Cytotoxic Polyketides from a Marine-derived Fungus *Aspergillus glaucus*

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Eight new aromatic polyketides (**2**, **4**−**6**, **8**, **14**, **16**, and **17**) together with eight known analogues (**3**, **7**, **9**−**13**, and **15**) were isolated from the marine-derived fungus *Aspergillus glaucus*. The structures and stereochemistry of the new compounds were elucidated by spectroscopic and chemical methods, and their cytotoxicities were evaluated against the HL-60 and A-549 cell lines.

Fungal polyketides constitute a large family of secondary metabolites endowed with a high degree of structural diversity and various biological activities.[1] These compounds include toxins such as aflatoxin B_1 produced by *Aspergillus* species, psychoactive compounds such as xenovulene, and pharmaceuticals such as the cholesterol-lowering drug lovastatin.[2] We have previously reported aspergiolide A (**1**), a novel cytotoxic anthraquinone derivative with a naphtho[1,2,3-*de*]chromene-2,7-dione skeleton, from cultures of the marine-derived fungus *Aspergillus glaucus*.[7] The *A. glaucus* group is well known for producing aromatic polyketide mycotoxins,[3] such as emodin, erythroglaucin, questin, physcion, physcion-9-anthrone, catenarin, rubrocristin,[4] physcion dianthranol, erythroglaucin,[5] kotanin, and desmethylkotanin.[6] In an effort to obtain more insight into the biosynthetic mechanisms and structure−activity relationships in this family of metabolites, our search for aromatic polyketides from this strain led to the discovery of eight new aromatic polyketides [namely, a new aspergiolide A analogue, aspergiolide B (**2**); three naphthyl ribofuranosides, isotorachrysone-6-*O*-α-D-ribofuranoside (**4**), 8-methoxy-3-methyl-1-naphthalenol-6-*O*-α-D-ribofuranoside (**5**), and 8-methoxy-1-naphthalenol-6-*O*-α-D-ribofuranoside (**6**); two anthracene derivatives, isoasperflavin (**8**) and (+)-variecolorquinones A (**14**); and two bianthrones, (*trans*)- and (*cis*)-emodin-physcion bianthrone (**16** and **17**)] and eight known analogues [isotorachrysone (**3**), asperflavin (**7**), emodin (**9**), physcion (**10**), questin (**11**), catenarin (**12**), rubrocristin (**13**), and physcion bianthrones (**15**)]. In this paper, we describe the isolation, structure, stereochemistry, and cytotoxicity against the HL-60 and A-549 cell lines of the new compounds.

Results and Discussion

Compound **2** was a new analogue of aspergiolide A (**1**), and both compounds had similar UV spectra [λ_{max} (log ε) 202 (4.36), 233 (4.31), 308 (4.01), 434 (3.58) for **2**; λ_{max} (log ε) 203 (4.25), 235 (4.19), 307 (3.90), 431 (3.61) nm for **1**[7]]. The HRESIMS of **2** (*m/z* 473.0868 $[M - H]^-$, calcd 473.0873) established the molecular formula as $C_{26}H_{18}O_9$. The ^{1}H and ^{13}C NMR data of **2** (Table 1) were similar to those of **1**[8] except that the signals of a phenolic hydroxy group in the structure of **1** were replaced by those of a methoxy group (δ_H 3.88, δ_C 56.1). The HMBC spectrum also supported the existence of the naphtho[1,2,3-*de*]chromene-2,7-dione skeleton (Figure 1), and the methoxy group was located at C-8 via the correlation between the δ_H 3.88 and δ_C 164.1 resonances. Therefore, compound **2** was identified as the 8-methoxy derivative of **1** and was named aspergiolide B (**2**).

Compound **4** was obtained as yellow needles. Its molecular formula $C_{19}H_{22}O_8$ was determined by HRESIMS (*m/z* 377.1254 $[M - H]^-$, calcd 377.1236). Comparison of the ^{1}H and ^{13}C NMR data (Table 2) with those of isotorachrysone (**3**)[9] suggested that they had the same naphthalene skeleton (Figure 1). Further comparison of their 1D NMR spectra indicated that compound **4**

Table 1. ^{1}H and ^{13}C NMR and HMBC Data for Compound **2**[a]

position	δ_H (*J*/Hz)	δ_C	HMBC (H→C)
1		159.1, qC	
2		131.7, qC[b]	
3		132.5, qC	
4		135.6, qC[b]	
5	7.00, s	109.8, CH	3, 6, 7, 9
6		163.4, qC	
7	6.75, s	102.5, CH	5, 6, 8, 9
8		164.1, qC	
9		111.7, qC	
10		184.6, qC[c]	
11		109.2, qC	
12		159.2, qC	
13	7.20, s	122.0, CH	11, 12, 15, 17
14		134.3, qC	
15		141.6, qC	
16		115.6, qC	
17	2.45, s	15.8, CH_3	13, 14, 15
18		191.2, qC[c]	
19		115.4, qC	
20		162.6, qC	
21	6.09, s	100.7, CH	19, 20, 22, 23
22		162.6, qC	
23	6.24, d (2.2)	111.8, CH	19, 21, 22, 25
24		145.1, qC	
25	2.54, s	23.1, CH_3	19, 23, 24
8-OCH_3	3.88, s	56.1, CH_3	8
6-OH	11.16, s		5, 6, 7
12-OH	13.84, s		11, 12, 13
20-OH	10.50 br, s		
22-OH	10.35, s		

[a] Spectra were recorded in DMSO-d_6 at 600 MHz for ^{1}H NMR and HMBC, and 150 MHz for ^{13}C NMR using TMS as internal standard. [b,c] Signals can be interchanged.

