 (s)		20, 24	1.58 (s)
22 (NH)		5.91 (brs)	19, 20, 21, 23	24, 28	5.65 (brs)

[a] Numbers represent carbon atoms that are observed to long-range CH couple with the proton(s) associated with this data row. [b] Enhanced proton signals as observed by difference NOE measurements. [c] Multiplicities by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

yielded compounds **1**−**5**. Compounds **1** and **2** are unusually substituted tetramic acid derivatives and most interesting as a result of their antimicrobial activity.



1 R = -$CH_2CH_2CH_2CH_3$ (C-24 to C-27)
2 R = -CH_3 (C-24)



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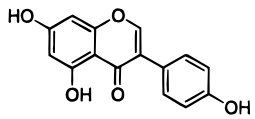

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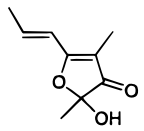

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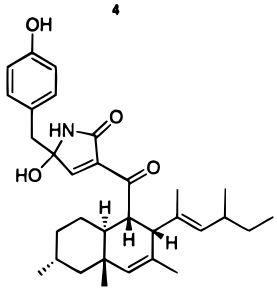
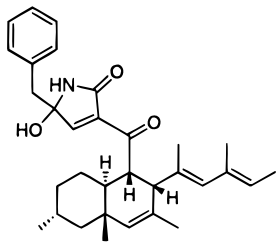
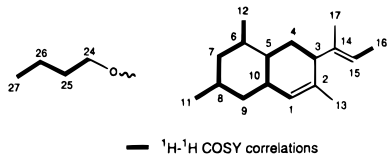


7 ZG-1494α



6 Oteromycin

Ascosalipyrrolidinone A (**1**) has the molecular formula $C_{27}H_{41}NO_3$ as deduced by accurate mass measurement. Its ^{13}C NMR spectrum contained 27 signals (see Table 1). From the ^{13}C NMR spectroscopic data (^{1}H decoupled and DEPT) it was evident that five of the eight elements



^{1}H-^{1}H COSY correlations

Figure 1. Selected partial structures of ascosalipyrrolidinone A (**1**) deduced from ^{1}H−^{1}H COSY correlations.

of unsaturation, indicated by the molecular formula of **1**, could be attributed to three carbon−carbon double bonds [δ 155.8 (d, C-20), 137.0 (s, C-19), 132.4 (s, C-2), 128.6 (d, C-1), 135.2 (s, C-14), 122.7 (d, C-15)] and two carbonyl groups [δ 201.9 (s, C-18), 167.5 (s, C-23)], these being the only multiple bonds within **1**; the molecule is thus tricyclic. The ^{1}H and ^{13}C NMR spectra further revealed the presence of five methylene groups [δ 19.2 (t, C-26), 32.0 (t, C-25), 38.5 (t, C-7), 39.0 (t, C-9)], one of which was attached to oxygen [δ 63.2 (t, C-24)]; six methine groups [δ 32.9 (d, C-8), 37.0 (d, C-6), 39.4 (d, C-10), 41.6 (d, C-5), 43.2 (d, C-4), 57.5 (d, C-3)], two of them adjacent to methyl groups [δ 0.67 (d, H_3-12); 0.91 (d, H_3-11)]; five methyl groups [δ 1.57 (s, H_3-28); 1.42 (brs, H_3-13); 0.89 (t, H_3-27); 1.42 (brs, H_3-16); 1.47 (brs, H_3-17)], and a quaternary carbon bound to oxygen and nitrogen [δ 87.1 (s, C-21)].

From the ^{1}H−^{1}H-COSY spectrum three fragments of **1** could be deduced (see Figure 1). Thus, ^{1}H−^{1}H couplings were observed between H_3-12 and H-6, between H-6 and H_2-7, between H_2-7 and H-8, between H-8 and H_2-9, between H_2-9 and H-10, between H-10 and H-1, between H-10 and H-5, between H-5 and H-4, between H-4 and H-3 and between H-8 and H_3-11. Cross-peaks between the resonances for H-15 and H_3-16 indicated these protons also to couple. Additionally, ^{1}H−^{1}H couplings

