Ophiobolin Sesterterpenoids and Pyrrolidine Alkaloids from the Sponge-Derived Fungus *Aspergillus ustus*

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Chemical examination of the fungus *Aspergillus ustus* isolated from the Mediterranean sponge *Suberites domuncula* yielded the five new ophiobolin-type sesterterpenoids **1**–**5** and the two new pyrrolidine alkaloids **6** and **7**, together with the known compound aurantiamine and cerebroside D. The structures of the new compounds were unambiguously elucidated on the basis of extensive spectroscopic-data analysis (1D- and 2D-NMR, MS, and UV) and comparison with literature data. All compounds were evaluated for their cytotoxicity against murine lymphoma cell line L5178Y.

Introduction. – In the last decade, marine microorganisms, such as bacteria, microalgae, and fungi, have become increasingly attractive as sources of new bioactive natural products [1][2]. Fungi have proven to be particularly prolific sources of new compounds compared to other microbial sources isolated from the sea. Several hundred new compounds have been described from marine-derived fungi making this group of microorganisms a prime target for marine-natural-product chemists. The highest incidence for discovery of new compounds lies apparently in the group of sponge-associated fungi that have received much attention in recent years [1][3]. There is nevertheless a continuing debate on the nature of sponge–fungal interactions since many if not most of these fungi are already well-known from the terrestrial environment including genera such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Phoma*, and *Fusariu*. Thus, a true marine origin of these fungal strains is frequently doubted [4][5]. It is indeed possible that many sponge-associated fungi originated from

terrestrial habitats (*e.g.*, soil) from which they were washed and survived as dormant spores in filter-feeding sponges. Attempts to detect fungal mycelia growing inside sponges have usually failed. On the other hand, the sponge *Suberites domuncula* has been shown to have receptor proteins for fungal-cell-wall components, such as (1 → 3)-β-D-glucan, providing evidence that at some time in its life cycle (or in its evolutionary history), the sponge, if not colonized by, at least has come into contact with fungi [6].

Regardless of the true nature of these interactions, sponge-derived fungi continue to be of interest as sources for new secondary constituents. An example is provided by a marine strain of *Aspergillus ustus* that had been isolated from the Mediterranean sponge *Suberites domuncula* [7]. This fungus is know from soil samples [8], as a pathogen on higher plants [9], but also from marine habitats such as Mangrove swamps [10], and from *S. domuncula* [7][11]. We were attracted to the below-described marine isolate of *A. ustus* mainly due to the structurally diverse terpenoids produced when cultivated *in vitro*. Previously, we have reported seven new drimane sesquiterpenoids from *A. ustus* [11]. Continued investigation now afforded five new sesterterpenes of the ophiobolin group and two new pyrrolidine alkaloids (*Fig. 1*) along with the known compounds aurantiamine [12] and cerebroside D [13]. The structure elucidation of the new compounds by one- and two-dimensional NMR and by HR-MS is described.

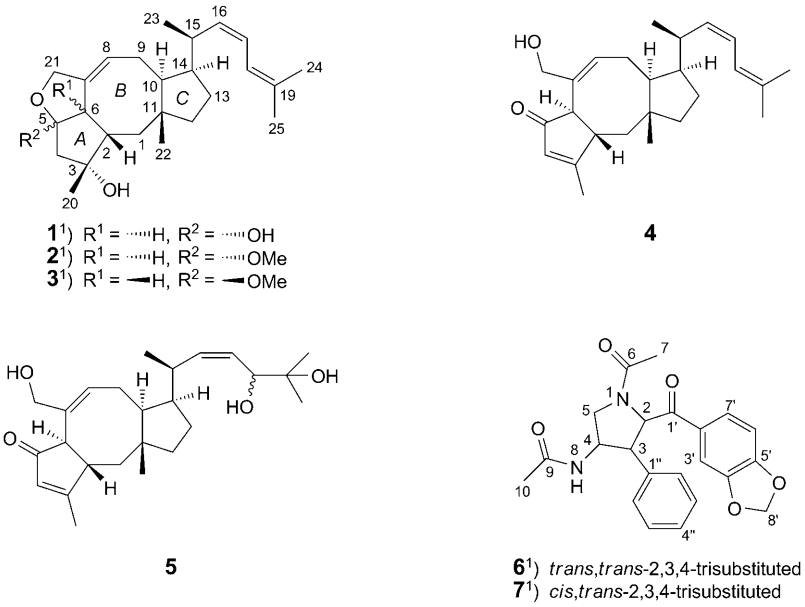

Fig. 1. *Compounds* **1**–**7** *isolated from* Aspergillus ustus

Results and Discussion. – The crude AcOEt extract of the fermentation broth of *A. ustus* was subjected to chromatographic separation including semi-prep. HPLC to yield

[1]) Trivial or arbitrary atom numbering; for systematic names, see *Exper. Part.*

five new ophiobolins **1**–**5**[1]) and two new pyrrolidine alkaloids **6**[1]) and **7**[1]), along with the known compounds aurantiamine and cerebroside D.

