Novel Chromone Derivatives from the Fungus *Aspergillus versicolor* Isolated from the Marine Sponge *Xestospongia exigua*[1]

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From the marine sponge *Xestospongia exigua* collected in Indonesia the fungus *Aspergillus versicolor* was isolated. Following cultivation in a seawater-based medium seven new angular tricyclic chromone derivatives (**1**−**7**) were obtained from the mycelia and culture filtrate. Compounds **2**−**7** contain an additional dihydropyran ring system which is replaced by a pyridine ring in **1**. The structures of the new natural products were established on the basis of extensive one- and two-dimensional NMR spectroscopic studies (^{1}H, ^{13}C, COSY, HMQC, HMBC, NOE difference spectra) as well as on mass spectral analysis.

In the search for new bioactive, marine compounds from the sea, increasing attention is being given to microorganisms such as bacteria and fungi as potential sources of new natural products.[2] Recently it was shown that filter feeding invertebrates such as sponges harbor a wealth of associated bacteria that do not merely reflect microbial communities of the seawater of the adjacent sediment but are apparently the result of more specialized interactions.[3] Whereas the nature of these associations between sponges and microorganisms is not yet fully understood, it has been repeatedly shown that sponge-associated fungi are an interesting source of new bioactive natural products.[4,5] Recent examples include biosynthetically diverse compounds such as asperic acid, hexylitaconic acid, malformin C, pyrophen, and asperazine, from a sponge-derived *Aspergillus niger*.[6] Polyketides representing cladospolide, padangolide, and patulolide derivatives were obtained from an unknown fungus isolated from an Indonesian encrusting sponge.[7] Similarly, the fungus *Paecilomyces* cf. *javanica*, isolated from the sponge *Jaspis* cf. *coriacea*, yielded a novel polyketide, deoxynortrichoharzin.[8] Examples from our group include hortein, a polyketide from the fungus *Hortaea werneckii* associated with the sponge *Aplysina aerophoba*,[9] macrolides, and a furan carboxylic acid derivative from the sponge-derived fungus *Cladosporium herbarum*,[10] or novel spiciferone derivatives from the fungus *Drechslera hawaiiensis* isolated from the marine sponge *Callyspongia aerizusa*.[11] The fungus *Microsphaeropsis* sp. obtained from the sponge *Aplysina aerophoba* yielded anthraquinones and betaenone derivatives that inhibit several protein kinases which constitute relevant pharmacological targets for anticancer therapy[12] as well as the γ-pyrones, microsphaerones A and B.[13]

In continuation of our studies on the chemistry of sponge-derived fungi we have obtained a strain of *Aspergillus versicolor* from the Indonesian sponge *Xestospongia exigua*. Isolates of *A. versicolor* obtained from terrestrial habitats have been extensively characterized by natural products chemists and have yielded a variety of secondary metabolites of different classes including xanthones such as sterigmatin[14] or sterigmatocystin,[15] anthraquinones such as averantin,[16] averufin,[17] or versicolorone,[18] a terpenoid, versiol,[19] and a cyclic peptide, aspercolorin.[20] It is interesting to note that an *A. versicolor* strain that had been isolated from the marine green alga *Penicillus capitatus* was shown to produce 9α,14-dihydroxy-6*β*-*p*-nitrobenzoylcinnamolide and related drimane lactones featuring an unusual nitrobenzoyloxy substituent.[21] Shortly before, the same compound, designated as insulicolide A, had been isolated from several strains of *Aspergillus insulicola* that were obtained from different green algae as well as a decaying leaf of the American Mangrove, *Rhizophora mangle*, collected in the Bahamas.[22] The isolate of *A. versicolor* from a marine sponge investigated in the present communication yielded none of the former metabolites but instead produced chromone derivatives (**1**−**7**) that proved to be new natural products. Structure elucidation of the new compounds by NMR spectroscopy and by mass spectrometry is reported.