were seen between the proton bound to nitrogen (H-22) and H-20 in the pyrollidinone and between H_2-24 and H_2-25, H_2-25 and H_2-26, and H_2-26 and H_3-27, indicating the presence of a butoxyl function. This information together with data obtained from the $^1H-^{13}C$ HMBC spectrum allowed the planar structure of **1** to be deduced. Thus, diagnostic HMBC correlations from the resonance for H_3-12 to those for C-5, C-6 and C-7 permitted the Decalin ring to be completed. Further proof of this system came from HMBC correlations between the resonance for H_3-13 and those of C-1, C-2 and C-3 clearly positioning CH_3-13 at C-2. HMBC correlations between the resonance for H_3-17 and those for C-3, C-14 and C-15 showed the 2-butenyl moiety to be placed at C-3. Further, $^1H-^{13}C$ HMBC correlations observed between the resonances for H-20 and the resonances for C-18, C-19, C-21, C-23, and C-28, and from the resonance for the NH proton (N-22) to those for all carbon atoms of the pyrrolidinone ring system, i.e., C-19, C-20, C-21, C-23 supported the existence of this moiety, as did the HMBC correlations between the resonances for H_3-28 and those for C-19, C-20, C-21 and C-24, thus completing the third ring within **1**. The linkage between the butoxyl group and C-21 was evident from the $^1H-^{13}C$ HMBC correlation between the resonances for H_2-24 and that for C-21. The remaining connectivities between between C-4, C-18 and C-19 followed by deduction.

The relative stereochemistry of **1** was determined from the results of NOE difference measurements. Thus, irradiation at the resonance frequency of H-5 caused enhancement of the resonance for H-10, and *vice versa*, and of the resonance for H-3, indicating the Decalin to be cis-fused and H-3 also to be on the same side of the molecule, *β*-oriented. Enhancement of the resonance for H-20 was caused by irradiation at the resonance frequency of H-15, indicating both these moieties to be on the same side of **1**. Irradiation at the resonance frequency of H-3 caused enhancement of the resonance for H-15 and *vice versa*, revealing the *E*-configuration of the $\Delta^{14,15}$ double bond. Irradiation at the resonance frequency of H-10 caused enhancement of the resonance for H-6 showing them also to be on the same side of the molecule. Finally, H_3-11 was deduced as having an α-orientation on the basis of a NOE interaction between H-8 and H-10. The configuration at C-21 remained unassigned as it could not be related in a relative sense to the other chiral centers in the molecule. In a relative sense, the new natural product ascosalipyrrolidinone A (**1**) is best described as (3*R**,4*S**,5*S**,6*S**,8*R**,10*R**)-3-[1,2,4a,5,6,7,8,8a-octahydro-3,6,8-trimethyl-2-[(*E*)-1-methyl-1-propenyl]-1-naphthalenyl]carbonyl-5-butoxy-1,5-dihydro-5-methyl-2*H*-pyrrol-2-one.

Compound **2** was purified in the same way as **1**. The small amount of **2** isolated precluded ^{13}C and 2D NMR spectral measurements. Comparison of its ^1H NMR data with those of compound **1** (Table 1), however, indicated the only difference between the two data sets to be the absence of the resonances for H_2-24, H_2-25, H_2-26 and H_3-27, the O-butyl moiety, in **2**, and the presence of a resonance for a methoxyl group instead (*δ* 3.1, 3H, s). This deduction was also supported by EIMS, IR and UV measurements. The molecule, ascosalipyrrolidinone B (**2**) is best described as (3*R**,4*S**,5*S**,6*S**,8*R**,10*R**)-3-[1,2,-4a,5,6,7,8,8a-octahydro-3,6,8-trimethyl-2-[(*E*)-1-methyl-1-propenyl]-1-naphthalenyl]carbonyl-1,5-dihydro-5-methoxy-5-methyl-2*H*-pyrrol-2-one.

Table 2. ^1H (300 MHz, $CDCl_3$) and ^{13}C (75.5 MHz, $CDCl_3$) NMR Data for Ascosalipyrone (3)

carbon	*δ* ^{13}C	*δ* ^1H	HMBC[a]	NOE[a]
2	167.6 s[c]			
3	99.8 s			
4	166.2 s			
5	101.6 d	6.21 (s)	3, 4, 6, 7, 8, 14	7, 9, 12, 13, O*H*
6	160.7 s			
7	49.3 d	3.77 (q, *J* = 7.0 Hz)	5, 6, 8, 12	
8	211.5 s			
9	47.1 d	2.68 (m)	8, 10, 11, 13	
10	26.1 t	1.69 (m), 1.38 (m)	8, 9, 11, 13	
11	11.6 q	0.82 (t, *J* = 7.3 Hz)	9, 10	
12	14.4 q	1.05 (d, *J* = 7.0 Hz)	6, 7, 8	
13	15.9 q	1.38 (d, *J* = 7.2 Hz)	8, 9, 10	
14	8.2 q	1.94 (s)	2, 3, 4	
OH		9.70 (brs)		

