Cottoquinazoline A and Cotteslosins A and B, Metabolites from an Australian Marine-Derived Strain of *Aspergillus versicolor*

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An Australian marine-derived isolate of *Aspergillus versicolor* (MST-MF495) yielded the known fungal metabolites sterigmatocystin, violaceol I, violaceol II, diorcinol, (−)-cyclopenol, and viridicatol, along with a new alkaloid, cottoquinazoline A (**1**), and two new cyclopentapeptides, cotteslosins A (**2**) and B (**3**). Structures for **1**−**3** and the known compounds were determined by spectroscopic analysis. The absolute configurations of **1**−**3** were addressed by chemical degradation and application of the C_3 Marfey's method. The use of "cellophane raft" high-nutrient media as a device for up-regulating secondary metabolite diversity in marine-derived fungi is discussed. The antibacterial properties displayed by *A. versicolor* (MST-MF495) were attributed to the phenols violaceol I, violaceol II, and diorcinol, while cotteslosins **2** and **3** were identified as weak cytotoxic agents.

In our ongoing pursuit of novel secondary metabolites from Australian microbes, we routinely employ a selection of media, evaluating extracts against a panel of bioassays and HPLC-DAD-ELSD-based dereplication techniques, to profile and compare secondary metabolite production. The search for new media is an iterative process, aimed at enhancing chemical diversity and productivity. In developing new media, increasing the concentration of nutrients is a vexed issue, as, for many microbes, increased nutrients provides luxuriant growth but at the expense of metabolite productivity and diversity. Indeed, it is generally accepted that secondary metabolite production is up-regulated by reducing, not increasing, nutrient availability. Nevertheless, our studies with fungi suggest that while this general premise is correct, for a small but significant proportion of fungi, increasing nutrients in malt extract agar (MEA) to 8× MEA can lead to increased secondary metabolite diversity. However, this enhanced diversity was achieved at a price, with extracts being less tractable to both bioassay and HPLC analysis due to the high concentrations of media extractables. To alleviate this problem, we introduced a "cellophane raft" to the agar, rationalizing that growth of fungi on a raft floating over nutrient-rich media would overcome the need to extract the media. While having little impact on terrestrial fungi, the presence of the cellophane raft did lead to increased metabolite production and diversity in over two-thirds of the 100 marine fungi investigated to date. The cellophane raft strategy has already been shown to successfully up-regulate the production of new diketopiperazines and polyketides by a marine-derived isolate of *Penicillium biliaii*[1] and new lipodepsipeptides by a marine-derived *Acremonium* sp.[2]

In this report, we describe our investigations into a marine isolate (MST-MF495) of a common terrestrial fungus, *Aspergillus versicolor*, recovered from beach sand. When grown on a cellophane raft over nutrient-rich media, this isolate up-regulated production of an array of chemically diverse metabolites that collectively exhibited antibacterial and cytotoxic activity. Comparison of the HPLC-DAD-ELSD profile obtained from this extract against an in-house database comprising (a) >1500 microbial metabolites, (b) extracts from ∼6000 microorganisms representative of the spectrum of known microbial metabolites, and (c) extracts from 50 000 "talented" microbes selected for their capacity to yield diverse metabolite profiles failed to return a match for several of the noteworthy *A. versicolor* (MST-MF495) metabolites. This report describes the isolation, characterization, and structure elucidation of these metabolites.

Results and Discussion

Solid-phase cellophane raft cultures (100 Petri dishes) of *A. versicolor* were pooled and extracted with MeOH. The resulting aqueous MeOH solution was concentrated *in vacuo* and then extracted with EtOAc. The combined EtOAc layers were dried and partitioned between *n*-hexane and MeOH. The MeOH-soluble portion was fractionated by sequential C_8 and C_{18} solid-phase extraction (SPE), followed by preparative and analytical HPLC, supported by HPLC-DAD-ESI(±)MS and on-flow HPLC-DAD-NMR analysis. Metabolites identified during this process included the known fungal natural products sterigmatocystin, violaceol I, violaceol II, diorcinol, (−)-cyclopenol, and viridicatol, together with a new alkaloid, cottoquinazoline A (**1**), and two new cyclopentapeptides, cotteslosins A (**2**) and B (**3**).