Results and Discussion

Following chromatographic separation of the combined EtOAc extracts obtained by extraction of the mycelium and by extraction of the aqueous culture filtrate seven compounds (**1**−**7**) were isolated. Spectroscopic analysis mainly by NMR spectroscopy and by mass spectrometry indicated these compounds were new natural products. They were identified to be novel chromones containing an additional annealed heterocyclic ring system which was a pyridine ring (in the case of **1**) or a dihydropyran ring (compounds **2**−**7**). The new natural products were shown to be members of two classes of hitherto undescribed angular chromones; thus we propose the names aspergillitine (**1**) and aspergiones A−F (**2**−**7**).

Aspergillitine (**1**) had the molecular formula $C_{15}H_{13}O_2N$, as indicated by HRESIMS, which was in agreement with the ^{1}H and ^{13}C NMR spectra. It was optically inactive but presented UV absorptions at λ_{max} 214, 245, 265, 295, 315, 370 nm, which were reminiscent of a chromone system (such as present in **2**, see below), but exhibiting a red-shift due to additional conjugative effects. The ^{1}H NMR spectrum displayed three aromatic methyl groups at δ 2.69 (H_3-

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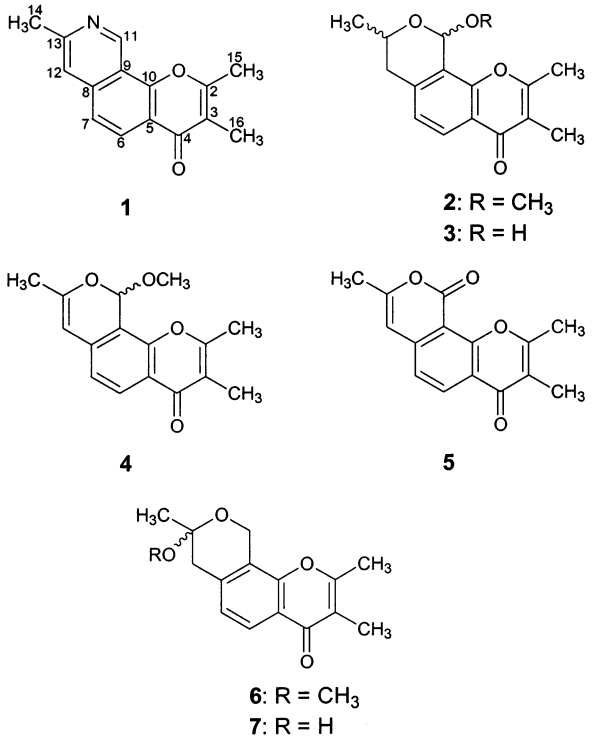

14), 2.38 (H_3-16), and 2.25 (H_3-15) and two aromatic proton singlets at δ 9.54 (H-11) and 7.83 (H-12), along with an AB spin system for two *ortho*-coupled aromatic protons at δ 7.82 (d, J = 8.6 Hz, H-6) and 7.60 (d, J = 8.6 Hz, H-7). Direct correlations observable in the HMBC spectrum allowed an unambiguous assignment of protons and protonated carbons (see Table 1). Two of the aromatic methyl groups at δ 2.25 (H_3-15) and 2.38 (H_3-16) showed correlations to two aromatic singlets at δ 144.4 (C-2) and 133.8 (C-3) and a carbonyl group at δ 181.0 (C-4), as well as to each other, thus revealing an α,β-methylated α,β-unsaturated carbonyl function. ^3J correlations from H-6 (δ 7.82) to the carbonyl group allowed assignment of the aromatic AB system to a ring system adjacent to the previously identified substructure. Further diagnostic correlations including H-6 to C-8 (δ 140.5) and the oxygen bearing C-10 (δ 163.0) as well as H-7 (δ 7.60) to both C-5 (δ 118.4) and C-9 (δ 113.5) clearly established the chromone substructure. The ^1J coupling constant of 189 Hz for H-11/C-11 indicative of a ring nitrogen atom attached to C-11 (δ 145.4) and, further, the low-field chemical shift of H-11 (δ 9.54) suggested a pyridine ring system attached to C-8 and C-9, respectively, thus accounting for the remaining degrees of unsaturation. A long-range correlation discernible in the ^{1}H−^{1}H COSY spectrum between H-7 and H-12 (δ 7.83) proved valuable for the further connection of substructures. The orientation of the pyridine ring and the position of the remaining aromatic methyl group were established on the basis of key HMBC correlations from H-11 to C-8, C-9, C-10, and C-13 (δ 156.4), as well as from H_3-14 (δ 2.69) to both C-13 and C-12 (δ 119.1).