[a] Numbers represent carbon atoms that are observed to long-range CH couple with the proton(s) associated with this data row. [b] Enhanced proton signals as observed by difference NOE measurements. [c] Multiplicities by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

Ascosalipyrone (**3**) was found to have the molecular formula $C_{13}H_{18}O_4$ by MS. Its ^1H and ^{13}C NMR spectrum contained resonances (Table 2) attributable to four methyl groups [*δ* 0.82 (t, H_3-11), 1.05 (d, H_3-12), 1.38 (d, H_3-13), 1.94 (s, H_3-14)], one methylene group [*δ* 26.1 (t, C-10)], three methine groups [*δ* 101.6 (d, C-5), 49.3 (d, C-7), 47.1 (d, C-9)], a quaternary olefinic carbon [*δ* 99.8 (s, C-3)], two oxygenated quaternary olefinic carbons [*δ* 160.7 (s, C-6, lactone), 166.2 (s, C-4, hydroxyl)], and two carbonyls [*δ* 167.6 (s, C-2, lactone), 211.5 (s, C-8, ketone)]. The pyrone ring of ascosalipyrone was established as follows. Diagnostic long-range $^1H-^{13}C$ heteronuclear couplings observed from the resonance for H_3-14 to the resonances for C-2, C-3 and C-4 revealed H_3-14 to be connected to C-3, which is further bonded to the lactone carbon C-2 and to C-4. Further HMBC correlations seen between the resonance for H-5 and those for C-3, C-4, and C-6 revealed C-5 to be bonded to C-4 and C-6, thus completing the 4-hydroxy-2-pyrone ring system.

The $^1H-^1H$ COSY spectrum of **3** contained cross-peaks between the resonance for H_3-11 and those for H_2-10, between the resonances for H_2-10 and those of H-9, and those for H-9 and for H_3-13, showing C-11 to be bonded to C-10, C-10 to C-9, and C-9 to C-13, generating an isobutyl moiety. Additionally, a $^1H-^1H$ COSY cross-peak between the resonances for H_3-12 and H-7 gave the C-7, C-12 ethyl moiety. Further analysis of the HMBC spectrum showed correlations from the resonances for H-7, H-9, H_2-10, H_3-12 and H_3-13 to C-8 indicated the C-8 carbonyl group to bond to both C-7 and C-9. As the resonance for H-7 showed HMBC correlations to those for C-6 and C-5, C-7 must bond to C-6 thus completing the planar structure of **3**. The proposed structure of **3** was supported by the results of NOE measurements. Thus, irradiation at the resonance frequency of H-5 caused enhancement of the resonances for H-7, H-9, and H_3-12, supporting the positioning of H-5. Therefore, compound **3** was unambiguously assigned as 6-(1,3-dimethyl-2-oxopentyl)-4-methyl-2*H*-pyran-2-one.

Compound **3** was most probably isolated as a mixture of diastereomers. This being evident from its ^{13}C NMR spectrum which contains double resonances for C-6 (*δ* 160.7 and 160.5), C-7 (*δ* 49.3 and 49.1), C-8 (*δ* 211.5 and 211.3), C-9 (*δ* 47.1 and 46.9), C-10 (*δ* 26.1 and 25.7), C-12 (*δ* 14.5 and 14.4) and C-13 (*δ* 16.4 and 15.9). Attempts

Table 3. Antibacterial, Antifungal, Enzyme Inhibitory (Tyrosine Kinase), and Antiplasmodial Activities of Compounds 1, 4, and 5

	antimicrobial activity		tyrosine kinase activity[c]		antiplasmodial activity (IC_{50})[d]	
compound	bacteria[a] 50 μg/disk	fungi[b] 50 μg/disk	200 μg/mL	40 μg/mL	K1, ng/mL	NF54, ng/mL
1	Bm 5 mm ti[e]	Mv 2 mm ti Mm 4 mm ti	23	70	736	378
4	na	na	7	55	9272	6054
5	na	Mv 1 mm gi Er 2 mm gi	9	56	1763	1760
chloroquine					97	4