The known fungal metabolites were identified by detailed spectroscopic analysis and, where possible, by comparison to literature data. First reported in 1956 from *A. versicolor*,[3] sterigmatocystin is a carcinogenic mycotoxin commonly encountered on moldy cheese crusts and has become synonymous with *A. versicolor*. Violaceol I was first described in 1978 as aspermutarubrol from *A. sydowi*[4] and again as ethericin A from *A. funiculosis*.[5] Noted as extremely air sensitive, violaceol I achieved its current nomenclature in 1982 when isolated, along with the isomer violaceol II, from *Emericella violacea*.[6,7] The violaceols have since been shown to equilibrate on standing in MeOH[8] and exhibit modest antibacterial properties.[7,8] The biosynthetically related diorcinol was first described in 1968 from *A. rugulosa*,[9] then in 1993 as the antibacterial principal from *E. falconensis*,[10] and again in 2002 as the antifungal principal from the marine-derived fungus *Keissleriella* sp. YS4108.[11] Of note, the bisphenolic violaceols and diorcinol were all reported in 2003[8] as co-metabolites from cultured lichen mycobionts and in 2007[12] as co-metabolites from the insect pathogenic fungus *Cordyceps* sp. (−)-Cyclopenol and viridicatol were first reported in 1963 from strains of *P. cyclopium*,[13] at which time cyclopenol was observed to undergo acid-mediated transformation into viridicatol. The benzodiazepine cyclopenol has since been reported[14] to be biosynthetically derived from anthranilic acid and phenylalanine, with subsequent processing by the enzyme cyclopenase yielding the quinoline viridicatol. We attribute the modest antibacterial and antifungal activities associated with *A.*

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Chart 1

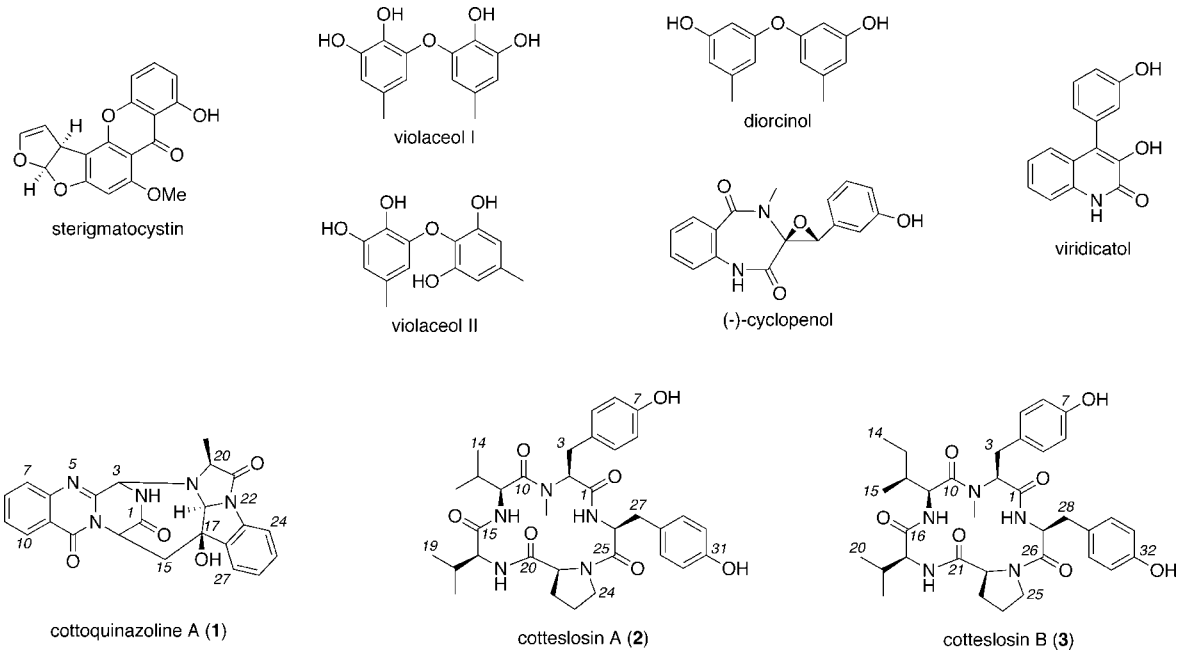

Table 1. NMR (DMSO-d_6, 600 MHz) Data for Cottoquinazoline A (**1**)[a]

pos	δ_C	δ_H, m (*J* in Hz)	COSY	$^1H-^{13}C$ HMBC	ROESY
1	167.8				
2		9.06, d (5.0)	3	3(w), 4, 14	3
3	65.4	5.24, d (5.0)	2	1, 14, 18	2, 20, 29
4	147.6				
6	146.8				
7	127.1	7.75, dd (8.2, 1.0)	8	9, 11	
8	134.4	7.85, ddd (8.2, 7,3, 1.4)	7, 9	10, 6	
9	127.1	7.56, ddd (8.0, 7.3, 1.0)	8, 10	7, 11	
10	126.1	8.14, dd (8.0, 1.4)	9	6, 8, 12	
11	120.6				
12	159.4				
14	53.6	5.27, dd (5.2, 2.3)	15a, 15b	17(w)	15a, 15b
15a	35.8	2.41, dd (14.9, 2.3)	14, 15b	1	14, 18
15b		3.07, dd (14.9, 5.2)	14, 15a	17, 18	14, 27
17	73.9				
18	79.7	4.90, d (1.8)	20(w)	3(w), 15(w), 17	15a, 20
20	63.2	4.08, dq (6.5, 1.8)	18(w), 29	21	3, 18
21	165.6				
23	135.8				
24	113.5	7.29, m		26	
25	129.2	7.29, m	26	23, 27	
26	129.5	7.10, ddd (7.6, 4.9, 3.7)	25, 27	24	
27	124.3	7.42, d (7.6)	26	17, 23, 25	15b
28	139.9				
29	14.6	1.47, d (6.5)	20	20, 21	3
17-OH		5.58, bs		15(w)	

[a] (w) denotes a weak correlation.

versicolor (MST-MF495) as due to cumulative levels of the phenolic violaceols and diorcinol.