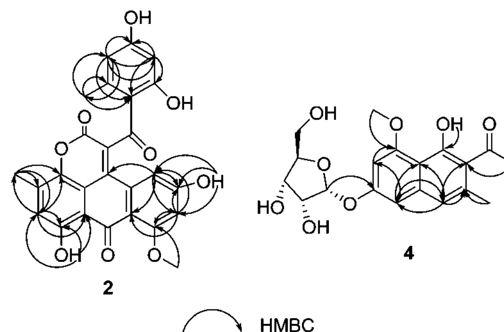

Figure 1. Selected HMBC correlations of compounds **2** and **4**.

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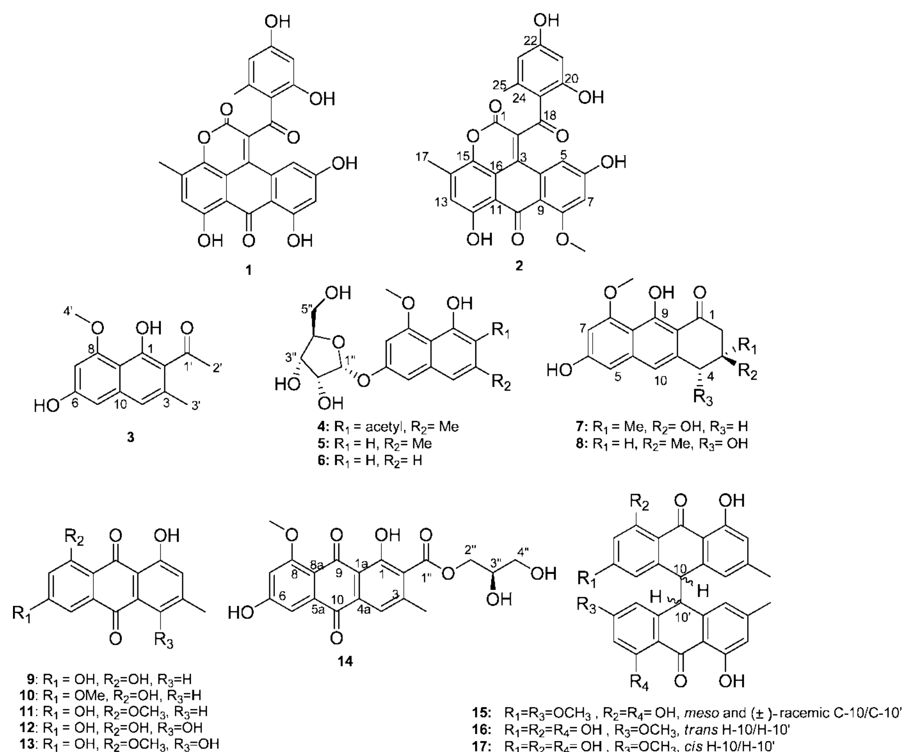
Table 2. ^{1}H and ^{13}C NMR Data for Compounds **3**−**6**[a]

	3[b]		4[c]		5[c]		6[c]	
position	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
1		154.0, qC		152.0, qC		153.7, qC		154.1, qC
2		122.9, qC		122.7, qC	6.47, s	110.1, CH	6.62, dd (7.8, 0.9)	108.3, CH
3		135.3, qC		133.9, qC		137.4, qC	7.25, t (8.2, 7.8)	127.9, CH
4	6.89, s	119.0, CH	7.01, s	118.8, CH	6.95, s	117.2, CH	7.16 br, d (7.3)	117.7, CH
5	6.66, d (2.2)	102.9, CH	6.90, d (1.8)	102.6, CH	6.86, d (1.8)	102.6, CH	6.96 d (2.2)	103.0, CH
6		159.0, qC		156.6, qC		155.4, qC		155.2, qC
7	6.57, s	98.4, CH	6.67, d (1.8)	98.6, CH	6.58, d (1.8)	97.8, CH	6.66, d (2.2)	98.6, CH
8		157.8, qC		157.3, qC		156.9, qC		156.9, qC
9		108.4, qC		108.6, qC		108.9, qC		110.7, qC
10		138.8, qC		136.8, qC		137.0, qC		136.9, qC
6-OH	8.84, s							
1-OH	9.75, s		9.73, s		9.12, s		9.22, s	
1′		204.0, qC		204.3, qC	2.31, s		3.99, s	
2′	2.49, s	32.3, CH_3	2.52, s	32.1, CH_3	3.97, s			
3′	2.24, s	20.2, CH_3	2.22, s	19.6, CH_3		21.2, CH_3		
4′	4.11, s	56.8, CH_3	4.01, s	56.4, CH_3		56.2, CH_3		56.3, CH_3
1″			5.73, d (4.8)	100.2, CH	5.67, d (4.8)	100.2, CH	5.70, d (4.8)	100.2, CH
2″			4.14, m	71.6, CH	4.09, m	71.5, CH	4.10, m	71.5, CH
3″			3.98, m	69.4, CH	3.94, m	69.3, CH	3.95, m	69.3, CH
4″			4.04, m	86.5, CH	3.99, m	86.3, CH	4.00, m	86.4, CH
5″			3.52, m	61.6, CH_2	3.48, m	61.5, CH_2	3.49, m	61.5, CH_2
2″-OH			4.75, d (8.7)		4.70, d (9.1)		4.70, d (9.1)	
3″-OH			4.96, d (5.5)		4.93, d (5.5)		4.93, d (5.5)	
5″-OH			4.86, t (5.5)		4.83, t (5.5)		4.83, t (5.5)	