The ^{1}H and ^{13}C NMR data for the heterocyclic part of the 2,3-dimethylated chromone in **1** were in excellent agreement with data published for the respective substructure in 6-acetyl-7-hydroxy-2,3-dimethylchromone, isolated from the roots of *Tithonia diversifolia*.[23] It is interesting to note that both aromatic methyl protons H_3-15 and H_3-16 displayed an identical set of HMBC correlations including an unusually strong ^4J correlation to each other's attached carbon atom. Thus, assignment of the respective positions in **1** was based on the analysis of HMBC correlations obtained for aspergiones A (**2**) and C (**4**), which exhibited identical substructures (see below). The ^{1}H and ^{13}C NMR of aspergillitine (**1**) are summarized in Table 1.

Aspergione A (**2**) was obtained as a colorless amorphous residue. The pseudomolecular ion peak at *m/z* 275 $[M + H]^+$ observed by ESIMS as well as the ^{1}H, ^{13}C, and DEPT NMR suggested the molecular formula $C_{16}H_{18}O_4$, which was further confirmed by HRESIMS. The characteristic UV absorption maxima observed at 235, 291, and 350 nm furthermore suggested the presence of a chromone moiety in **2**,[24,25] but without the pronounced red-shift as observed for aspergillitine (**1**).

Comparison of the 1D NMR data indicated that **2** contained the same 8,9-disubstituted 2,3-dimethylated chromone backbone as in **1**, while small but significant differences in the ^{13}C NMR signals attributable to the phenyl ring, together with a pronounced upfield shift for the AB system of H-6 and H-7 (δ 6.85 and δ 7.59, J = 7.9), indicated a different substitution pattern at positions 8 and 9 (see Table 1). From the ^{1}H−^{1}H COSY spectrum, an additional spin system comprising H_2-12 (δ 2.75 and 2.69), H-13 (δ 4.32), and H_3-14 (δ 1.38) as well as an additional methoxyl group (δ 3.62) were readily identified. A long-range correlation of H-12 to H-7 in conjunction with HMBC correlations from H-12 to both C-7 and C-9 allowed positioning of this substituent at C-8 (see Table 1). The

Table 1. NMR Spectral Data of Aspergillitine (**1**)[a] and Aspergione A (**2**)[b]

	1			**2**		
	δ_H (J Hz)	δ_C	HMBC[c]	δ_H (J Hz)	δ_C	HMBC[c]
2		144.4 s			145.4 s	
3		133.8 s			132.0 s	
4		181.0 s			183.1 s	
5		118.4 s			122.0 s	
6	7.82 d (*8.6*)	123.5 d	4, 5w, *6*, 8, 10	7.59 d (*7.9*)	123.7 d	8, 10
7	7.60 d (*8.6*)	120.9 d	5, 6w, *7*, 8, 9, 12	6.85 d (*7.9*)	122.6 d	5, *7*, 9, 12
8		140.5 s			143.8 s	
9		113.5 s			119.1 s	
10		163.0 s			162.2 s	
11	9.54 s	145.4 d	8, 9, 10, *11*, 13	5.70 br s	94.8 d	8, 13, OCH_3
12	7.83 s	119.1 d	7, 9, *12*, 13	A 2.75 dd (*17.2, 3.8*)	36.0 t	7, 8, 9, *12*, 13
				B 2.69 dd (*17.2, 10.4*)		
13		156.4 s		4.32 ddq (*10.4, 6.2, 3.8*)	62.9 d	
14	2.69 s	24.0 q	12, 13, *14*	1.38 d (*6.2*)	21.1 q	12, 13, *14*
15	2.25 s	19.7 q	2, 3, 4, *15*, 16	2.10 s	20.2 q	2, 3, *15*, 16
16	2.38 s	16.6 q	2, 3, 4, 15, *16*	2.35 s	17.4 q	2, 3, 4w, 15, *16*
OCH_3				3.62 s	55.7 q	11, OCH_3