Compound **1** had the molecular formula $C_{25}H_{38}O_3$ as determined from HR-ESI-MS (*m*/*z* 409.2720 ($[M+Na]^+$)) in association with NMR data. The ^{1}H-NMR spectrum (*Table 1*) exhibited four olefinic H-atoms at δ(H) 5.19 (*dd*, *J* = 9.5, 9.5 Hz, H–C(16)), 5.33 (br. *s*, H–C(8)), 6.03 (*d*, *J* = 11.7 Hz, H–C(18)), and 6.07 (*dd*, *J* = 11.7, 9.5 Hz, H–C(17)), four Me *s*s at δ(H) 0.85 (Me(22)), 1.10 (Me(20)), 1.70 (Me(24)), and 1.78 (Me(25)), one Me *d* at δ(H) 0.91 (*d*, *J* = 6.7 Hz, Me(23)), and two exchangeable H-atoms at δ(H) 4.09 (*s*, OH–C(3)) and 5.86 (*s*, OH–C(5)). The ^{13}C-NMR spectrum (*Table 2*) displayed in total 25 resonances, including those of six olefinic C-atoms, one ketal C-atom (δ(C) 116.9 (C(5))) and two O-bearing C-atoms, in addition to those of five Me groups and eleven sp^3 C-atoms. The gross structure of **1** was very similar to that of the known compound ophiobolin H (= (1*R*,2a*S*,6a*S*,7*R*,9a*R*,10a*S*,10b*S*)-7-[(1*S*,2*Z*)-1,5-dimethylhexa-2,4-dien-1-yl]-1,2,4,6,6a,7,8,9,9a,10,10a,10b-dodecahydro-1,9a-dimethyl-2a*H*-3-oxacyclopenta[5,6]cycloocta[1,2,3-*cd*]pentalene-1,2a-diol), previously isolated from the same fungus, based on 2D-NMR data analysis (*Fig. 2*) and by comparison with literature data [14][15]. However, the ROESY interactions H–C(6)/H–C(10), H–C(10)/H_α–C(9), H–C(2)/Me(22), and Me(22)/H_β–C(9), and the absence of a ROESY correlation H–C(6)/H–C(2) (*Fig. 3*), suggested the same orientation of H–C(6) and H–C(10), and of H–C(2) and Me(22), respectively. Thus, rings *A*/*B* and *B*/*C* are *trans*-fused. The ROESY cross-peaks OH–C(3)/H–C(6), and OH–C(5)/H–C(6) confirmed the *cis* geometry of rings *A*/*D* and furthermore indicated OH–C(5) to be spatially close to H–C(6) (*Fig. 3*). Thus, compound **1** was identified as the new (5α,6α)-isomer of ophiobolin H.

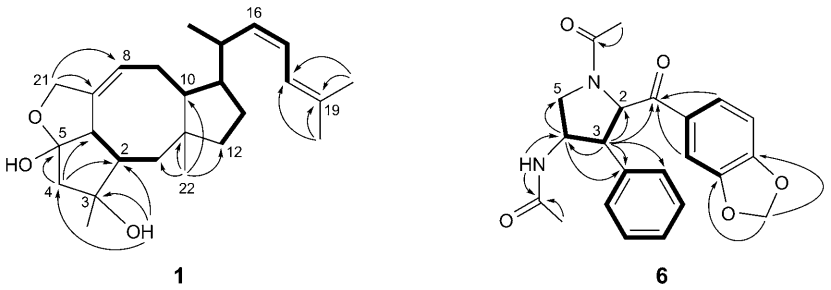

Fig. 2. *Key COSY* (—) *and HMBCs* (H → C) *of compound* **1**[1]) *and* **6**[1])

The molecular formula of **2** was determined as $C_{26}H_{40}O_3$ based on HR-ESI-MS data (*m*/*z* 423.2880 ($[M+Na]^+$)). The ^{1}H- and ^{13}C-NMR data of **2** (*Tables 1* and *2*) corresponded to those of **1**, except for the presence of an additional Me group as a MeO substituent (δ(H) 3.07 (*s*, 3 H); δ(C) 49.0). The HMBC data allowed the placement of the MeO group at C(5). The ROESY signals of **2**, similar to those of **1**, indicated that **2** is (5α,6α)-5-*O*-methylophiobolin H.

Table 1. *^{1}H-NMR Data* (600 MHz, (D_6)DMSO) *of Compounds* **1**–**5**[1]). δ in ppm, J in Hz[a]).