[a] Spectra were recorded at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR using TMS as internal standard. [b] Measured in acetone-d_6. [c] Measured in DMSO-d_6.



was a furanoside. The connection between the sugar and naphthalene moieties through the O bond was established by the key HMBC correlation from H-1″ (δ_H 5.73) to C-6 (δ_C 156.6). The ribose residue was confirmed through comparing the ^{13}C NMR data with those of several furanosides, such as methyl ribofuranosides[10] and asperflavin ribofuranoside.[11] D-Ribose in **4** was established by measurement of its optical rotation following acid hydrolysis ($[\alpha]^{20}_{D}$ −18.5, *c* 0.085, H_2O).[12] The sugar moiety was further determined as α-D-ribofuranose by comparison of the $J_{1'',2''}$ value (4.8 Hz) with those of the methyl-α-D-ribofuranoside ($J_{1,2}$ = 4.3 Hz) and methyl-β-D-ribofuranoside ($J_{1,2}$ = 1.2 Hz).[13] Thus, compound **4** was identified as isotorachrysone-6-*O*-α-D-ribofuranoside.

Compounds **5** and **6** were both analogues of **4**, and their molecular formulas were established by HRESIMS as $C_{17}H_{20}O_7$ and $C_{16}H_{18}O_7$, respectively. Careful comparison of their ^{1}H and ^{13}C NMR spectra with those of **4** revealed that the structures contained the same sugar residue and the only substituent differences appeared on the naphthalene moiety. The resonances of the acetyl group in **4** were absent in **5**, and a new *meta*-coupled aromatic proton resonance (δ 6.47) appeared at this position. The corresponding C-2 carbon shifted upfield from δ 122.7 to δ 110.1. In compound **6**, both the resonances of the acetyl group and CH_3-3′ were absent and two *ortho*-coupled aromatic proton signals (δ 6.62 and 7.25) appeared correspondingly at C-2 (δ 108.3) and C-3 (δ

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **7** and **8**[a]

	7		**8**	
position	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
1		203.2, qC		202.5, qC
2	2.82, d (16.9)	51.4, CH_2	2.72, dd (4.6, 17.4)	43.0, CH_2
	2.62, dd (16.9, 1.8)		2.51, dd (10.5, 17.4)	
3		69.4, qC	2.07, m	36.3, CH
4	2.96, d (16.0)			
	2.87, d (14.6)	42.7, CH_2	4.30 br, t (7.2, 7.7)	71.9, CH
5	6.54, d (2.2)	101.9, CH	6.62, d (2.2)	102.6, CH
6		160.4, qC		160.6, qC
7	6.41, d (2.2)	97.8, CH	6.45, d (1.8)	98.1, CH
8		161.2, qC		161.2, qC
9		165.0, qC		165.2, qC
10	6.79, s	115.7, CH	7.10, d (0.8)	113.8, CH
3-CH_3	1.26, s	28.9, CH_3	1.05, d (6.4)	17.8, CH_3
8-OCH_3	3.84, s	55.7, CH_3	3.85, s	55.7, CH_3
4a		137.8, qC		141.6, qC
8a		108.9, qC		108.3, qC[b]
9a		108.1, qC		108.0, qC[b]
10a		141.6, qC		142.2, qC
3-OH	4.83, s			
4-OH			5.53, d (6.9)	
6-OH	10.28, s		10.34, s	
9-OH	14.97, s		15.10 (s)	

[a] Spectra were recorded in DMSO-d_6 at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR using TMS as internal standard. [b] Signals can be interchanged.

127.9). Therefore, **5** and **6** were identified as 8-methoxy-3-methyl-1-naphthalenol-6-*O*-α-D-ribofuranoside and 8-methoxy-1-naphthalenol-6-*O*-α-D-ribofuranoside, respectively. To date, less than 10 naphthyl furanosides have been identified as natural products, and only one naphthyl ribofuranoside has been reported previously.[14,15]

Compound **8** had the same molecular formula, $C_{16}H_{16}O_5$, established by the HRESIMS (*m/z* 287.0912 [M − H]$^-$, calcd 287.0919), as the known pigment asperflavin (**7**).[16,17] Careful comparison of their 1D NMR data (Table 3) indicated that they had the same skeleton and only OH-3 in **7** shifted to C-4 in **8**. This was confirmed by analyzing the peak shapes and *J* values of H-2 (δ 2.72, dd, $J = 4.6, 17.4$; δ 2.51, dd, $J = 10.5, 17.4$), H-3 (δ 2.07, m), and H-4 (δ 4.30, brt, $J = 7.2, 7.7$). The $J_{3,4}$ (7.7 Hz) value helped to establish the *trans* configuration of C-3/C-4.[18] The absolute configuration was thus determined to be 3*R*, 4*S* by the maximal negative Cotton effect at 281.0 nm and the maximal positive effect at 223.4 nm in the CD spectrum.[19] Therefore, the structure of **8** was established, and the compound was named isoasperflavin.