[a] Recorded in DMSO-d_6. [b] Recorded in $CDCl_3$. [c] H to C, $^1J_{C-H}$ italic, w = weak.

Table 2. ^{1}H NMR Spectral Data[a] of Aspergiones B−F (**3**−**7**)

	3[b]	**4**[b]	**5**[b]	**6**[c]	**7**[d]
6	7.61 d (*7.8*)	7.63 d (*7.8*)	7.94 d (*8.0*)	7.55 d (*8.0*)	7.47 d (*7.9*)
7	6.87 d (*7.8*)	6.77 d (*7.8*)	7.02 d (*8.0*)	6.99 d (*8.0*)	6.94 d (*7.9*)
11	6.27 d (*4.0*)	6.33 s		A 5.05 br d (*16.0*)	A 4.85 br d (*15.6*)
				B 4.94 br d (*16.0*)[e]	B 4.79 br d (*15.6*)
12	A 2.76 dd (*17.4, 3.8*)	5.86 br s	6.31 d (*0.8*)	A 3.07 br d (*17.3*)	A 2.92 br d (*17.2*)
	B 2.70 dd (*17.4, 10.4*)			B 2.97 br d (*17.3*)	B 2.82 br d (*17.2*)
13	4.45 ddq (*10.4, 6.2, 3.8*)				
14	1.39 d (*6.2*)	2.08 s	2.32 d (*0.8*)	1.58 s	1.46 s
15	2.13 s	2.10 s	2.25 s	2.17 s	2.08 s
16	2.34 s	2.34 s	2.40 s	2.39 s	2.30 s
OCH_3		3.58 s		3.35 s[e]	
OH	2.94 d (*4.0*)				6.08 s

[a] δ_H (*J* Hz). [b] Recorded in $CDCl_3$. [c] Recorded in MeOH-d_4. [d] Recorded in DMSO-d_6. [e] (Partially) obscured by solvent or residual water signal.

remaining broad proton singlet (δ 5.70, H-11) was connected to a hemiacetal carbon (δ 94.8), as indicated by the chemical shifts. Correlations to C-8, C-13, and the methoxyl carbon allowed elucidation of the structure of **2** as depicted. Thus, aspergione A (**2**) exhibited the same carbon framework as aspergillitine (**1**), with the exception of the pyridine ring in **1**, which was replaced by a dihydropyran ring in **2**. Attempts to clarify the relative stereochemistry at positions 11 and 13 were unsuccessful, since no significant NOEs could be observed between the respective positions by 1D NOE or NOE difference experiments. It should be noted that the optical rotation measured for aspergione A (**2**) was rather small, thus suggesting that the compound was possibly not isolated as a single stereoisomer, but rather as an enantiomerically enriched mixture. Similar findings also hold true for the other optically active congeners reported in the present study, aspergiones B (**3**), C (**4**), E (**6**), and F (**7**).

As was observed for aspergillitine (**1**), in **2** both aromatic methyl protons H_3-15 and H_3-16 also displayed unusually strong ^4J correlations to each other's attached carbon atom. However, in **2** an additional, albeit weak ^3J correlation from one methyl group to the carbonyl group (C-4) was discernible; thus the respective signal was assigned to H_3-16 (see Table 1).