	1	**2**	**3**	**4**	**5**[b])
H_α–C(1)	1.46–1.50[c])	1.45–1.50[c])	1.31 (*m*)	1.07 (*t*, *J* = 12.9)	1.04 (*t*, *J* = 12.9)
H_β–C(1)	1.46–1.50[c])	1.45–1.50[c])	1.44 (*dd*, *J* = 14.2, 3.2)	1.95 (*dd*, *J* = 13.1, 3.3)	1.95 (*dd*, *J* = 13.2, 3.5)
H–C(2)	1.50–1.55 (*m*)	1.50–1.55 (*m*)	2.00 (*m*)	2.75 (br. *d*, *J* = 13.2)	2.75 (br. *d*, *J* = 12.6)
H_α–C(4) or H–C(4)	1.97 (*d*, *J* = 14.1)	2.07 (*d*, *J* = 14.2)	2.05 (*d*, *J* = 13.2)	5.84 (*d*, *J* = 1.4)	5.84 (*d*, *J* = 1.3)
H_β–C(4)	1.93 (*d*, *J* = 14.1)	1.71 (*d*, *J* = 14.2)	1.81 (*d*, *J* = 13.2)		
H–C(6)	2.91 (*d*, *J* = 9.8)	2.97 (*d*, *J* = 10.2)	3.01 (*d*, *J* = 10.7)	3.35[c])	3.35[c])
H–C(8)	5.33 (br. *s*)	5.34 (br. *s*)	5.47 (*d*, *J* = 7.1)	5.48 (*d*, *J* = 4.7)	5.55 (*d*, *J* = 4.4)
H_α–C(9)	2.15 (*m*)	2.12 (*m*)	2.38 (*dd*, *J* = 13.6, 8.5)	2.32 (br. *s*)	2.62 (*m*)
H_β–C(9)	1.75 (*m*)	1.71 (*m*)	1.67 (*m*)	1.86 (*m*)	1.81 (*m*)
H–C(10)	2.22 (*m*)	2.19 (*m*)	1.49 (*m*)	2.55 (*m*)	2.50[d])
H_α–C(12)	1.34 (*m*)	1.34 (*m*)	1.31 (*m*)	1.36 (*m*)	1.35 (*m*)
H_β–C(12)	1.39 (*m*)	1.38 (*m*)	1.31 (*m*)	1.44 (*m*)	1.43 (*m*)
H_α–C(13)	1.57 (*m*)	1.56 (*m*)	1.64 (*m*)	1.54 (*m*)	1.53 (*m*)
H_β–C(13)	1.20 (*m*)	1.17 (*m*)	1.49 (*m*)	1.23 (*m*)	1.23 (*m*)
H–C(14)	1.82 (*m*)	1.80 (*m*)	2.02 (*m*)	1.85 (*m*)	1.81 (*m*)
H–C(15)	2.50[d])	2.49[d])	2.67 (*m*)	2.55 (*m*)	2.55 (*m*)
H–C(16)	5.19 (*dd*, *J* = 9.5, 9.5)	5.18 (*dd*, *J* = 9.5, 9.5)	5.21 (*dd*, *J* = 9.8, 9.8)	5.19 (*m*)	5.27 (*m*)
H–C(17)	6.07 (*dd*, *J* = 11.7, 9.5)	6.06 (*dd*, *J* = 11.7, 9.5)	5.98 (*dd*, *J* = 11.3, 9.8)	6.05[c])	5.23 (*m*)
H–C(18)	6.03 (*d*, *J* = 11.7)	6.02 (*d*, *J* = 11.7)	6.01 (*d*, *J* = 11.7)	6.04[c])	3.93 (*dd*, *J* = 8.5, 6.0)
Me(20)	1.10 (*s*)	1.11 (*s*)	1.09 (*s*)	2.00 (*s*)	2.00 (*s*)
H_α–C(21)	4.29 (*d*, *J* = 11.7)	4.23 (*d*, *J* = 11.7)	4.52 (*d*, *J* = 11.3)	3.70 (*d*, *J* = 12.0)	3.70 (*d*, *J* = 11.0)
H_β–C(21)	4.37 (*d*, *J* = 11.7)	4.33 (*d*, *J* = 11.7)	4.27 (*d*, *J* = 12.0)	3.94 (br. *s*)	3.99 (*m*)
Me(22)	0.85 (*s*)	0.84 (*s*)	0.86 (*s*)	0.96 (*s*)	0.97 (*s*)
Me(23)	0.91 (*d*, *J* = 6.7)	0.90 (*d*, *J* = 6.6)	0.82 (*d*, *J* = 6.9)	0.88 (*d*, *J* = 6.6)	0.88 (*d*, *J* = 6.6)
Me(24)	1.70 (*s*)	1.69 (*s*)	1.67 (*s*)	1.69 (*s*)	1.01 (*s*)
Me(25)	1.78 (*s*)	1.77 (*s*)	1.76 (*s*)	1.78 (*s*)	1.02 (*s*)
	OH–C(3): 4.09 (*s*)	OH–C(3): 4.19 (*s*)	OH–C(3): 3.78 (*s*)	OH–C(21): 4.34 (br. *s*)	OH–C(21): 4.33 (*dd*, *J* = 9.8, 3.1)
	OH–C(5): 5.86 (*s*)	MeO–C(5): 3.07 (*s*)	MeO–C(5): 3.08 (*s*)		OH–C(18): 4.31 (*d*, *J* = 6.0)