The (+)HRESIMS analysis of cottoquinazoline A (**1**) returned a pseudomolecular ion $[M + Na]^+$ indicative of a molecular formula of $C_{23}H_{19}N_5O_4$ (Δmmu = 0.7), requiring 17 double-bond equivalents (DBE). Examination of the 1D and 2D NMR (DMSO-d_6) data (Table 1) for **1** revealed spectroscopic and structural features comparable to those of the fumiquinazoline class of fungal metabolite, previously reported from a marine-derived strain of *A. fumigatus* separated from the gastrointestinal tract of a marine fish[15] and from a marine-derived *Acremonium* sp. obtained from the surface of a Caribbean tunicate.[16] More specifically, the NMR data for **1** were indicative of a *nor* analogue of the known fumiquinazoline D, in which the C-3 tertiary methyl (δ_H 2.02, s) was replaced by a C-3 methine proton (δ_H 5.24, d) showing coupling to the 2-NH (δ_H 9.06, d). This *nor*-methyl fumiquinazoline structure represents a new carbon skeleton. The remaining planar structure for **1** was assigned on the basis of NMR analysis (Table 1), with a ROESY NMR correlation confirming a *cis* configuration between H-18 and H-20 and a *cis* configuration between H-3 and H-14 being defined by the steric constraints imposed by the heterocyclic ring closure from N-19 to C-3. Whereas an NOE correlation was used to define a *cis* configuration between H-18 and 17-OH in fumiquinazoline D,[15] the ROESY data for **1** did not exhibit a comparable correlation, but instead revealed an H-18 to H-14 correlation consistent with an H-18 to 17-OH *trans* configuration, unique among all known fumiquinazolines. Steric considerations required that the C-14 and C-3 substituents defining the heterocyclic ring spanning C-15, C-17, C-18, and N-19 be *cis* disposed. However, all spectroscopic efforts at defining the relative configuration between the two subunits defined by C-17, C-18 and C-19, and C-3 and C-14, respectively, proved inconclusive. Attempts at hydrolyzing **1**, with a view to recovering Ala followed by Marfey's analysis to assign absolute configuration, were unsuccessful. The partial relative stereostructure for **1** remains as shown.

Particularly noteworthy metabolites encountered in our investigations of *A. versicolor* (MST-MF495) were two new cyclic pentapeptides, cotteslosins A (**2**) and B (**3**). The (+)HRESIMS analysis of **2** returned a pseudomolecular ion $[M + Na]^+$ indicative of a molecular formula of $C_{34}H_{45}N_5O_7$ (Δmmu = 0.2) requiring 15 DBE. Examination of the NMR (DMSO-d_6) data (Table 2) revealed resonances consistent with five amino acids, including three amide NH [δ_H 6.82 (d), 7.29 (d), and 8.21 (d)], an amide *N*-methyl [δ_H 2.66 (s), δ_C 30.3], and two phenolic OH [δ_H 9.22 (s)] resonances that were identified by C_3 Marfey's analysis[2] as L-Tyr, *N*-Me-L-Tyr, L-Pro, L-Val, and L-Val (Table 4), accounting for 14 DBE and requiring that **2** be monocyclic. Diagnostic 2D NMR (DMSO-d_6) HMBC correlations (Table 2) from 26-NH to C-1, 2-NMe to C-10, 11-NH to C-15, and 16-NH to C-20 established the sequence L-Tyr-*N*-Me-L-Tyr-L-Val-L-Val-L-Pro and required cyclization from the L-Pro nitrogen to the L-Tyr carboxyl. It was determined that the L-Pro residue in **2** adopted a *cis* configuration, as indicated by the ^{13}C NMR data $\Delta\delta_{\beta\gamma}$ 9.7 ppm,[17] leading to assignment of the structure as shown. By comparison, the (+)HRESIMS analysis **3** returned a pseudomolecular ion $[M + Na]^+$ indicative of a molecular formula of $C_{35}H_{47}N_5O_7$ (Δmmu = 0.1), consistent with a CH_2 homologue of **2**. Indeed, detailed examination of the NMR (DMSO-d_6) data for **3** (Table 3), including diagnostic HMBC