Compound **14** was a yellow pigment with the molecular formula $C_{20}H_{18}O_9$ established by HRESIMS (*m/z* 403.1021 [M + H]$^+$, calcd 403.1029). Both the UV spectra [λ_{max} (log ε) 440 (3.45), 286 (3.88), 251 (3.75), 223 (4.05)] and the 1D NMR data (Table 4) were consistent with those of the known compound variecolorquinone A, which was isolated from the metabolites produced by the halotolerant fungal strain *Aspergillus variecolor* B-17.[20] Because they had opposite specific rotations ($[\alpha]^{20}_D = +16.8$ for **14**; $[\alpha]^{20}_D = -18.0$ for variecolorquinone A[20]), the absolute configuration of C-3″ in **14** was deduced as *R* and **14** was named (+)-variecolorquinone A.

The new bianthrone isomers (**16** and**17**) had UV spectra (λ_{max} 239, 278, and 360) characteristic of bianthrones.[21] Their HRESIMS (*m/z* 523.1409 and 523.1400 [M − H]$^-$, calcd 523.1393) established the same molecular formula of $C_{31}H_{24}O_8$. According to their ^{1}H NMR spectra (Table 4), they both had eight *meta*-coupled aromatic protons, two methyl groups, five phenolic hydroxy groups, and one methoxy group. Comparison of their 1D NMR data (Table 4) with those of the physcion bianthrones (**15**),[22] emodin bianthrones,[23,24] and the anthracene monomers physcion and emodin[25] indicated they were two C-10/C-10′ isomers of physcion-emodin bianthrones. Furthermore, in the ^{1}H NMR spectra of **16** and **17**, the chemical shifts of OH-1/1′ were more upfield and the shifts of OH-6 and OH-8/8′ were more downfield in **16** than in **17**. Comparison with the literature data indicated that **16** and **17** respectively had a *trans* and a *cis* relationship between H-10/H-10′.[21,23,24]

Aromatic polyketides differ from other polyketides by their characteristic polycyclic aromatic structures.[26] They are produced by repetitive Claisen condensations of a starter unit (a dedicated FAS, another PKS or an acyl CoA) with malonyl-CoA elongation units. The biosynthesis of polyketides in fungi is governed by iterative type I polyketide synthases (PKS), which are multifunctional proteins consisting of domains for individual enzyme activities.[1,2,26] Aspergiolide B (**2**) may have a biosynthetic pathway similar to aspergiolide A (**1**), probably using the same PKS but with a different anthraquinone precursor, rubrocristin (**13**).[7] Compound **2** may also be formed through postmodification of compound **1**, such as *O*-methylation by *S*-adenosylmethionine (Figure 2, A). The biogenetic relationships of the other polyketides (**3**−**17**) isolated herein are postulated (Figure 2, B). With the accumulation of knowledge about the enzymology and the genome of *A. glaucus*, investigation strategies employing precursor-directed biosynthesis[27] and mutational biosynthesis[28] become feasible for further studies on aspergiolide polyketides.

The new compounds were evaluated for their cytotoxicities against the HL-60 cell line by the MTT method[29] and the A-549 cell line by the SRB method.[30] Aspergiolide B (**2**) showed potent cytotoxicities against the HL-60 and A-549 cell lines with IC_{50} values of 0.51 and 0.24 μM, respectively, indicating that the *O*-methylation of OH-8 did not negatively impact its activities.[7] Compound **16**, (*trans*)-emodin-physcion bianthrone, showed moderate cytotoxicities against the HL-60 and A-549 cell lines with IC_{50} values of 7.8 and 9.2 μM, respectively. Its *cis*-isomer, compound **17**, showed comparable cytotoxicities, and the IC_{50} values were 44.0 and 14.2 μM, respectively. It appears that the isomerization of the C-10/C-10′ axis does not impact the activities of the emodin-physcion bianthrones. The other new compounds showed no cytotoxicity at 100 μM against either cell line, suggesting that the naphtho[1,2,3-*de*]chromene-2,7-dione skeleton may be important for cytotoxicity and warrants further investigation to identify the molecular targets for the aspergiolides.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-500D micromelting point apparatus and were

Table 4. ^{1}H and ^{13}C NMR Data for Compounds **14**−**17**[a]