[a]) The ^{1}H-NMR data were assigned by 2D-NMR (COSY, HMBC, and ROESY). [b]) The chemical shift of OH–C(19) was not assigned. [c]) Overlapping signals. [d]) Overlapping with solvent signals.

Table 2. *^{13}C-NMR Data* (150 MHz, (D_6)DMSO) *of Compounds* **1**–**5**[1]). δ in ppm.

	1[a])	**2**	**3**	**4**	**5**
C(1)	41.8 (*t*)	41.7 (*t*)	35.7 (*t*)	45.4 (*t*)	45.6 (*t*)
C(2)	50.7 (*d*)	49.8 (*d*)	50.0 (*d*)	50.0 (*d*)	50.0 (*d*)
C(3)	80.2 (*s*)	80.2 (*s*)	77.9 (*s*)	179.0 (*s*)	179.1 (*s*)
C(4)	53.6 (*t*)	48.0 (*t*)	50.7 (*t*)	129.4 (*t*)	129.4 (*t*)
C(5)	116.9 (*s*)	119.8 (*s*)	119.7 (*s*)	207.3 (*s*)	207.5 (*s*)
C(6)	55.2 (*d*)	54.3 (*d*)	50.9 (*d*)	51.6 (*d*)	51.7 (*d*)
C(7)	137.4 (*s*)	136.6 (*s*)	141.2 (*s*)	134.3 (*s*)	133.6 (*s*)
C(8)	116.0 (*d*)	120.1 (*d*)	120.2 (*d*)	127.4 (*d*)	127.7 (*d*)
C(9)	27.2 (*t*)	27.5 (*t*)	24.2 (*t*)	28.2 (*t*)	28.1 (*t*)
C(10)	41.8 (*d*)	41.9 (*d*)	55.2 (*d*)	42.9 (*d*)	43.1 (*d*)
C(11)	43.2 (*s*)	43.2 (*s*)	43.0 (*s*)	44.6 (*s*)	44.7 (*s*)
C(12)	45.0 (*t*)	44.8 (*t*)	43.1 (*t*)	43.8 (*t*)	43.8 (*t*)
C(13)	27.2 (*t*)	27.2 (*t*)	26.0 (*t*)	27.2 (*t*)	27.2 (*t*)
C(14)	51.3 (*d*)	51.6 (*d*)	46.7 (*d*)	51.4 (*d*)	51.4 (*d*)
C(15)	32.3 (*d*)	32.1 (*d*)	34.5 (*d*)	31.8 (*d*)	31.9 (*d*)
C(16)	136.0 (*d*)	136.0 (*d*)	137.9 (*d*)	136.8 (*d*)	138.9 (*d*)
C(17)	123.2 (*d*)	123.1 (*d*)	121.5 (*d*)	122.9 (*d*)	128.2 (*d*)
C(18)	120.1 (*d*)	120.1 (*d*)	120.3 (*d*)	120.2 (*d*)	73.6 (*d*)
C(19)	134.9 (*s*)	134.9 (*s*)	134.4 (*s*)	134.9 (*s*)	71.5 (*s*)
C(20)	26.4 (*q*)	26.5 (*q*)	26.3 (*q*)	16.9 (*q*)	16.9 (*q*)
C(21)	[b])	73.6 (*t*)	72.3 (*t*)	64.2 (*t*)	64.5 (*t*)
C(22)	22.8 (*q*)	22.7 (*q*)	18.2 (*q*)	22.4 (*q*)	22.5 (*q*)
C(23)	21.2 (*q*)	21.1 (*q*)	19.9 (*q*)	21.1 (*q*)	20.9 (*q*)
C(24)	18.0 (*q*)	17.9 (*q*)	17.9 (*q*)	26.1 (*q*)	26.1 (*q*)
C(25)	26.1 (*q*)	26.1 (*q*)	26.1 (*q*)	17.9 (*q*)	25.1 (*q*)
*Me*O–C(5)	–	49.0 (*q*)	49.4 (*q*)	–	–

[a]) The ^{13}C-NMR data were obtained from HMBC. [b]) The chemical shift data of C(21) of **1** was not assigned.