Table 2. NMR (DMSO-d_6, 600 MHz) Data for Cotteslosin A (**2**)

pos	δ_C	δ_H, m (*J* in Hz)	COSY	TOCSY	$^1H-^{13}C$ HMBC
			N-Me-L-Tyr		
1	168.4				
2	61.6	4.13, d (11.7)	3a, 3b	3a, 3b	1, 3, 2-NMe
3a	33.0	3.18, m	2, 3b	2, 3b	1, 2, 4, 5/9
3b		2.63, t (12.6)	2, 3a	2, 3a	2, 4, 5/9
4	127.1				
5/9	130.1	6.94, d (8.3)	6/8	6/8	3, 5/9, 6/8, 7
6/8	115.2	6.68, m	5/9	5/9	4, 5/9, 7, 6/8
7	156.2				
2-NMe	30.3	2.66, s			10
7-OH		9.22, s			6/8, 7
			L-Val[1]		
10	170.5				
11	52.5	3.61, t (8.1)	12	12, 13, 14, 11-NH	10, 12, 13, 14
12	29.9	1.75, m	11, 13, 14	11, 13, 14	10, 11, 13, 14
13	19.1	0.44, d (6.6)	12, 14	11, 12, 14	11, 12, 14
14	17.9	0.61, d (6.6)	12, 13	11, 12, 13	11, 12, 13
11-NH		8.21, d (8.9)	11	11, 12, 13, 14	15, 11
			L-Val[2]		
15	169.5				
16	61.3	4.01, m	17	17, 18, 19, 16-NH	15, 17
17	30.9	1.75, m	16, 18, 19	16, 18, 19	16, 19
18	19.4	0.85, d (6.6)	17, 19	16, 17, 19	16, 17, 19
19	19.0	0.78, d (6.5)	17, 18	16, 17, 18	16, 17, 18
16-NH		7.29, d (10.1)	16	16, 17, 18, 19	20
			L-Pro		
20	170.2				
21	61.0	4.01, m	22	22, 23b, 24	20, 23, 24
22	31.4	1.91, m	21, 23a, 23b	21, 23a, 23b, 24	20, 21, 23
23a	21.7	1.75, m	22, 23b	22, 23b, 24	22, 24
23b		1.61, m	21, 23a, 24	21, 22, 23a, 24	22, 24
24	46.2	3.48, m	23a, 23b	21, 22, 23a, 23b	23
			L-Tyr		
25	168.8				
26	52.9	4.64, m	27a, 27b	27a, 27b, 26-NH	
27a	37.3	2.96, dd (12.9, 9.4)	26, 27b	26, 27b	25, 26, 28, 29/33
27b		2.81, dd (13.2, 4.4)	26, 27a	26, 27a	26, 28, 29/33
28	127.1				
29/33	130.1	7.01, d (8.3)	30/32	30/32	27, 29/33, 30/32, 31
30/32	115.0	6.68, m	29/33	29/33	28, 29/33, 30/32, 31
31	155.9				
26-NH		6.82, d (7.5)	26	26, 27a, 27b	1, 26
31-OH		9.22, s			30/32, 31

correlations from 27-NH to C-1, 2-NMe to C-10, 11-NH to C-17, and 17-NH to C-21, supported by C_3 Marfey's analysis (Table 4),[2] established **3** as the L-Val[1] to L-*allo*-Ile variant of **2**, as shown (with the L-Pro adopting a *cis* configuration, $\Delta\delta_{\beta\gamma}$ 9.7 ppm).[17]

Terrestrial isolates of *A. versicolor* are well-known producers of novel natural products, with the archetypal metabolite being sterigmatocystin.[3] Nevertheless, despite intensive investigations over many decades, leading to multiple scientific publications, *A. versicolor* isolates continue to yield new chemistry, as illustrated by a 2007 report describing a series of aroyl uridines.[18] As a noteworthy extension of this potential, the past decade has seen published accounts of several marine-derived isolates of *A. versicolor* (recovered from a green alga, two sponges, and a sea urchin) returning an array of unusual metabolites including nitrobenzyl esters,[19] oxindole-diterpene conjugates,[20] chromones,[21,22] hydroindenoisopyrans,[23] and polyketides.[24] Our latest installment to the *A. versicolor* story only serves to emphasize the continuing molecular potential that exists even within seemingly well-known and well-studied fungi. The use of a cellophane raft approach also demonstrates a valuable new method for up-regulating the production and simplifying the recovery of secondary metabolites in marine-derived fungal strains.

Cotteslosin A (**2**) was assessed for antitumor properties and exhibited weak cytotoxicity against human melanoma (MM418c5, EC_{50} 66 μg/mL), prostate (DU145, EC_{50} 90 μg/mL), and breast (T47D, EC_{50} 94 μg/mL) cancer cell lines. It is interesting to note the structural similarities between the cotteslosins and the cyclic pentadepsipeptide sansalvamide A[25] and its *N*-methyl analogue,[26] both of which were obtained from marine-derived fungi. Structure−activity relationship studies based around synthetic sansalvamide analogue libraries have established that the anticancer properties of the sansalvamides can be enhanced (70-fold improved potency and 250-fold greater selectivity) by conversion to a cyclic pentapeptide, sansalvamide A peptide, and through the inclusion of adjacent Tyr and *N*-Me-Phe residues and the incorporation of a single D-amino acid,[27−35] leading to nM potency against pancreatic (PL-45, BxPC3)[35] and colon (HCT-116) cancer cell lines.[34]

Our investigations into as yet unidentified minor co-metabolites of *A. versicolor* (MST-MF495) continue, as we seek to isolate and identify the elusive, but nevertheless potent and selective cytotoxic agent(s) evident from our bioassay profile of the crude extract. Furthermore, the cellophane raft, high-nutrient media strategy employed in this study represents a valuable new approach to accessing "hidden" secondary metabolite potential within the genome of marine-derived fungi and is worthy of wider application against other fungi.