	14[b]		**15**[c,f,g]		**16**[d]		**17**[d]	
position	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
1		158.5, qC		161.9, qC/161.8, qC		161.7, qC		162.7, qC
1′						161.7, qC		162.7, qC
2		128.7, CH	6.10, s/6.12, s	116.9, CH/117.0, CH	6.38 br, s	116.4, CH	6.34 br, s	117.2, CH
2′					6.31 br, s	116.4, CH	6.27 br, s	117.2, CH
3		142.1, qC		146.8, qC/146.7, qC		146.5, qC/146.6, qC[f]		147.5, qC/147.8, qC[f]
3′								
4	7.45 br, s	118.7, CH	6.68, s/6.70, s	120.8, CH/120.7, CH	6.62, s	121.1, CH	6.66, s	122.0, CH/122.1, CH[f]
4′					6.62, s	121.1, CH	6.69, s	
5	7.15, d (2.2)	109.6, CH	6.38, d (2.3)/6.35, d (2.3)	107.8, CH/107.7, CH	6.31 br, s	108.9, CH	6.26, d (2.3)	109.9, CH
5′					6.40, d (2.2)	107.5, CH	6.34, d (2.3)	108.4, CH
6		169.0, qC		165.1, qC/165.2, qC		165.7, qC		166.3, qC
6′						nd[e]		167.8, qC
7	6.78, d (2.2)	105.2, CH	6.01 br, s/5.97 br, s	100.1, CH/100.1, CH	6.10 br, s	101.7, CH	6.15 br, s	102.6, CH
7′					6.14 br, s	100.3, CH	6.18 br, s	101.2, CH
8		164.1, qC		164.5, qC/164.6, qC		164.3, qC/164.5, qC[f]		165.2, qC/165.4, qC[f]
8′								
9		184.7, qC		190.3, qC/190.3, qC		190.2, qC/190.4, qC[f]		191.1, qC/191.4, qC[f]
9′								
10		182.5, qC	4.36, s/4.34, s	56.5, CH/56.5, CH	4.56, s	55.8, CH/55.9, CH[f]	4.56, s	56.7, CH/56.8, CH[f]
10′					4.56, s		4.56, s	
1a		115.0, qC		114.2, qC/114.3, qC		114.0, qC/113.9, qC[f]		115.0, qC/115.1, qC[f]
1a′								
4a		132.2, qC		140.1, qC/140.5, qC		140.2, qC		141.7, qC/142.0, qC[f]
4a′						140.2, qC		
5a		136.4, qC		142.8, qC/143.2, qC		144.1, qC/144.4, qC[f]		144.2, qC/144.7, qC[f]
5a′								
8a		109.9, qC		110.8, qC/110.9, qC		110.3, qC/110.9, qC[f]		111.0, qC/111.5, qC[f]
8a′								
3-CH_3	2.36, s	19.4, CH_3	2.29, s/2.31, s	22.0, CH_3/22.0, CH_3	2.23, s/2.24, s[g]	20.9, CH_3	2.27, s/ 2.29, s[g]	21.8, CH_3/21.9, CH_3[f]
3′-CH_3						20.9, CH_3		
6-OCH_3			3.84, s/3.82, s	55.6, CH_3/55.6, CH_3				
6′-OCH_3					3.89, s	55.3, CH_3	3.84, s	56.1, CH_3
8-OCH_3	3.89, s	55.9, CH_3						
1″		166.0, qC						
2″	4.22, dd (6.4, 10.9)							
	4.37, dd (4.1, 10.9)	66.8, CH_2						
3″	3.78, m	69.2, CH						
4″	3.42, m	62.5, CH_2						
1-OH	13.65, s		11.83, s/11.89, s		11.75, s		11.82, s	
1′-OH					11.70, s		11.79, s	
6-OH					9.79, s		9.75, s	
8-OH			12.19, s/12.14, s		12.05, s		11.97, s	
8′-OH					12.02, s		11.97, s	
3″-OH	5.06 br, s							
4″-OH	4.76 br, s							

[a] Spectra were recorded in DMSO-d_6 at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR using TMS as internal standard. [b] Measured in DMSO-d_6. [c] Measured in $CDCl_3$. [d] Measured in acetone-d_6. [e] Signals not detected. [f] Signals for C_x and C_x' ($x = 1, 2, 3,...$) can be interchanged. [g] Signals for H_x and H_x' ($x = 1, 2, 3,...$) can be interchanged.