The structure of aspergione B (**3**) followed readily from interpretation of the NMR and MS spectra, with the latter indicating a loss of 14 mass units compared to aspergione A (**2**). The ^{1}H and ^{13}C NMR spectra of **3** clearly indicated that the methoxyl group at C-11 in **2** was replaced by a hydroxyl function, resulting in a downfield shift of the signal of H-11 to δ 6.27 (d, J = 4.0 Hz) compared to δ 5.70 (br s) for the same proton in the ^{1}H NMR spectrum of **2**. The remaining proton and carbon signals of **3** closely resembled those of **2** (see Tables 2 and 3).

The molecular weight of aspergione C (**4**) was 2 mass units smaller than that of aspergione A (**2**), suggesting the loss of two protons, which was confirmed by HRESIMS. Comparison of ^{1}H and ^{13}C NMR data of both compounds showed that signals assigned to the chromone moiety were very similar, but revealed an additional broad olefinic proton singlet at δ 5.86 (H-12) and an olefinic methyl singlet at δ 2.08 (H_3-14) in **4** instead of a methyl doublet for H_3-14 and the two geminal protons for H_2-12 in **2**. These findings indicated the presence of a double bond at positions 12 and 13 in **4**. The structure of **4** was also confirmed by HMBC correlations (data not shown), which were virtually identical to those observed for aspergione A (**2**). The only differences were with regard to the lack of the correlation between H_2-12 and C-8 as well as to the direct correlation of the methoxyl group. Again, both aromatic methyl protons H_3-15 and H_3-16 displayed unusually strong ^4J correlations to C-16 and C-15, respectively. Besides a strong ^3J correlation from H_3-16 a weak ^4J correlation from H_3-15 to C-4 was observed, thus supporting the assignment of both methyl groups as described above for aspergione C (**2**). The ^{1}H and ^{13}C NMR data are summarized in Tables 2 and 3.

Table 3. ^{13}C NMR Spectral Data of Aspergiones B−F (**3**−**7**)

	3[a]	**4**[a]	**5**[a]	**6**[b]	**7**[c]
2	144.5 s	145.5 s	145.5 s	146.5 s	144.3 s
3	131.1 s	131.3 s	130.3 s	134.3 s	131.8 s
4	182.5 s	182.7 s	182.0 s	182.5 s	182.2 s
5	122.7 s	121.7 s	115.8 s	121.9 s	120.1 s
6	122.5 d	125.2 d	130.3 d	124.8 d	123.6 d
7	122.0 d	118.2 d	119.1 d	122.5 d	121.1 d
8	142.0 s	138.6 s	147.2 s	143.6 s	142.5 s
9	119.1 s	108.1 s	113.1 s	120.1 s	118.7 s
10	162.5 s	161.2 s	159.1 s	160.9 s	159.6 s
11	87.5 d	94.7 d	166.3 s	58.0 t	56.1 t
12	36.5 t	101.3 d	104.1 d	40.1 t	40.1 t
13	62.2 d	155.2 s	159.2 s	95.4 s	93.6 s
14	21.0 q	20.4 q[d]	20.9 q	28.8 q	28.5 q
15	20.1 q	20.2 q[d]	20.1 q	20.2 q	19.8 q
16	17.0 q	17.4 q	17.7 q	17.5 q	16.8 q
OCH_3		55.4 q		49.6 q[e]	

[a] Recorded in $CDCl_3$. [b] Recorded in MeOH-d_4. [c] Recorded in DMSO-d_6. [d] Signals may be interchanged. [e] Obscured by solvent signal.

