Spiro Fused Diterpene—Indole Alkaloids from a Creek-Bottom-Derived Aspergillus terreus

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Four metabolites, teraspiridoles A-D (2-5), formed from the merger of a diterpene and modified indole scaffold were obtained from an *Aspergillus terreus* isolate. The structures and absolute configurations of these natural products were established using NMR, mass spectrometry, Marfey's method, VCD, and ECD data. Teraspiridole B (3) exhibited weak inhibition of planaria regeneration/survival.

Natural products containing a tricyclic 9,9a-dihydro-1*H*-imidazo[1,2-*a*]indol-3(2*H*)-one scaffold (1) (Figure 1) are quite rare with most examples originating from just a few fungal genera (e.g., *Penicillium* and *Aspergillus*).¹ In most cases, 1 is linked to substituted quinazolinones as exemplified by the tremorgenic mycotoxins fumiquinazoline A,² tryptoquivaline,³ and others⁴ (Figure 1). Scaffold 1 also cryptically appears as part of a tetracyclic subunit in the sponge-derived kapakahines,⁵ as well as the fungal-derived metabolite chaetominine⁶ (Figure 1). The virtual absence of **1** among other groups of natural products is surprising given the modular architecture of secondary-metabolite biosynthetic pathways¹ and the frequency with which genes from divergent clusters are swapped/duplicated and integrated into new pathways.⁷ Incorporating compounds containing under-represented natural-product scaffolds like **1** into natural-product libraries is important for enhancing the likelihood of discovering new bioactive molecules.

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While preparing natural products for our pure compound library, we encountered a biosynthetically talented *Aspergillus terreus* isolate. This specimen was obtained from the sediment of a small creek bed (Cedar Creek) near Buffalo Valley, Oklahoma. After culturing the fungus on Cheerios breakfast cereal,⁸ LC-PDA ESIMS revealed several metabolites with molecular weights between 560 and 580 Da (Supporting Information (SI), Figure S1). Our investigation of each compound's ESIMS and UV data revealed that these metabolites were not attributable to any substance previously reported from *A. terreus*. However,

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virtually all other compounds were readily dereplicated as metabolites common for A. terreus, thus supporting the ITS-based taxonomic assignment of this isolate. Massguided purification of the unknown metabolites led to the identification of teraspiridoles A-D (2-5) (Figure 2), which constitute a new class of spiro-linked diterpeneindole alkaloids.



Figure 1. Tricyclic 9,9a-dihydro-1H-imidazo[1,2-a]indol-3(2H)one scaffold (1) and selected metabolites containing 1; note that none of the reported compounds containing scaffold 1 are linked to a diterpene.



Figure 2. Teraspiridoles A–D (2–5) isolated from A. terreus.

Teraspiridole A $(2)^9$ was obtained as a pale yellow solid. Its molecular formula was determined to be $C_{33}H_{42}N_2O_6$ based on HRESIMS, which indicated 14 degrees of unsaturation. Analysis of the ¹³C NMR and HSQC data showed that 2 contained 10 quaternary carbons (3 carbonyls), 12 methines, 4 methylenes, and 7 methyls (Table 1). The HMBC spectrum showed correlations from H-1 to C-3/5/10/18, as well as from H-5/20/21 to C-4. Together, these data suggested the presence of an α,β -unsaturated ε -caprolactone subunit similar to that observed in the planar structure of toonayunnanin C.¹⁰ Further examination of the HMBC (correlations from H-18 to C-10/9/5; H-19 to C-7/8/14/9; H-17 to C-12/13/14; H-33/7 to C-32) and COSY (correlations between H-1 and H-2, H-5 and H-6, H-6 and H-7, H-9 and H-11, H-11 and H-12, H-14 and H-15) data provided evidence that the α,β -unsaturated ε -caprolactone was part of a tetracyclic diterpene fragment (Fragment A, Figure 3).