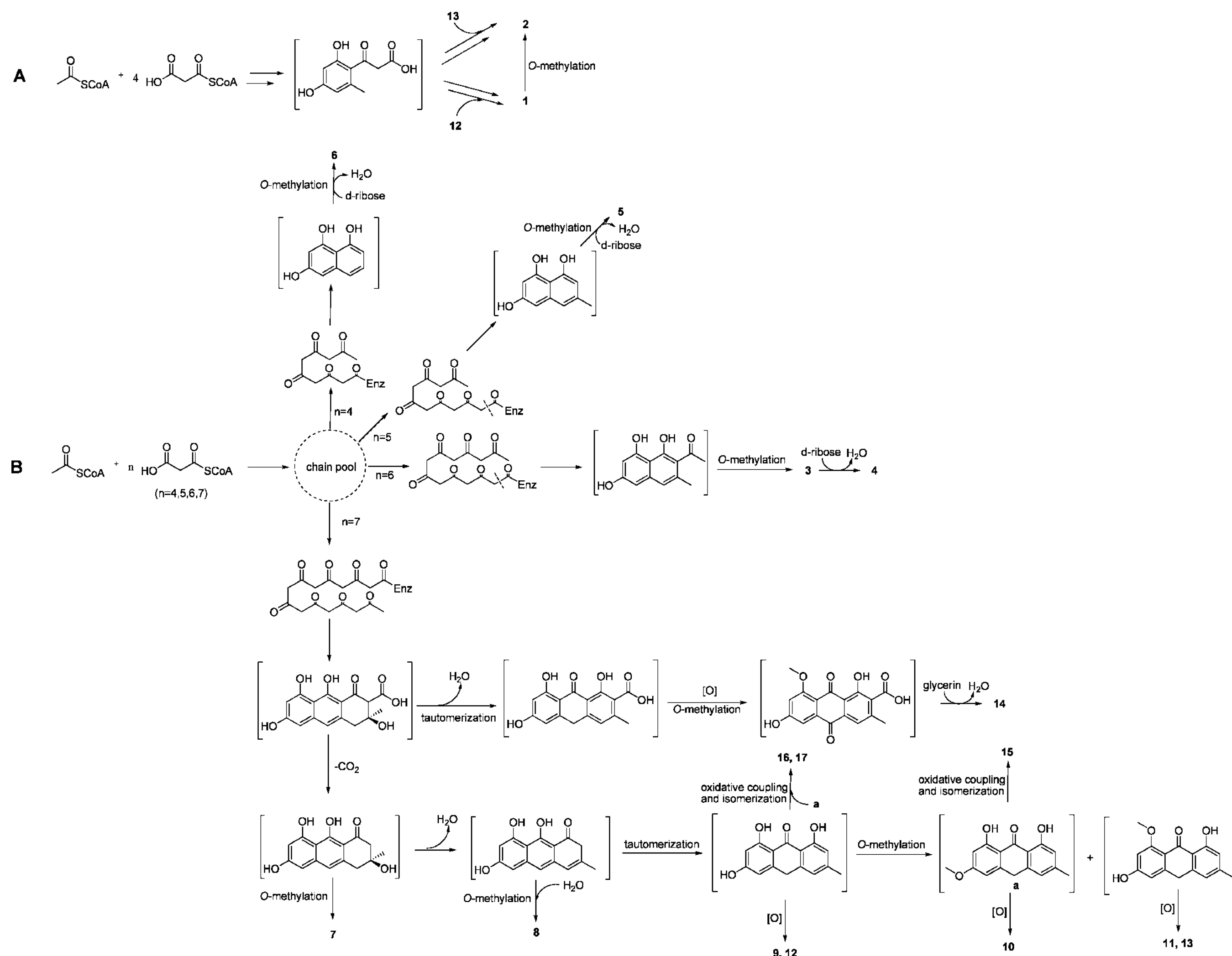


Figure 2. Postulated biosynthetic pathways for aspergiolide B (**2**, A) and polyketides **3**−**17** (B).

uncorrected. Specific rotations were obtained on a Jasco P-1020 digital polarimeter. UV spectra were recorded on Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. ^{1}H NMR, ^{13}C NMR, and DEPT spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semiprepartive HPLC was performed using an ODS column [YMC-pak ODS-A, 10 × 250 mm, 5 μm, 4 mL/min].

Fungal Material. The fungus *Aspergillus glaucus* was obtained from marine sediment surrounding mangrove roots collected in the Fujian Province, China. It was identified by Prof. Li Tian, the First Institute of Oceanography, SOA, Qingdao, China, and preserved in the China Center for Type Culture Collection (patent depositary number CCTCC M 206022). Working stocks were prepared on potato dextrose agar slants stored at 4 °C.

Fermentation and Extraction. Spores were directly inoculated into 500 mL Erlenmeyer flasks containing 100 mL fermentation media (mannitol 20 g, maltose 20 g, glucose 10 g, monosodium glutamate 10 g, KH_2PO_4 0.5 g, $MgSO_4\cdot 7H_2O$ 0.3 g, yeast extract 3 g, and corn steep liquor 1 g, dissolved in 1 L of seawater, pH 6.5). The flasks were incubated on a rotatory shaker at 165 rpm at 28 °C. After 9 days of cultivation, 15 L of whole broth was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with EtOAc, while the latter was extracted with acetone. The acetone extract was evaporated under reduced pressure to afford an aqueous solution and then extracted with EtOAc. The two EtOAc extracts were combined and concentrated in vacuo to give a crude gum (30 g).

Purification of the New Compounds. The crude gum (30 g) was subjected to silica gel column chromatography ($CHCl_3$/MeOH, v/v, gradient), and the active fractions 3, 4, 5, and 6 eluted with the solvent $CHCl_3$/MeOH (40:1, 20:1, 10:1, and 9:1) were separately subjected to repeated Sephadex LH-20 column chromatography ($CHCl_3$/MeOH, 1:1). The active subfractions 5-5-2, 6-2-2, 5-5-1-4, and 4-6-6-1 were further purified respectively by HPLC using a reversed-phase C18 column (50% MeOH, 30% CH_3CN, 35% CH_3CN, and 75% CH_3CN, 4.0 mL/min), to give compounds **4** (21.3 mg), **5** (2.8 mg), **6** (4.5 mg), **8** (3.0 mg), **16** (3.2 mg), and **17** (4.1 mg). Another two subfractions, 5-5-2-5 and 6-3-4, were respectively subjected to repeated Sephadex LH-20 column chromatography (MeOH) to give compounds **2** (10 mg) and **14** (24 mg).