The molecular formula of aspergione D (**5**) was determined as $C_{15}H_{12}O_4$ as shown by ESIMS, which yielded the pseudomolecular ion at *m/z* 257 $[M + H]^+$ and by HRESIMS. The ^{1}H NMR spectrum of **5** was similar to that of **4** with the exception of the signals of H-11 and the methoxyl group, which were absent in the spectrum of **5** (see Table 2). In addition, the methoxyl carbon signal was absent in the ^{13}C NMR spectrum, while the signal of C-11 (δ 166.3, s) in **5** had suffered a large downfield shift compared to the respective signal in **4** (δ 94.7, d, see Table 3). Thus, in aspergione D (**5**) the methoxyl substituent at C-11 of aspergione C (**4**) had been replaced by an oxo group, which also accounts for the observed loss of 16 mass units in the molecular weight of **5** compared to **4**.

The molecular weight of aspergione E (**6**) was the same as that of compound **2**, indicating that both chromone derivatives were isomers. Instead of the broad singlet observed for H-11 in **2**, the ^{1}H NMR spectrum of **6** exhibited signals of two geminal protons displaying a coupling constant of 16.0 Hz (see Table 2). The chemical shifts (δ 5.05, H-11A and 4.94, H-11B) and the lack of further coupling indicated that these protons were bound to an isolated carbon carrying an oxygen function. Since the proton spectrum of **6** lacked the signal for H-13 and since the signals of H-12A and H-12B were only split into doublets (J = 17.3 Hz) due to geminal coupling (as opposed

to further coupling to H-13 as observed for **2**), the methoxyl function had to reside at the methyl-bearing carbon C-13. The $^1H-^1H$ COSY spectrum (data not shown) and the ^{13}C NMR data (see Table 3) were also in agreement with the structure of **6** as depicted.

The molecular weight of aspergione F (**7**) was 14 mass units smaller than that of aspergione E (**6**), indicating the loss of a methylene group, which was confirmed by HRESIMS. Inspection of the ^1H NMR spectrum of **7** revealed the loss of the methoxyl signal observed previously for **6** at δ 3.35 (see Table 2). Instead, the signal of a hydroxyl group was detected that showed a weak correlation with the geminal protons H-12A and H-12B in the $^1H-^1H$ COSY spectrum of **7** (data not shown). The remaining ^1H and ^{13}C data of **7** were very similar to those obtained for **6** (see Tables 2 and 3). Thus, **7** was identified as the *O*-demethyl derivative of **6**.

The chromatographic isolation of the putative precursors **3** and **7** failed to give the methylated congeners **2** and **6** as byproducts even though it proceeded in the presence of methanol. This finding suggests in our view that **2** and **6** are indeed true natural products rather than artifacts.

Aspergillitine (**1**) and aspergiones A−F (**2**−**7**) are unusual new natural products in several regards. First, 2,3-methylated chromones are only rarely encountered as natural products. Examples include chaetochromin D, a bis(naphtho-γ-pyrone) derivative from the fungus *Chaetomium gracile*,[26] 5-hydroxy-2,3-dimethyl-7-methoxychromone from mycobiont cultures of the lichen *Graphis scripta*,[27] and 6-acetyl-7-hydroxy-2,3-dimethylchromone from the plant *Tithonia diversifolia*.[23] Moreover, aspergillitine (**1**) features an additional annealed pyridine ring, which is replaced by a dihydropyran ring in aspergiones A−F (**2**−**7**), yielding an angular tricyclic chromone ring system that, to the best of our knowledge, is unprecedented in the natural products literature. In this context it is interesting to note that the fungal chromone fulvic acid[28] contains an additional dihydopyran ring that is identical to the one present in aspergione F (**7**), but annealed at positions corresponding to 2 and 3 in aspergillitine or aspergiones, respectively, to yield a linear but not angular tricyclic skeleton. The incorporation of a nitrogen atom (in **1**) instead of an oxygen atom (in **2**−**7**) into the hetercyclic systems of fungal-derived polyketides is only rarely observed in nature. One example is the structural analogy of bostrycoidin[29] to fusarubin,[30] two naphthoquinones with an additional annealed six-membered ring, isolated from *Fusarium bostrycoides* and *F. javanicum*, respectively. Interestingly, in the case of bostrycoidin, its 2-methyl pyridine ring is identical to the one present in aspergillitine (**1**), while the partially saturated 2-hydroxy-2-methylpyran ring in fusarubin is also encountered in aspergione F (**6**).