Compound **3** had the same molecular formula as **2** from its HR-ESI-MS data. The ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC, and ROESY data revealed that **3** was closely related to ophiobolin H [15] and thus differed with regard to the relative configuration at C(5) and C(6) from the core structure of **1** and **2** (*Fig. 3*). The only structural difference between **3** and ophiobolin H was the presence of a MeO group in **3** which was located at C(5) based on the HMBC cross-peak between MeO (δ(H) 3.08 (*s*, 3 H)) and C(5). Accordingly, compound **3** was identified as 5-*O*-methylophiobolin H.

Compound **4** had the molecular formula $C_{25}H_{36}O_2$ according to HR-ESI-MS (*m*/*z* 391.2610 ($[M+Na]^+$)) and NMR data. A comparison of its ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) with those of **1** revealed that it shared a partial structure with the latter compound. In the ^{13}C-NMR spectrum of **4**, three C-atom signals at δ(C) 179.0 (C(3)), 129.4 (C(4)), and 207.3 (C(5)) characteristic of an α,β-unsaturated ketone similar to ophiobolin G (=(3a*S*,6a*S*,7*R*,9a*R*,10a*S*)-7-[(1*S*,2*Z*)-1,5-dimethylhexa-2,4-dien-1-yl]-3,3a,6,6a,7,8,9,9a,10,10a-decahydro-1,9a-dimethyl-3-oxodicyclopenta[*a*,*d*]cyclooctene-4-carboxaldehyde) were detected [14][15]. Comparison of the ^{1}H- and ^{13}C-NMR data of **4** with those of ophiobolin G [15] indicated that the two compounds differed by the

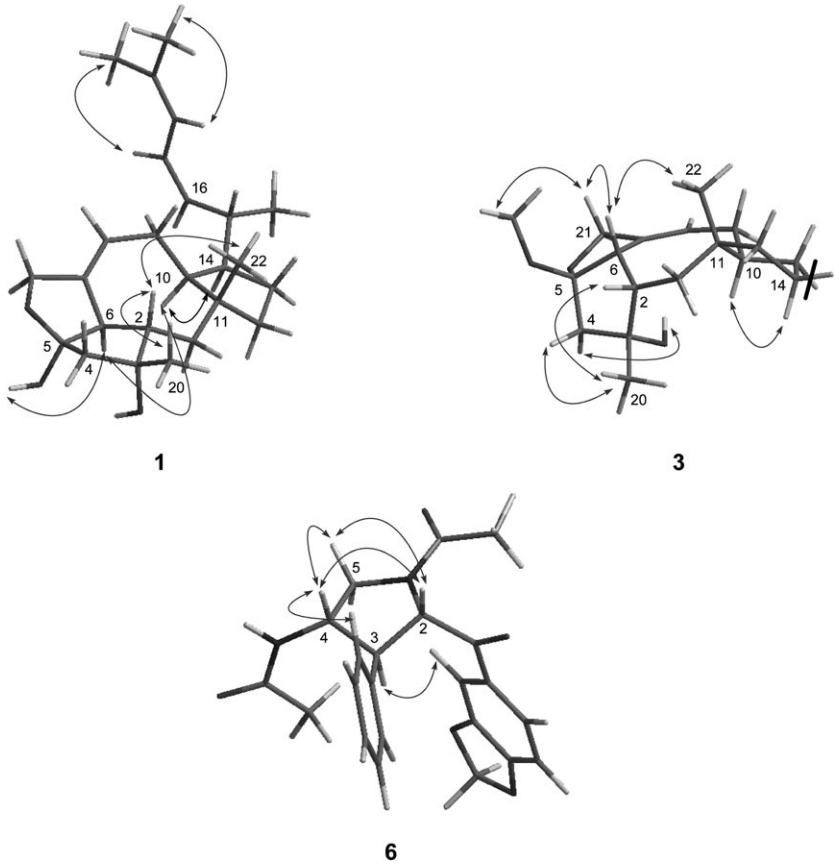

Fig. 3. *Key ROESY correlations of compounds* **1**[1]*),* **3**[1]*), and* **6**[1])

presence of an OH–CH_2(21) group (δ(H) 3.94 and 3.70; δ(C) 64.2) in **4** instead of the terminal CH(21)=O group in ophiobolin G. The ^{1}H,^{1}H-COSY data (CH_2(21)/H–C(8) and CH_2(21)/OH–C(21)), and the HMBCs CH_2(21)/C(6) and H–C(8)/C(21) supported this assignment. The ROESY correlations H–C(2)/Me(22) and H_β–C(1), and H–C(6)/H–C(10) and H_α–C(1), and the absence of a correlation H–C(6)/H–C(2) indicated the *trans*-junction of rings *A*/*B*. Hence, compound **4** was determined as (6α)-21,21-*O*-dihydroophiobolin G.