Acid Hydrolysis of 4 and Determination of the Configuration of the Ribofuranose. A solution of **4** (9 mg) in 6 mol/L HCl (1 mL) was reacted for 3 h at 100 °C. The reaction mixture was extracted with EtOAc repeatedly to remove the aglycone fraction, which was identical to isotorachrysone (**3**).[7] The H_2O layer was then concentrated to furnish the sugar residue (1.7 mg). The rotation recorded for the ribose isolated in this study was $[\alpha]^{20}_{D}$ −18.5 (*c* 0.085, H_2O), which closely matched that for the D-ribose (lit. −20).[9]

Biological Assays. Cytotoxic activity was evaluated using the HL-60 cell line by the MTT method[29] and the A-549 cell line by the SRB method.[30] In the MTT assay, the cell line was grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cell suspensions (200 μL) at a density of 5 × 10^4 cells/mL were plated in 96-well microtiter plates and incubated for 24 h. The test compound solutions (2 μL in MeOH) at different concentrations were added to each well and further incubated for 72 h under the same conditions. MTT solution (20 μL of a 5 mg/mL solution in IPMI-1640 medium) was added to each well and incubated for 4 h. Old medium (150 μL) containing MTT was then gently replaced by

DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm.

In the SRB assay, cell suspensions (200 μL) were plated in 96-cell plates at a density of 2×10^5 cells/mL. Then the test compound solutions (2 μL in MeOH) at different concentrations were added to each well and further incubated for 24 h. Following drug exposure, the cells were fixed with 12% trichloroacetic acid and the cell layer was stained with 0.4% SRB. The absorbance of SRB solution was measured at 515 nm. Dose−response curves were generated, and the IC_{50} values were calculated from the linear portion of log dose response curves.

Aspergiolide B (2): red solid (MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.36), 233 (4.31), 308 (4.01), 434 (3.58); IR (KBr) ν_{max} cm^{-1} 3133, 2925, 1678, 1588, 1439, 1377, 1337, 1236, 1204, 1173, 1083 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 473.0868 $[M - H]^-$ (calcd for $C_{26}H_{17}O_9$, 473.0873).

Isotorachrysone-6-*O*-α-D-ribofuranoside (4): yellow needles (MeOH); mp 174−176 °C; $[\alpha]^{20}_D$ +178.6 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 245 (3.41), 308 (3.33), 337 (3.29); IR (KBr) ν_{max} 3390, 3289, 2966, 2924, 2850, 1642, 1619, 1576, 1456, 1394, 1258, 1157, 1106, 1017, 803 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 377.1254 $[M - H]^-$ (calcd for $C_{19}H_{21}O_8$, 377.1236).

8-Methoxy-3-methyl-1-naphthalenol-6-*O*-α-D-ribofuranoside (5): yellow needles (MeOH); mp 176−178 °C; $[\alpha]^{20}_D$ +110.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 237 (4.00), 303 (3.43), 318 (3.36), 333 (3.35); IR (KBr) ν_{max} 3409, 2962, 2927, 2853, 1638, 1614, 1583, 1455, 1385, 1362, 1253, 1164, 1102, 1012, 849 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 359.1118 $[M + Na]^+$ (calcd for $C_{17}H_{20}O_7Na$, 359.1107).

8-Methoxy-1-naphthalenol-6-*O*-α-D-ribofuranoside (6): yellow oil (MeOH); $[\alpha]^{20}_D$ +137.7 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.81), 299 (3.63), 321 (3.51), 335 (3.52); IR (KBr) ν_{max} 3402, 2926, 1633, 1618, 1591, 1454, 1402, 1376, 1317, 1252, 1166, 1124, 1042, 1003 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 345.0943 $[M + Na]^+$ (calcd for $C_{16}H_{18}O_7Na$, 345.0950).

Isoasperflavin (8): yellow solid (MeOH); $[\alpha]^{20}_D$ +34.1 (*c* 0.075, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 223.4 (0.9), 281.0 (−0.5); UV (MeOH) λ_{max} (log ε) 233 (3.94), 268 (3.06), 318 (3.33), 331 (3.27), 392 (3.62); IR (KBr) ν_{max} 3367, 2923, 2853, 1681, 1595, 1368, 1260, 1202, 1091, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRESIMS *m/z* 287.0912 $[M - H]^-$ (calcd for $C_{16}H_{15}O_5$, 287.0919).

(+)-Variecolorquinones A (14): yellow solid (MeOH); $[\alpha]^{25}_D$ +16.8 (*c* 0.03, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 440 (3.45), 286 (3.88), 251 (3.75), 223(4.05); IR (KBr) ν_{max} 3425, 1718, 1672, 1635, 1598, 1574 cm^{-1}; ^1H and ^{13}C NMR, see Table 4; HRESIMS *m/z* 403.1021 $[M + H]^+$ (calcd for $C_{20}H_{19}O_9$, 403.1029).

(*trans*)-Emodin-physcion bianthrone (16): yellow solid (MeOH); $[\alpha]^{20}_D$ −8.4 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (4.20), 278 (4.14), 360 (4.23); IR (KBr) ν_{max} 3354, 2971, 2926, 2854, 1638, 1619, 1595, 1486, 1456, 1362, 1327, 1277, 1259, 1164, 1066 cm^{-1}; ^1H and ^{13}C NMR, see Table 4; HRESIMS *m/z* 523.1409 $[M - H]^-$ (calcd for $C_{31}H_{23}O_8$, 523.1393).