Aspergillitine (**1**) displayed only moderate antibacterial activity against *Bacillus subtilis*,[31] while it was inactive against *Escherichia coli* and *Saccharomyces cerevisiae*. In the same assay systems, aspergiones C (**4**) and E (**6**) were inactive.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer Model 341 LC polarimeter. UV spectral data were obtained from online UV spectra measured by photodiode array detection (Gynkotek, Germany). ^1H and ^{13}C NMR (chemical shifts in ppm) spectra were recorded on Bruker ARX 400 or DRX 500 NMR spectrometers in $CDCl_3$ (unless stated otherwise). EIMS were measured on a Finnigan MAT 8430 spectrometer. ESIMS spectra were recorded on a Finnigan MAT TSQ-7000 mass spectrometer. High-resolution ESIMS were recorded on a Micromass Q-Tof-2 mass spectrometer using peak matching.

Isolation and Cultivation of the Fungus. *Aspergillus versicolor* (Vuil.) Tirab. (deuteromycota) was isolated from a fresh sample of the marine sponge *Xestospongia exigua* (family Petrosiidae) that had been collected by scuba diving at Mengangan Island, Bali, Indonesia, in September 1997. Under sterile conditions, a tissue sample was removed from the inside of the sponge body and subsequently inoculated on malt agar slants, containing malt extract, agar, and artificial sea salt. Cultures were incubated at 27 °C, and from the growing cultures, pure strains of *Aspergillus versicolor* were isolated by reinoculation on malt agar plates. The fungal strain was identified by Dr. R. A. Samson (Institute of the Royal Netherlands Academy of Arts and Sciences at Baarn, The Netherlands). A voucher strain (no. HBI-2) is deposited at the same laboratory as species identification.

For natural product analysis cultures of *A. versicolor* were grown for five weeks (without shaking) in 1.5% malt extract broth (ME) made up with distilled water supplemented with 2.44% of SERA artificial sea-salts mixture at room temperature.

Extraction and Isolation. The mycelia and culture filtrate were extracted with EtOAc. The extract was evaporated under reduced pressure to obtain 13.0 g of a crude residue. This crude extract was subjected to vacuum liquid chromatography (VLC) on a short silica gel column employing the solvent system hexane/acetone (2:1). For monitoring 50 μL of each fraction was subjected to TLC on premade silica gel plates (Merck, Germany) using the same solvent system. Each fraction obtained was subjected to HPLC analysis on a reversed-phase column using a photodiode array detector (Gynkotek, Germany) employing a linear gradient of water (adjusted to pH 2.0 by addition of phosphoric acid) and methanol. Interesting fractions were subjected to further chromatographic separation using Sephadex LH-20 columns and methanol as solvent. If needed, final purification was achieved by semipreparative reversed-phase HPLC. Yields of compounds were as follows: **1** (152 mg), **2** (12.0 mg), **3** (1.5 mg), **4** (2.5 mg), **5** (3.0 mg), **6** (8.0 mg), **7** (9.0 mg).

Aspergillitine (1): yellow powder (MeOH); UV (MeOH−H_2O) λ_{max} 214, 245, 265, 295, 315, 370 nm; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 239 $[M]^+$ (100), 224, 210, 196, 185, 168, 141, 129, 115, 113, 98, 89, 88, 69, 63, 51, 43; ESIMS *m/z* 240 $[M + H]^+$, 218, 204, 115; HRESIMS *m/z* 240.1029 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_2$, 240.1025).