Experimental Section

General Experimental Procedures. Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell. NMR spectra were obtained on a Bruker Avance DRX600 spectrometer, in the solvents indicated and referenced to residual ^1H signals in the deuterated solvents. Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes. High-resolution ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 μL/min using sodium formate clusters as an internal calibrant. HPLC was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series diode array and/or multiple wavelength detectors

Table 3. NMR (DMSO-d_6, 600 MHz) Data for Cotteslosin B (**3**)

pos	δ_C	δ_H, m (*J* in Hz)	COSY	TOCSY	$^1H-^{13}C$ HMBC
			N-Me-L-Tyr		
1	168.4				
2	61.6	4.13, m	3a, 3b	3a, 3b	3, 2-NMe
3a	32.9	3.18, dd (3.3, 0.3)	2, 3b	2, 3b	4, 5
3b		2.63, m	2, 3a	2, 3a	4, 5
4	127.1				
5/9	130.1	6.94, d (8.5)	6/8	6/8	3, 5/9, 6/8, 7
6/8	115.2	6.67, m	5/9	5/9	4, 5/9, 6/8, 7
7	156.2				
2-NMe	30.3	2.66, s			10, 2
7-OH		9.25, s			
			L-*allo*-Ile		
10	170.5				
11	59.3	4.13, m	12, 11-NH	12, 15, 11-NH	17, 12, 13, 15
12	37.1	1.56, m	11, 13a, 13b, 15	11, 13a, 13b, 15	
13a	25.4	1.35, m	13b, 14	12, 13b, 14	
13b		1.05, m	12, 13a	12, 13a	
14	10.6	0.84, t (7.3)	13a, 13b	13a, 13b	12, 13
15	15.0	0.77, d (6.9)	12	11, 12, 11-NH	11, 12, 13
11-NH		7.30, d (9.9)	11	11, 15	10
			L-Val		
16	169.7				
17	52.5	3.62, m	18, 17-NH	18, 19, 20, 17-NH	21
18	29.8	1.74, m	17	17, 19, 20	21
19	17.8	0.61, d (6.9)	18	17, 18, 20	17, 18, 20
20	19.1	0.45, d (6.7)	18	17, 18, 19	17, 18, 19
17-NH		8.25, d (9.1)	17	17	21
			L-Pro		
21	170.2				
22	61.0	4.02, t (5.1)	23	23, 24a, 24b	21, 23, 24, 25
23	31.4	1.91, m	22, 24b	22, 24a, 24b, 25a, 25b	21
24a	21.7	1.74, m	23, 24b, 25a	22, 23, 24b, 25b	
24b		1.62, m	23, 24a, 25b	22, 23, 24a, 25b	
25	46.2	3.47, m	24a, 24b	23, 24a, 24b	
			L-Tyr		
26	168.8				
27	52.9	4.64, m	28a, 28b, 27-NH	28a, 28b, 27-NH	
28a	37.3	2.97, dd (13.2, 9.4)	27, 28b	27, 28b, 27-NH	26, 27, 29, 30/34
28b		2.81, dd (13.1, 4.4)	27, 28a	27, 23a, 27-NH	26, 27, 29, 30/34
29	127.1				
30/34	130.1	7.02, d (8.5)	31/33	31/33	28, 30/34, 31/33, 32
31/33	115.0	6.67, m	30/34	30/34	30/34, 31/33, 33
32	155.9				
27-NH		6.84, d (7.4)	27	27, 28a, 28b	1
32-OH		9.25, s			

Table 4. C_3 Marfey's Analysis of Cotteslosins A and B (**2**, **3**)

amino acid	t_{rD} (min) analyte[a]	t_{rL} (min) standard[b]	t_{rD} (min) standard[c]
	Cotteslosin A (**2**)		
Pro	21.2	19.0	21.2
Val	36.2	27.5	36.2
Tyr[d]	29.9	24.6	29.9
Tyr[e]	53.9	47.1	53.7
N-Me-Tyr[d]	22.9	21.6	22.9
N-Me-Tyr[e]	48.1	47.5	48.1
	Cotteslosin B (**3**)		
Ile	not detected	33.3	42.2
allo-Ile	41.1	32.4	41.1
Pro	21.3	19.0	21.2
Val	36.2	27.5	36.2
Tyr[d]	30.5	24.6	29.9
Tyr[e]	54.0	47.1	53.7
N-Me-Tyr[d]	not detected	21.6	22.9
N-Me-Tyr[e]	48.1	47.5	48.1

[a] Retention times from a hydrolyzed sample of cotteslosin A or B derivatized with D-FDAA. [b] Retention time of L-amino acid standards derivatized with L-FDAA. [c] Retention time of L-amino acid standards derivatized with D-FDAA. [d] Monoderivatized amino acid. [e] Diderivatized amino acid.

and Agilent 1100 Series fraction collector, controlled using ChemStation Rev.9.03A and Purify version A.1.2 software.