Compound **5** had the molecular formula $C_{25}H_{38}O_4$ as evident from the HR-ESI-MS (*m*/*z* 425.2670 $[M+Na]^+$)). The NMR data of **5** (*Tables 1* and *2*) were very similar to those of **4**, with the exception of the side chain where two hydroxylated C-atoms were observed. The ^{1}H,^{1}H-COSY and HMBC data allowed to position of the two OH groups at C(18) and C(19). Compound **5** was thus determined as (6α)-18,19,21,21-*O*-tetrahydro-18,19-dihydroxyophiobolin G.

Compound **6** was isolated as a white powder, and its molecular formula $C_{22}H_{22}N_2O_5$ was determined from the HR-ESI-MS (*m*/*z* 395.1620 $[M+H]^+$)). The UV spectrum

showed absorption maxima at 235, 283, and 319 nm, characteristic of a conjugated chromophore. The ^{1}H-NMR spectrum (*Table 3*) exhibited eight aromatic H-atoms, two Me groups (δ(H) 1.72 (Me(10)) and 2.00 (Me(7))), and one exchangeable H-atom at δ(H) 8.12 (*d*, $J = 8.5$ Hz, H–N(8)). The ^{13}C-NMR spectrum (*Table 3*) displayed 22 C-atoms, including one ketone C=O group (δ(C) 197.0 (C(1′))), two amide C=O groups (δ(C) 167.9 (C(6)) and 169.2 C(9))), twelve aromatic C-atoms for two benzene moieties, one acetal C-atom (δ(C) 101.6 (C(8′))), two Me groups, and four sp^3 C-atoms. The ^{1}H,^{1}H-COSY and HMBC data of **6** (*Fig. 2*) indicated the presence of a pyrrolidine moiety, in which a Ph group was linked at C(3), while a 3,4-(methylenedioxy)phenone moiety was attached to C(2). The presence of an Ac group at the N-atom was deduced from the HMBC H–C(5)/C=O (Ac). In addition, the presence of an acetamide group at position C(4) was confirmed by the HMBC H–C(4)/C=O (δ(C) 169.2). The ROESY correlations (*Fig. 3*) H–C(4)/H–C(2), H_b–C(5) and H–C(2″), and H–C(2)/H_b–C(5) indicated that H–C(4) and H–C(2) had the same orientation which was opposite to that of H–C(3). Compound **6** was named aspergillamide A. Structurally similar pyrrolidine alkaloids such as preussin are known from *Aspergilli* and have been reported previously, *e.g.*, from *A. ochraceus* [16].

Table 3. *^{1}H- and ^{13}C-NMR Data* ((D$_6$)DMSO, 500 and 125 MHz, resp.) *of Compounds* **6** *and* **7**[1]). δ in ppm, J in Hz.

	6		**7**	
	δ(C)	δ(H)	δ(C)	δ(H)
H–C(2)	61.8 (*d*)	5.88 (*d*, $J = 9.1$)	64.4 (*d*)	5.32 (*d*, $J = 9.5$)
H–C(3)	50.1 (*d*)	3.80 (*dd*, $J = 11.5, 9.3$)	54.1 (*d*)	3.18 (*dd*, $J = 9.9, 9.9$)
H–C(4)	48.3 (*d*)	5.12 (*m*)	53.3 (*d*)	4.62 (*m*)
CH_2(5)	51.1 (*t*)	4.14 (*dd*, $J = 8.7, 8.7$, H_a), 3.35 (*m*, H_b)	51.7 (*t*)	4.10 (*dd*, $J = 8.7, 8.7$, H_a), 3.40 (*m*, H_b)
C(6)	167.9 (*s*)		167.4 (*s*)	
Me(7)	21.5 (*q*)	2.00 (*s*)	21.4 (*q*)	1.98 (*s*)
H–N(8)	–	8.12 (*d*, $J = 8.5$)	–	8.15 (*d*, $J = 8.2$)
C(9)	169.2 (*s*)		168.9 (*s*)	
Me(10)	22.4 (*q*)	1.72 (*s*)	22.2 (*q*)	1.68 (*s*)
C(1′)	197.0 (*s*)		195.5 (*s*)	
C(2′)	131.2 (*s*)		130.3 (*s*)	
H–C(3′)	107.2 (*d*)	7.04 (*d*, $J = 1.3$)	107.0 (*d*)	7.08 (br. *s*)
C(4′)	146.8 (*s*)		145.8 (*s*)	
C(5′)	150.7 (*s*)		151.1 (*s*)	
H–C(6′)	107.3 (*d*)	6.76 (*d*, $J = 8.2$)	107.4 (*d*)	6.78 (*d*, $J = 8.2$)
H–C(7′)	124.5 (*d*)	7.25 (*dd*, $J = 8.2, 1.3$)	124.1 (*d*)	7.14 (br. *d*, $J = 8.2$)
CH_2(8′)	101.6 (*t*)	6.02 (*s*)	101.7 (*t*)	6.05, 6.04 (2*s*)
C(1″)	134.1 (*s*)		137.1 (*s*)	
H–C(2″,6″)	128.6 (*d*)	7.12 (*d*, $J = 7.6$)	128.3 (*d*)	7.27[a])
H–C(3″,5″)	127.7 (*d*)	7.06 (*dd*, $J = 7.6, 7.2$)	127.7 (*d*)	7.22[a])
H–C(4″)	126.9 (*d*)	7.01 (*dd*, $J = 7.2, 7.2$)	127.2 (*d*)	7.22 – 7.27[a])