(*cis*)-Emodin-physcion bianthrone (17): yellow solid (MeOH); $[\alpha]^{20}_D$ +9.8 (*c* 0.06, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (4.07), 278 (3.98), 360 (4.03);^1H and ^{13}C NMR, see Table 4; IR (KBr) ν_{max} 3353, 2980, 2924, 2853, 1637, 1618, 1595, 1487, 1457, 1361, 1329, 1259, 1189, 1160, 1065 cm^{-1}; HRESIMS *m/z* 523.1400 $[M - H]^-$ (calcd for $C_{31}H_{23}O_8$, 523.1393).

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References and Notes

(1) Julia, S.; Christian, H. *J. Biotechnol.* **2006**, *124*, 690–703.
(2) Cox, R. *J. Org. Biomol. Chem.* **2007**, *5*, 2010–2026.
(3) Ashley, J. N.; Raistrick, H.; Richards, T. *Biochem. J.* **1939**, *33*, 1291–1303.
(4) Heidrun, A.; Ilham, K.; Hans, Z.; Hartmut, L. *Arch. Microbiol.* **1980**, *126*, 223–230.
(5) Bachmann, M.; Luthy, J.; Schlatter, C. *J. Agric. Food Chem.* **1979**, *27*, 1342–1347.
(6) George, B.; Dieter, H. K.; Shank, R. C.; Steven, M. W.; Wogan, G. N. *J. Org. Chem.* **1971**, *36*, 1143–1147.
(7) Du, L.; Zhu, T.; Fang, Y.; Liu, H.; Gu, Q.; Zhu, W. *Tetrahedron* **2007**, *63*, 1085–1088.
(8) Du, L.; Zhu, T.; Fang, Y.; Liu, H.; Gu, Q.; Zhu, W. *Tetrahedron* **2008**, *64*, 4657.
(9) Lin, C. N.; Lu, C. M.; Lin, H. C.; Ko, F. N.; Teng, C. M. *J. Nat. Prod.* **1995**, *58*, 1934–1940.
(10) Thomas, E. W.; Harry, P. C. H.; Terry, E. N.; Nick, A. M. *Biochemistry* **1974**, *13*, 2650–2655.
(11) Li, Y.; Li, X.; Lee, U.; Kang, J. S.; Choi, H. D.; Sona, B. W. *Chem. Pharm. Bull.* **2006**, *54*, 882–883.
(12) Robert, K. N.; Harry, W. D.; Hewitt, G. F., Jr. *J. Am. Chem. Soc.* **1954**, *76*, 763–767.
(13) Anthony, S. S.; Robert, B. *J. Org. Chem.* **1984**, *49*, 3292–3300.
(14) Antkowiak, W. Z.; Krzyzosiak, W. J.; Biernat, J.; Ciesiolka, J.; Gornicki, P. *Bull. Acad. Pol. Sci., Ser. Sci. Chim.* **1977**, *25*, 173–178.
(15) Antkowiak, W. Z.; Krzyzosiak, W. J. *Bull. Acad. Pol. Sci., Ser. Sci. Chim.* **1977**, *25*, 421–425.
(16) Fujimoto, H.; Fujimaki, T.; Okuyama, E.; Yamazaki, M. *Chem. Pharm. Bull.* **1999**, *47*, 1426–1432.
(17) John, F. G. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2406–2411.
(18) Bringmann, G.; Münchbach, M.; Messer, K.; Koppler, D.; Michel, M.; Schupp, O.; Wenzel, M.; Louis, A. M. *Phytochemistry* **1999**, *51*, 693–699.
(19) Bringmann, G.; Messer, K.; Saeb, W.; Peters, E. M.; Peters, K. *Phytochemistry* **2001**, *56*, 387–391.
(20) Wang, W.; Zhu, T.; Tao, H.; Lu, Z.; Fang, Y.; Gu, Q.; Zhu, W. *J. Antibiot.* **2007**, *60*, 603–607.
(21) Politi, M.; Sanogo, R.; Ndjoko, K.; Guilet, D.; Wolfender, J. L.; Hostettmann, K.; Morelli, I. *Phytochem. Anal.* **2004**, *15*, 355–364.
(22) Monache, G. D.; Rosa, M. C.; Scurria, R.; Monacelli, B.; Pasqua, G.; Olio, G. D.; Botta, B. *Phytochemistry* **1991**, *30*, 1849–1854.
(23) Mai, L. P.; Gueritte, F.; Dumontet, V.; Tri, M. V.; Hill, B.; Thoison, O.; Guenard, D.; Sevenet, T. *J. Nat. Prod.* **2001**, *64*, 1162–1168.
(24) Donald, W. C.; John, S. E.; Warwick, D. R. *Aust. J. Chem.* **1976**, *29*, 1535–1548.
(25) Danielsen, K.; Aksnes, D. W. *Magn. Reson. Chem.* **1992**, *30*, 359–360.
(26) Shen, B. *Top. Curr. Chem.* **2000**, *209*, 1–51.
(27) Thiericke, R.; Rohr, J. *Nat. Prod. Rep.* **1993**, *10*, 265–289.
(28) Weist, S.; Süssmuth, R. D. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 141–150.
(29) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(30) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

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