Bioassay Details. See Capon et al., 2005.[36]

Fungal Culture. MST-MF495 was isolated from a beach sand sample collected at low tide from Cottesloe, Western Australia. It was identified on morphological grounds as a typical isolate of *Aspergillus versicolor*. This classification was further confirmed by the presence of sterigmatocystin, a diagnostic secondary metabolite of *A. versicolor*. A sample of this strain has been deposited in the culture collection of Microbial Screening Technologies, Sydney, Australia.

Fermentation. A spore suspension of the fungus was spread onto a single sheet of sterile cellophane cut to rest snugly on the surface of the Petri plate containing malt extract agar (8× MEA) (malt extract (16%), peptone (0.8%), glucose (16%), agar (2%); 100 × 15 g). The plates were incubated for 21 days at 24 °C.

Extraction and Isolation. The cellophane rafts on which the mycelia were growing (100 Petri plates) were collected, pooled, and extracted with MeOH (2 × 2 L). An aliquot (10 mL) of the MeOH extract was dried *in vacuo* (43 mg), allowing the dry mass of the entire MeOH extract to be calculated (17.2 g). The remaining aqueous MeOH extract was concentrated *in vacuo* (~400 mL) and then extracted with EtOAc (3 × 400 mL). The combined EtOAc layers were dried *in vacuo* (5.41 g) and then partitioned between MeOH (100 mL) and *n*-hexane (100 mL). The MeOH-soluble fraction was dried *in vacuo* (1.27 g) and fractionated by C_{18} SPE (stepwise gradient 10−100% MeOH/water, 10 fractions). Fractions 3 and 4 were fractionated by preparative HPLC (Agilent Zorbax C_8 column, 250 × 21.2 mm, 7 μm, 20 mL/min, isocratic elution, 45% MeOH, 55% water, 0.01% TFA). Selected subfractions were further fractionated by semipreparative HPLC (Agilent Zorbax C_8 column, 250 × 9.4 mm, 5 μm, 4.2 mL/min, linear gradient elution, 10−100% MeCN/water, 0.01% TFA over 45 min), supported by HPLC-DAD-(±)ESIMS and on-flow HPLC-DAD-NMR analysis. Metabolites identified during this process included sterigmatocystin (150 mg, 0.87%), violaceol I (11.2 mg, 0.065%) and II (1.8 mg, 0.010%), diorcinol (2.8 mg, 0.016%), (−)-cyclopenol (1 mg, 0.006%), and viridicatol (<1 mg, <0.006%), along with a new alkaloid,

cottoquinazoline A (**1**, 0.6 mg, 0.003%), and two new cyclopentapeptides, cotteslosins A (**2**, 4.6 mg, 0.027%) and B (**3**, <1 mg, <0.006%). Order of elution from the C_8 column was viridicatol, (−)-cyclopenol, violaceol II, violaceol I, **2**, diorcinol, **1**, **3**, and sterigmatocystin with percent yields calculated relative to the initial 17.2 g, methanol-soluble fraction.

Sterigmatocystin: $[\alpha]^{23}_D$ −350 (*c* 0.05, MeOH); NMR data (DMSO-d_6, 600 MHz) see Table S1, Supporting Information; HRESI(+)MS *m/z* 325.0692 (calcd for $C_{18}H_{13}O_6$, 325.0707). Also confirmed by literature comparison.[37]

Violaceol I: NMR data (MeOH-d_4, 600 MHz) see Table S2, Supporting Information; HRESI(+)MS *m/z* 285.0737 (calcd for $C_{14}H_{14}O_5Na$, 285.0739). Also confirmed by literature comparison.[8]

Violaceol II: NMR data (MeOH-d_4, 600 MHz) see Table S3, Supporting Information; HRESI(+)MS *m/z* 285.0742 (calcd for $C_{14}H_{14}O_5Na$, 285.0739). Also confirmed by literature comparison.[8]

Diorcinol: NMR data (MeOH-d_4, 600 MHz) see Table S4, Supporting Information; ESI(±)MS (100 kV) *m/z* 231 $[M + H]^+$. Also confirmed by literature comparison.[10]

(−)-Cyclopenol: $[\alpha]^{23}_D$ −220 (*c* 0.03, MeOH); NMR data (DMSO-d_6, 600 MHz) see Table S5, Supporting Information; HRESI(+)MS *m/z* 333.0864 (calcd for $C_{17}H_{14}N_2O_4Na$, 333.0851). Also confirmed by literature comparison.[38]

Viridicatol: NMR data (DMSO-d_6, 600 MHz) see Table S6, Supporting Information; ESI(±)MS (100 kV) *m/z* 254 $[M + H]^+$.