Aspergione A (2): colorless amorphous residue (MeOH); $[\alpha]^{20}_D$ +8.83° (*c* 0.28, MeOH); UV (MeOH−H_2O) λ_{max} 235, 291, 350; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 275 $[M + H]^+$, 261, 236, 227, 204, 195, 172, 163, 140, 129; HRESIMS *m/z* 275.1293 $[M + H]^+$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

Aspergione B (3): colorless amorphous residue (MeOH): $[\alpha]^{20}_D$ +1.89° (*c* 1.6, MeOH); UV (MeOH−H_2O) λ_{max} 231, 291, 352 nm; ^1H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 261 $[M + H]^+$; HRESIMS *m/z* 261.1118 $[M + H]^+$ (calcd for $C_{15}H_{17}O_4$, 261.1127).

Aspergione C (4): yellow amorphous residue (MeOH); $[\alpha]^{20}_D$ +1.90° (*c* 0.58, MeOH); UV (MeOH−H_2O) λ_{max} 236, 280, 290, 350, 390 nm; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 272 $[M]^+$, 258, 241, 229, 217, 216, 201, 187, 177, 160, 157, 141, 131, 115, 103, 91, 77, 69, 60, 43; ESIMS *m/z* 273 $[M + H]^+$, 163, 106; HRESIMS *m/z* 273.1147 $[M + H]^+$ (calcd for $C_{16}H_{17}O_4$, 273.1127).

Aspergione D (5): colorless amorphous residue (MeOH); UV (MeOH−H_2O) λ_{max} 211, 270, 325, 335, 390 nm; ^1H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 257 $[M + H]^+$, 243, 227, 204, 195, 172, 163, 140, 129, 107; HRESIMS *m/z* 257.0793 $[M + H]^+$ (calcd for $C_{15}H_{13}O_4$, 257.0814).

Aspergione E (6): colorless amorphous residue (MeOH); $[\alpha]^{20}_D$ +2.50° (*c* 0.60, MeOH); UV (MeOH−H_2O) λ_{max} 235, 291, 350 nm; ^1H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 275 $[M + H]^+$, 261, 236, 227, 204, 195, 172, 163, 140, 129; HRESIMS *m/z* 275.1263 $[M + H]^+$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

Aspergione F (7): colorless amorphous residue (MeOH); $[\alpha]^{20}_D$ −1.70° (*c* 1.50, MeOH); UV (MeOH−H_2O) λ_{max} 235, 291, 350 nm; ^1H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 261 $[M + H]^+$, 236, 227, 204, 195, 172, 163, 140, 129; HRESIMS *m/z* 261.1131 $[M + H]^+$ (calcd for $C_{15}H_{17}O_4$, 261.1127).

Agar Diffusion Assay. Susceptibility disks (5 mm in diameter) were impregnated with 5 and 10 μg of the isolated compounds dissolved in MeOH or in CH_2Cl_2, respectively, and placed on LB agar plates inoculated with the test bacteria: *Bacillus subtilis* DSM 2109 (=ATCC 11774), *Escherichia coli* DSM 10290 (=ATCC 15766). The plates were observed for zones of inhibition, after 24 h of incubation at 37 °C. The compounds were also assayed using *Saccharomyces cerevisiae* DSM 1333 (=ATCC 9763) as test organism, which was inoculated on YM agar plates, and zones of inhibition were recorded after 24 h of incubation at 27 °C. In all cases, for the controls containing only the respective amount of solvent, no zones of inhibition were observed.

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References and Notes

(1) Aspergiones A−F (**2**−**7**) had been previously reported as a preliminary communication (Lin, W. H.; Fu, H. Z.; Li, J.; Proksch, P. *Chin. Chem. Lett.* **2001**, *12*, 235-238), but due to limited spectral data, the wrong structures had been assigned. Here we report the full experimental data for the above-mentioned compounds.
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(31) The respective diameters of the observed zones of inhibition were as follows. *Bacillus subtilis*, aspergillitine (**1**) 5 μg: 7 mm, 10 μg: 8 mm; gentamycin sulfate (positive control) 5 μg: 12 mm, 10 μg: 15 mm; aspergiones C (**4**) and E (**6**): no inhibition.

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