[a]) Overlapping signals.

Compound **7** had the same molecular formula as **6** as established by HR-ESI-MS data (*m/z* 395.1620 ($[M+H]^+$)). ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMQC, and HMBC Data revealed that the gross structure of **7** is identical to that of **6**. However, ROESY correlations (*Fig. 3*) between H–C(4)/H–C(2′) and H–C(2)/H–N(8) confirmed that **7** is the C(2) epimer of **6**, for which we propose the name aspergillamide B.

All of the compounds were tested (at a concentration of 10 μg/ml) for cytotoxic activity against murine L5178Y lymphoma cells. However, none of the compounds reduced survival of cells by more than 10–20% compared to controls.

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Experimental Part

General. Solvents were distilled before use, and spectral-grade solvents were used for spectroscopic measurements. CC = Column chromatography. HPLC: *Dionex-P580* system coupled to a photodiode-array detector (*UVD340S*); routine detection at 235, 254, 280, and 340 nm; column *Eurospher-10 C_{18}* (125 × 4 mm (i.d.); *Knauer*, Germany); linear gradient 0.02% H_3PO_4 in H_2O and MeOH. Semi-prep. HPLC: *LaChrom-Merck-Hitachi* machine (pump *L-7100*); column *Eurospher-100 C_{18}* (8 mm length; *Knauer*, Germany); flow rate 5.0 ml/min. UV Spectra: *L-7400* UV detector; λ_{max} in nm). Optical rotations: *Perkin-Elmer-241-MC* polarimeter. 1D- and 2D-NMR Spectra: *Bruker-ARX-500* or *Avance-DMX-600* spectrometers; at 500 or 600 MHz (^{1}H) and 125 or 150 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz; DUL plus probes. ESI-MS: *Finnigan-LCQ-Deca* mass spectrometer; in *m/z* (rel. %). HR-ESI-MS: *Micromass-Q-Tof* mass spectrometer; in *m/z* (rel. %).

Fungal Material. The fungus *Aspergillus ustus*, internal strain 8009, was isolated from the marine sponge *Suberites domunculus*, which had been collected from the Adriatic Sea and then kept in an aquarium until fungi were isolated from sponge segments as previously described [7]. The fungus was identified both according to morphological and molecular attributes [7].

Cultivation and Extraction. For production of secondary metabolites, the fungus was cultivated at 22° for 21 d on both biomalt agar [4] and on barley-spelt solid media [7]. For initial analysis of the natural products, the cultures were lyophilized and extracted with AcOEt, and the dried residues were defatted by petroleum ether extraction.

Isolation. The AcOEt extract of *A. ustus* (3.66 g) was fractionated by VLC (silica gel, CH_2Cl_2 with increasing amounts of MeOH). *Fraction 4* (CH_2Cl_2/MeOH 98:2; 681 mg) was further purified by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1) and then subjected to prep. HPLC (MeOH/H_2O 80:20): **1** (2.4 mg), **2** (2.5 mg), **3** (3.6 mg), and **4** (1.9 mg). *Fr. 6* (CH_2Cl_2/MeOH 96:4; 262 mg) was further purified by CC (*Sephadex LH-20,* CH_2Cl_2/MeOH 1:1) and prep. HPLC (MeOH/H_2O 80:20): **7** (1.9 mg) and aurantiamide (6.0 mg). *Fr. 7* (CH_2Cl_2/MeOH 94:6; 377 mg) was further purified by CC (*Sephadex LH-20*, 100% MeOH) and prep. HPLC (MeOH/H_2O 80:20): **5** (2.2 mg) and **6** (6.0 mg). *Fr. 8* (CH_2Cl_2/MeOH 90:10; 366 mg) was further purified by CC (*Sephadex LH-20*, 100% MeOH): cerebroside D (10.0 mg).