[a] Tested against *Bacillus megaterium* (Bm) and *Escherichia coli* (Ec); benzylpenicillin and streptomycin sulfate were positive controls. Inhibition zones of benzylpenicillin, 2 mm (Ec), 17 mm (Bm); inhibition zones of streptomycin sulfate, 2 mm (Ec), 10 mm (Bm). Test concentration: 50 μg/disk. [b] Tested against *Eurotium repens* (Er), *Fusarium oxysporum* (Fo), *Microbotryum violacea* (Mv), and *Mycotypha microspora* (Mm); miconazol was positive control. Inhibition zones, 25 mm (Mv), 25 mm (Er), 3 mm (Fo), and 7 mm (Mm), respectively. Test concentration: 50 μg/disk. [c] Values give % residual tyrosine kinase ($TKp56^{lck}$) activity; lck = lymphocytic kinase. [d] Antiplasmodial activity was measured against two reference strains of *Plasmodium falciparum* K1 (Thailand; resistant to chloroquine and pyrimethamine) and NF 54 (an airport strain of unknown origin; susceptible to standard antimalarials). [e] na = not active; ti = total inhibition zone in mm, no colonies of the test organism were growing within the inhibition zone; gi = growth inhibition zone in mm, some colonies of the test organism were growing within the inhibition zone. Inhibition zones were measured from the edge of the filter disks.

to separate the isomers by GC-MS proved unsuccessful. The structure of **3** resembles that of phomapyrone B which was isolated from the phytopathogenic fungus *Leptosphaeria maculans*, the asexual stage of *Phoma lignam*.[18] The only difference between the two molecules being the presence of the methyl group at C-9 in the side chain of **3**.

Compound **4** was identified as genistein by comparison of its spectroscopic data with published values.[19] Isoflavone compounds such as **4** are known to occur commonly in higher plants, but they have also been isolated from bacteria,[20−23] and from a culture of the fungus *Aspergillus niger*.[24] In most cases, as in this study, the fermentation media contain plant-based nutrients, and so it is not clear if **4** was produced by the microorganism or was isolated as an original component of the medium. On several other occasions during our studies the same cultivation medium was used and genistein was never found. It can, however, be speculated that genistein may be present in the medium in a glycosidic form that is normally not extracted with ethyl acetate and that some fungi, e.g., *Ascochyta salicorniae*, are able to enzymatically hydrolyze the glycosidic bonds. This is a possible explanation as to why we have never found this compound before. Genistein was reported to inhibit the enzymes β-galactosidase,[22] dopa decarboxylase, histidine decarboxylase, and catechol-O-methyltransferase.[24]

Compound **5**, 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-*trans*-propenylfuran-3-one, was identified by comparison of its ^1H and ^{13}C NMR data with published values. This compound was first isolated from culture filtrates of the phytopathogenic fungus *Stemphylium radicinum*.[25]

Compounds **1** and **2** are deoxy tetramic acid derivatives and as such belong to an extremely rare group of natural products. Two compounds similar to **1** and **2** have been reported from the fermentation broth of other fungi. Oteromycin (**6**), an antagonist of the ET_B receptor, was obtained from two strains of an unidentified fungus,[26] and ZG-1494α (**7**), an inhibitor of platelet-activating factor (PAF) acetyltransferase was isolated from *Penicillium rubrum*.[27] Significant structural differences between ascosalipyrrolidinones A and B (**1** and **2**), and oteromycin and ZG-1494α are the substitution and stereochemistry of the Decalin ring system and the presence of a methyl group in **1** and **2** at C-21, instead of the phenyl or 4-hydroxyphenyl moiety found in oteromycin and ZG-1494α, respectively. The finding of the new and structurally unusual compounds reported here from *A. salicorniae* further supports the proposition that marine derived fungi are an extremely interesting and valuable source of new and novel natural products.