Cottoquinazoline A (1): $[\alpha]^{23}_D$ +98 (*c* 0.03, MeOH); UV (1:1 MeCN/H_2O) λ_{max} (rel intensity) 230 (1), 258 (0.52), 268 (0.45), 279 (0.37), 303 (0.13), 316 (0.098) nm; NMR data (DMSO-d_6, 600 MHz) see Table 1; HRESI(+)MS *m/z* 452.1322 (calcd for $C_{23}H_{19}N_5O_4Na$, 452.1329).

Cotteslosin A (2): $[\alpha]^{23}_D$ −98 (*c* 0.11, MeOH); UV (1:1 MeCN/H_2O) λ_{max} (rel intensity) 221 (1), 277 (0.14) nm; NMR data (DMSO-d_6, 600 MHz) see Table 2; HRESI(+)MS *m/z* 658.3219 (calcd for $C_{34}H_{45}N_5O_7Na$, 658.3211).

Cotteslosin B (3): UV (1:1 MeCN/H_2O) λ_{max} (rel intensity) 222 (1), 277 (0.11) nm; NMR data (DMSO-d_6, 600 MHz) see Table 3; HRESI(+)MS *m/z* 672.3368 (calcd for $C_{35}H_{47}N_5O_7Na$, 672.3367).

C_3 Marfey's Analysis. The use of both L-FDAA and D-FDAA allows preparation of diastereomeric amino acid derivatives representative of both the L- and D-amino acid antipodal series using only L-amino acid standards. Therefore, L-amino acid standards were derivatized with both L-FDAA and D-FDAA. L-FDAA (1% w/v in acetone; 100 μL) and aqueous sodium bicarbonate (1 M; 20 μL) were added to an aqueous solution of the amino acid to be analyzed (50 mM; 50 μL). A second reaction mixture was prepared using D-FDAA in place of L-FDAA. The solutions were incubated for 60 min at 37 °C before being quenched by addition of HCl (1 M; 20 μL) and diluted with MeCN (810 μL). An aliquot of each derivatized amino acid solution (5 μL) was injected onto a Zorbax StableBond C_3 HPLC column (150 × 4.6 mm, 5 μm) maintained at 50 °C. The column was developed with a linear gradient of 15−60% MeOH/water (+ isocratic 5% of a 1% formic acid solution in MeCN) over 55 min. Detection was by diode array detection monitoring at 340 nm or ESIMS monitoring total ion current. Samples of **2** and **3** (50 μg each) were hydrolyzed in HCl (6 M; 200 μL) at 100 °C overnight, reduced to dryness under a stream of nitrogen, and analyzed as per the amino acid standards.