(5α,6α)-Ophiobolin H (= *(1*R,*2a*R,*6a*S,*7*R,*9a*R,*10a*S,*10b*R*)-7-[(1*S,*2*Z*)-1,5-Dimethylhexa-2,4-dien-1-yl]-1,2,4,6,6a,7,8,9,9a,10,10a,10b-dodecahydro-1,9a-dimethyl-2a*H*-3-oxacyclopenta[5,6]cycloocta[1,2,3-*cd*]pentalene-1,2a-diol*; **1**): White amorphous powder. $[\alpha]_D^{20} = +25$ ($c = 0.1$, $CHCl_3$). UV (HPLC, mobile phase): 242. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 409.2720 ($[M+Na]^+$, $C_{25}H_{38}NaO_3^+$; calc. 409.2719).

(5α,6α)-5-O-*Methylophiobolin H* (= *(1*R,*2a*R,*6a*S,*7*R,*9a*R,*10a*S,*10b*R*)-7-[(1*S,*2*Z*)-1,5-Dimethylhexa-2,4-dien-1-yl]-2,2a,4,6,6a,7,8,9,9a,10,10a,10b-dodecahydro-2a-methoxy-1,9a-dimethyl-1*H*-3-oxacyclopenta[5,6]cycloocta[1,2,3-*cd*]pentalen-1-ol*; **2**): White amorphous powder. $[\alpha]_D^{20} = +99$ ($c = 0.1$, $CHCl_3$). UV (HPLC, mobile phase): 236. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 423.2880 ($[M+Na]^+$, $C_{26}H_{40}NaO_3^+$; calc. 423.2875).

5-O-*Methylophiobolin H* (= *(1*R,*2a*S,*6a*S,*7*R,*9a*R,*10a*S,*10b*S)*-7-[(1*S,*2*Z)*-1,5-Dimethylhexa-2,4-dien-1-yl]-2,2a,4,6,6a,7,8,9,9a,10,10a,10b-dodecahydro-2a-methoxy-1,9a-dimethyl-1*H*-3-oxacyclopenta[5,6]cycloocta[1,2,3-*cd*]pentalen-1-ol*; **3**): White amorphous powder. $[\alpha]_D^{20} = +33$ ($c = 0.1$, $CHCl_3$). UV (HPLC, mobile phase): 242. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-TOF-MS: 423.2860 ($[M+Na]^+$, $C_{26}H_{40}NaO_3^+$; calc. 423.2875).

(6α)-21,21-O-*Dihydroophiobolin G* (= *(3a*R,*6a*S,*7*R,*9a*R,*10a*S*)-7-[(1*S,*2*Z*)-1,5-Dimethylhexa-2,4-dien-1-yl]-6,6a,7,8,9,9a,10,10a-octahydro-4-(hydroxymethyl)-1,9a-dimethyldicyclopenta[*a,d*]cycloocten-3(3a*H*)-one*; **4**): White amorphous powder. $[\alpha]_D^{20} = +49$ ($c = 0.1$, $CHCl_3$). UV (HPLC, mobile phase): 236. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 391.2610 ($[M+Na]^+$, $C_{25}H_{36}NaO_2^+$; calc. 391.2613).

(6α)-18,19,21,21-O-*Tetrahydro-18,19-dihydroxyophiobolin G* (= *(3a*R,*6a*S,*7*R,*9a*R,*10a*S*)-7-[(1*S,*2*Z*)-4,5-Dihydroxy-1,5-dimethylhex-2-en-1-yl]-6,6a,7,8,9,9a,10,10a-octahydro-4-(hydroxymethyl)-1,9a-dimethyldicyclopenta[*a,d*]cycloocten-3(3a*H*)-one*; **5**): White amorphous powder. $[\alpha]_D^{20} = +27$ ($c = 0.1$, $CHCl_3$). UV (HPLC, mobile phase): 232. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 425.2670 ($[M+Na]^+$, $C_{25}H_{38}NaO_4^+$; calc. 425.2668).

Aspergillamide A (= N-*[(3*S,*4*S,*5*S*)-1-Acetyl-5-(1,3-benzodioxol-5-ylcarbonyl)-4-phenylpyrrolidin-3-yl]acetamide*; **6**): White amorphous powder. $[\alpha]_D^{20} = -76$ ($c = 0.1$, MeOH). UV (HPLC, mobile phase): 235, 283, 319. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 395.1620 ($[M+H]^+$, $C_{22}H_{23}N_2O_5^+$; calc. 395.1607).

Aspergillamide B (= N-*[(3*S,*4*S,*5*R*)-1-Acetyl-5-(1,3-benzodioxol-5-ylcarbonyl)-4-phenylpyrrolidin-3-yl]acetamide*; **7**): White amorphous powder. $[\alpha]_D^{20} = +45$ ($c = 0.1$, MeOH). UV (HPLC, mobile phase): 233, 282, 316. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 395.1620 ($[M+H]^+$, $C_{22}H_{23}N_2O_5^+$; calc. 395.1607).

Cell Proliferation Assays. Cytotoxicity was tested against L5178Y cells by using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay as described previously [11].

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