The antimicrobial, antialgal, nematicidal, antiplasmodial, antitrypanosomal and cytotoxic properties as well as brine shrimp lethality of all compounds, except **2**, were assessed. The HIV-1 reverse transcriptase and tyrosine kinase ($p56^{lck}$) inhibitory activities of these compounds were also investigated using ELISA (Table 3). Ascosalipyrrolidinone A (**1**) was found to exhibit antimicrobial activity in agar diffusion assays against *Bacillus megaterium* (5 mm), *Mycotypha microsporum* (4 mm) and *Microbotryum violaceum* (2 mm) at a concentration of 50 μg/filter disk. It also inhibited the enzyme tyrosine kinase to 70% of its activity at a concentration of 40 μg/mL and to 23% at a concentration of 200 μg/mL. In bioassays for antiplasmodial activity compound **1** was found to inhibit the growth of two strains of *Plasmodium falciparum* at levels consistent with it being considered as a moderate antiplasmodial agent (see Table 3). Compound **1** exhibited significant activity against *Trypanosoma cruzi* and also against *Trypanosoma brucei* subsp. *rhodesiense*, as well as having cytotoxic activity against rat skeletal muscle myoblast cells and mouse peritoneal macrophages (Table 4). In the applied test systems ascosalipyrone (**3**) demonstrated no activities. The two known compounds, genistein (**4**) and 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-trans-propenylfuran-3-one (**5**) we found to inhibit the enzyme tyrosine kinase, an activity already reported for

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Table 4. Activities against *Trypanosoma brucei* subsp. *rhodesiense* and *Trypanosoma cruzi* and Cytotoxicity of Compounds 1 and 5

			cytotoxicity	
compd	T .b. rhodesiense MIC, μg/mL	*T. cruzi* MIC, μg/mL	L-6[a] MIC μg/mL	macrophage[b] IC_{50}, μg/mL
1	30	1.1	3.7	2.2
5	90	30	10	
melarsoprol[c]	0.072			
benznidazole[c]		30		

[a] Rat skeletal muscle myoblast cells. [b] Mouse peritoneal macrophages. [c] Positive controls.

genistein.[23,28] Additionally, compound **5** exhibited activity against *Tr. brucei* subsp. *rhodesiense* and *Tr. cruzi* and also weak antifungal activity against *Microbotryum violaceum* and *Eurotium repens*. In assays with *Artemia salina* (brine shrimp) and *Caenorrhabditis elegans* (nematode), none of the tested compounds had any effects.

Experimental Section

General Experimental Procedures. These were performed as previously reported.[29]

Isolation and Taxonomy of the Fungus. Algal material was collected from the North Sea, Tönning, Germany. After sterilization with 70% ethanol algal samples were rinsed with sterile seawater and pressed onto agar plates to detect any residual fungal spores on the surface of the algae. Sterilized algae were then cut into small pieces and placed on agar plates containing isolation medium: 30 g/L blended *Ulva* sp., 15 g/L agar, and 1000 mL of seawater from the sample collecting site. After autoclaving, the antibiotics benzylpenicillin and streptomycin sulfate were added by sterile filtration. Fungal colonies growing out of the algal tissue were transferred to medium for sporulation (1.0 g of glucose, 0.1 g of yeast extract, 0.5 g of peptone from meat, enzymatic digest, 15 g of agar, and 1000 mL of seawater, pH 8) in order to enable taxonomy of the isolates. Fungal strains were identified by Dr. S. Draeger, Institute for Microbiology, Technical University of Braunschweig.

Cultivation. The microorganism was cultured at 20 °C for 40 days in 8 L of solid medium containing 20 g/L biomalt extract, 8 g/L agar and 80% artificial seawater. The composition of the artificial seawater was as described by Höller et al.[30]

Biological Activity. Activity of compounds was tested in agar diffusion assays against the bacteria *Bacillus megaterium*, *Escherichia coli*, the fungi *Microbotryum violaceum*, *Eurotium repens*, *Fusarium oxysporum*, *Mycotypha microsporum* and the alga *Chlorella fusca*.[31] Assays performed with *Artemia salina* and *Caenorrhabditis elegans* at a concentration of 17 μg/mL were done according to methods of Peters et al.[32] ELISA-based enzyme inhibition assays against tyrosine kinase $p56^{lck}$ (tyrosine kinase assay kit, nonradioactively, no. 1534513) and reverse transcriptase HIV-1[33] followed the methods of Boehringer Mannheim. Antiplasmodial activity was determined as described by Desjardins.[34] Activity against hemoflagellates that cause human sleeping sickness (*Trypanosoma brucei* subsp. *rhodesiense*) and Chagas disease (*Trypanosoma cruzi*) and also cytotoxic activity were assessed as described by Kaminsky and Brun.[35]