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Supporting Information Available: NMR spectra and tabulated data for all compounds and UV−vis spectra for **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Capon, R. J.; Stewart, M.; Ratnayake, R.; Lacey, E.; Gill, J. H. *J. Nat. Prod.* **2007**, *70*, 1746–1752.
(2) Ratnayake, R.; Fremlin, L. J.; Lacey, E.; Gill, J. H.; Capon, R. J. *J. Nat. Prod.* **2008**, *71*, 403–408.
(3) Davies, J. E.; Roberts, J. C.; Wallwork, S. C. *Chem. Ind.* **1956**, 178–179.
(4) Shibata, K.; Kamikawa, T.; Kaneda, N.; Taniguchi, M. *Chem. Lett.* **1978**, 797–798.
(5) Koenig, W. A.; Pfaff, K. P.; Loeffler, W.; Schanz, D.; Zaehner, H. *Liebigs Ann. Chem.* **1978**, 1289–1296.
(6) Yamazaki, M.; Maebayashi, Y. *Chem. Pharm. Bull.* **1982**, *30*, 509–513.
(7) Yamazaki, M.; Maebayashi, Y. *Chem. Pharm. Bull.* **1982**, *30*, 514–518.
(8) Takenaka, Y.; Tanahashi, T.; Nagakura, N.; Hamada, N. *Chem. Pharm. Bull.* **2003**, *51*, 794–797.
(9) Ballantine, J. A.; Hassall, C. H.; Jones, B. D. *Phytochemistry* **1968**, *7*, 1529–1534.
(10) Itabashi, T.; Nozawa, K.; Nakajima, S.; Kawai, K. *Chem. Pharm. Bull.* **1993**, *41*, 2040–2041.
(11) Liu, C. H.; Meng, J. C.; Zou, W. X.; Huang, L. L.; Tang, H. Q.; Tan, R. X. *Planta Med.* **2002**, *68*, 363–365.
(12) Bunyapaiboonsri, T.; Yoiprommarat, S.; Intereya, K.; Kocharin, K. *Chem. Pharm. Bull.* **2007**, *55*, 304–307.
(13) Birkinshaw, J. H.; Luckner, M.; Stickings, C. E.; Mohammed, Y. S.; Mothes, K. *Biochem. J.* **1963**, *89*, 196–202.
(14) Luckner, M. *Eur. J. Biochem.* **1967**, *2*, 74–78.
(15) Takahashi, C.; Matsushita, T.; Doi, M.; Minoura, K.; Shingu, T.; Kumeda, Y.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2345–2353.
(16) Belofsky, G. N.; Anguera, M.; Jensen, P. R.; Fenical, W.; Kock, M. *Chem.−Eur. J.* **2000**, *6*, 1355–1360.
(17) Siemion, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem., Int. Ed.* **1975**, *14*, 702–703.
(18) Jiao, P.; Mudur, S. V.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2007**, *70*, 1308–1311.
(19) Belofsky, G. N.; Jensen, P. R.; Renner, M. K.; Fenical, W. *Tetrahedron* **1998**, *54*, 1715–1724.
(20) Yamada, K.; Doi, M.; Yamada, T.; Minoura, K.; Numata, A. *Tennen Yuki Kagobutsu* **2000**, *42*, 397–402.
(21) Lin, W. H.; Fu, H. Z.; Li, J.; Proksch, P. *Chin. Chem. Lett.* **2001**, *12*, 235–238.
(22) Lin, W. H.; Brauers, G.; Ebel, R.; Wray, V.; Berg, A.; Sudarsono; Proksch, P. *J. Nat. Prod.* **2003**, *66*, 57–61.
(23) Lin, W. H.; Li, J.; Fu, H. Z.; Proksch, P. *Chin. Chem. Lett.* **2001**, *12*, 435–438.
(24) Lee, Y. M.; Mansoor, T. A.; Hong, J.; Lee, C.-O.; Bae, K. S.; Jung, J. H. *Nat. Prod. Sci.* **2007**, *13*, 90–96.
(25) Belofsky, G. N.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1999**, *40*, 2913–2916.
(26) Cueto, M.; Jensen, P. R.; Fenical, W. *Phytochemistry* **2000**, *55*, 223–226.
(27) Gu, W. X.; Liu, S. X.; Silverman, R. B. *Org. Lett.* **2002**, *4*, 4171–4174.
(28) Carroll, C. L.; Johnston, J. V. C.; Kekec, A.; Brown, J. D.; Parry, E.; Cajica, J.; Medina, I.; Cook, K. M.; Corral, R.; Pan, P. S.; McAlpine, S. R. *Org. Lett.* **2005**, *7*, 3481–3484.
(29) Liu, S. X.; Gu, W. X.; Lo, D.; Ding, X. Z.; Ujiki, M.; Adrian, T. E.; Soff, G. A.; Silverman, R. B. *J. Med. Chem.* **2005**, *48*, 3630–3638.
(30) Rodriguez, R. A.; Pan, P. S.; Pan, C. M.; Ravula, S.; Lapera, S.; Singh, E. K.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; McAlpine, S. R. *J. Org. Chem.* **2007**, *72*, 1980–2002.
(31) Otrubova, K.; McGuire, K. L.; McAlpine, S. R. *J. Med. Chem.* **2007**, *50*, 1999–2002.
(32) Styers, T. J.; Kekec, A.; Rodriguez, R.; Brown, J. D.; Cajica, J.; Pan, P. S.; Parry, E.; Carroll, C. L.; Medina, I.; Corral, R.; Lapera, S.; Otrubova, K.; Pan, C. M.; McGuire, K. L.; McAlpine, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 5625–5631.
(33) Ujiki, M. B.; Milam, B.; Ding, X. Z.; Roginsky, A. B.; Salabat, M. R.; Talamonti, M. S.; Bell, R. H.; Gu, W. X.; Silverman, R. B.; Adrian, T. E. *Biochem. Biophys. Res. Commun.* **2006**, *340*, 1224–1228.
(34) Otrubova, K.; Lushington, G.; Velde, D. V.; McGuire, K. L.; McAlpine, S. R. *J. Med. Chem.* **2008**, *51*, 530–544.
(35) Davis, M. R.; Styers, T. J.; Rodriguez, R. A.; Pan, P. S.; Vasko, R. C.; McAlpine, S. R. *Org. Lett.* **2008**, *10*, 177–180.
(36) Capon, R. J.; Ratnayake, R.; Stewart, M.; Lacey, E.; Tennant, S.; Gill, J. H. *Org. Biomol. Chem.* **2005**, *3*, 123–129.
(37) Cole, R. J., Schweikert, M. A. *Handbook of Secondary Fungal Metabolites*; Academic Press: San Diego, 2003; Vol. 1.
(38) Hodge, R. P.; Harris, C. M.; Harris, T. M. *J. Nat. Prod.* **1988**, *51*, 66–73.

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