Extraction and Isolation. Fungal mycelia were separated mechanically from the culture agar and extracted first with MeOH (6.5 L) and then with EtOAc (6.5 L) after being blended with an Ultra Turrax T25 at 8000 min^{-1}. Prior to extraction with 24 L of EtOAc followed by 24 L of *n*-butanol the solid medium was diluted with H_2O and mixed using the Ultra Turrax T 25. The resultant EtOAc extract (11.4 g) was purified employing a combination of chromatographic techniques. First, it was passed over normal phase silica (vacuum liquid chromatography, VLC) using a gradient starting with CH_2Cl_2 then EtOAc and finally MeOH as eluent to yield 17 fractions each of 250 mL. VLC fraction 4 (85 mg, eluted with EtOAc/CH_2Cl_2 1:9) in which the antimicrobial activity was concentrated was subjected to further normal phase VLC employing EtOAc/MeOH 95:5 as eluent to give 115 fractions. Fractions 4−6 were combined after TLC examination (32 mg), and yielded compounds **1** (8.7 mg, 1.1 mg/L) and **2** (0.8 mg, 0.1 mg/L) after purification by RP-18 HPLC (Spherisorb ODS S2 5μm, 8 mm × 25 cm) using acetonitrile/water 85:15 as eluent. VLC fraction 8 from the first separation was further investigated on the basis of its ^{1}H NMR spectrum, which contained a number of interesting resonances in the δ 4.0−10.4 range. Separation of this fraction by VLC, using normal phase silica and a gradient starting with CH_2Cl_2 then EtOAc and finally MeOH as eluent gave 13 fractions. Fractions 4 and 5 were combined after TLC examination and further separated by RP-18 HPLC using acetonitrile:water 60:40 as eluent to yield compound **3** (19 mg, 2.4 mg/L). Compounds **4** (2 mg, 0.25 mg/L), and **5** (11 mg, 1.4 mg/L) were obtained by normal phase HPLC separation of fraction 3 from VLC fraction 8 using hexane/acetone 70:30 as eluent.

Ascosalipyrrolidinone A (1): a colorless amorphous powder; $[\alpha]^{20}_D = -51.3°$ ($c = 0.16$, EtOH); UV (EtOH) λ_{max} (log ϵ) 211 (4.1), 225 sh (3.89), 267 (3.08) nm; IR (film) ν_{max} 3300, 1710, 1630 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; EIMS *m/z* 427 [M^+] (88), 409 (26), 392 (44), 354 (72), 239 (54), 231 (60), 201 (88), 175 (44), 159 (72), 135 (54), 124 (100), 105 (92), 91 (66), 69 (58), 55 (80); HREIMS *m/z* 427.309 (calcd for $C_{27}H_{41}NO_3$ 427.309).

Ascosalipyrrolidinone B (2): a colorless powder; $[\alpha]^{20}_D = 0.0°$ ($c = 0.05$, EtOH); UV (EtOH) λ_{max} (log ϵ) 207 (4.38), 225 (4.08), 273 (3.24) nm; IR (film) ν_{max} 3580, 1725, 1605 cm^{-1}; ^{1}H NMR data see Table 1; EIMS *m/z* 385 [M^+], 352 (40), 336 (32), 293 (36), 239 (38), 231 (45), 201 (52), 175 (28), 159 (36), 149 (100), 124 (46), 109 (42), 71 (44), 57 (58); HREIMS *m/z* 385.261 (calcd for $C_{24}H_{35}NO_3$ 385.261).

Ascosalipyrone (3): a yellow amorphous powder; UV (EtOH) λ_{max} (log ϵ) 210 (3.81), 290 (3.51) nm; IR (film) ν_{max} 2965, 1715, 1630 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; EIMS 238 [M^+] (18), 154 (100), 85 (18), 57 (54); HREIMS *m/z* 238.120 (calcd for $C_{13}H_{18}O_4$ 238.121)

Genistein (4): has physical and spectroscopic properties as previously reported.[19,21,36]

2,3-Dihydro-2-hydroxy-2,4-dimethyl-5-*trans*-propenylfuran-3-one (5): with $[\alpha]^{20}_D = +1.8°$ ($c = 0.29$, EtOH); lit.[25] $[\alpha]^{22}_D = \pm 2°$ ($c = 0.5$); ^{13}C NMR 202.5 (s), 176.9 (s), 139.7 (d), 118.9 (d), 106.9 (s), 101.9 (s), 22.2 (q), 19.0 (q), 5.4 (q) ppm; and other physical and spectroscopic details as previously reported.[25]

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Supporting Information Available: Spectroscopic data for compounds **1**-**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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