Table 1.	¹³ C NMR	Data (100	MHz) of	Teraspirid	loles A-	D
(2 - 5) in	CD ₃ OD					

	2	3	4	5
position	$\delta_{ m C}$, mult	$\delta_{ m C}$, mult	$\delta_{ m C}$, mult	δ_{C} , mult
1	$158.3\mathrm{CH}$	$158.2~\mathrm{CH}$	$38.8 \mathrm{CH}_2$	$38.8 \mathrm{CH}_2$
2	$120.2~\mathrm{CH}$	$120.2~\mathrm{CH}$	$31.5~\mathrm{CH}_2$	$31.5~\mathrm{CH}_2$
3	$168.8~\mathrm{C}$	$168.7~\mathrm{C}$	$175.9~\mathrm{C}$	$175.9~\mathrm{C}$
4	$85.4~\mathrm{C}$	$85.4~\mathrm{C}$	$85.6~\mathrm{C}$	$85.6~\mathrm{C}$
5	$53.7~\mathrm{CH}$	$53.7 \mathrm{CH}$	$50.1 \mathrm{CH}$	$50.1~\mathrm{CH}$
6	$27.4~\mathrm{CH}_2$	$27.3 \ \mathrm{CH}_2$	$27.6 \mathrm{CH}_2$	$27.5 \mathrm{CH}_2$
7	$80.0 \mathrm{CH}$	$80.1 \mathrm{CH}$	$80.4~\mathrm{CH}$	$80.4~\mathrm{CH}$
8	40.8 C	$40.7~\mathrm{C}$	$40.7~\mathrm{C}$	40.6 C
9	$55.9 \mathrm{CH}$	$55.9~\mathrm{CH}$	$59.1 \mathrm{CH}$	$59.0~\mathrm{CH}$
10	$42.8~\mathrm{C}$	$42.8~\mathrm{C}$	38.6 C	$38.6~\mathrm{C}$
11	$20.9 \mathrm{CH}_2$	$21.0~\mathrm{CH}_2$	$19.7 \ \mathrm{CH}_2$	$19.9~{ m CH}_2$
12	$38.9 \mathrm{CH}_2$	$39.1 \ \mathrm{CH}_2$	$39.1 \mathrm{CH}_2$	$39.3~\mathrm{CH}_2$
13	$82.3~\mathrm{C}$	$82.4~\mathrm{C}$	82.1 C	$82.3~\mathrm{C}$
14	$58.7~\mathrm{CH}$	$58.2 \mathrm{CH}$	$58.8~\mathrm{CH}$	$58.3\mathrm{CH}$
15	$32.2\mathrm{CH}_2$	$33.0 \ \mathrm{CH}_2$	$32.3 \mathrm{CH}_2$	$33.1~\mathrm{CH}_2$
16	89.0 C	89.3 C	88.9 C	$89.2~\mathrm{C}$
17	$22.7 \mathrm{CH}_3$	$23.1~\mathrm{CH}_3$	$22.6 \mathrm{CH}_3$	$22.9\mathrm{CH}_3$
18	$13.8~\mathrm{CH}_3$	$13.8~\mathrm{CH}_3$	$16.5~\mathrm{CH}_3$	$16.4~\mathrm{CH}_3$
19	$11.4 \mathrm{CH}_3$	$11.6 \mathrm{CH}_3$	$11.1 \mathrm{CH}_3$	$11.2~\mathrm{CH}_3$
20	$25.9~\mathrm{CH}_3$	$25.9~\mathrm{CH}_3$	$25.8~\mathrm{CH}_3$	$25.8\mathrm{CH}_3$
21	$31.2\mathrm{CH}_3$	$31.1~\mathrm{CH}_3$	$29.4 \mathrm{CH}_3$	$29.4~\mathrm{CH}_3$
22	$84.9 \mathrm{CH}$	$86.1 \mathrm{CH}$	84.9 CH	$86.1\mathrm{CH}$
23	$59.2\mathrm{CH}$	$45.4~\mathrm{C}$	$59.2~\mathrm{CH}$	$45.4~\mathrm{C}$
24	$173.0~\mathrm{C}$	177.0 C	$173.0~\mathrm{C}$	$177.0~\mathrm{C}$
25	$136.2~\mathrm{C}$	$137.6~\mathrm{C}$	$136.2~\mathrm{C}$	$137.6~\mathrm{C}$
26	$114.6~\mathrm{CH}$	$116.0~\mathrm{CH}$	$114.6~\mathrm{CH}$	$116.0~\mathrm{CH}$
27	$128.8\mathrm{CH}$	$128.7~\mathrm{CH}$	$128.8\mathrm{CH}$	$128.7~\mathrm{CH}$
28	$125.6\mathrm{CH}$	$126.0 \mathrm{CH}$	$125.5 \mathrm{CH}$	$125.9~\mathrm{CH}$
29	$124.1\mathrm{CH}$	$123.7~\mathrm{CH}$	$123.9 \mathrm{CH}$	$123.6~\mathrm{CH}$
30	$140.3~\mathrm{C}$	$139.7~\mathrm{C}$	$140.4~\mathrm{C}$	$139.8~\mathrm{C}$
31	$17.6 \mathrm{CH}_3$	$11.4~\mathrm{CH}_2$	$17.6 \mathrm{CH}_3$	$11.5~\mathrm{CH}_2$
31a		$16.2~\mathrm{CH}_2$		$16.1 \mathrm{CH}_2$
32	$170.8~\mathrm{C}$	$170.7~\mathrm{C}$	170.9 C	$170.8~\mathrm{C}$
33	$19.9~{ m CH}_3$	$20.0~{ m CH}_3$	$19.9~{ m CH}_3$	$20.0~{ m CH}_3$

The remaining atoms in 2 were arranged similar to those observed in metabolites bearing a methylated 1 [HMBC correlations from H-22/29 to C-16, from H-26 to C-30, from H-22 to C-24 (in CDCl₃), from H-23 to C-24, and from H-31 to C-24]. Further evidence for the methyl group was deduced from the splitting pattern of the C-23 methine proton (δ 4.27), which appear as a double quartet (1.1 and 6.8 Hz) in CDCl₃ (SI, Table S1). With fragments A and B deciphered, we noted that these assignments resulted in a single quaternary carbon at δ 89.0 (C-16) that we had incorporated into both partial structures. Since our analysis had already accounted for all the atoms in the proposed molecular formula, we interpreted this to mean

⁽⁹⁾ Teraspiridole A (2): pale yellow solid; $[\alpha]^{24}_{D}$ +68.0 (*c* 0.2, CHCl₃); (9) Teraspiridole A (2): pare yenow solid; [a] $_{\rm D}$ +06.0 (c 0.2, C11C13), UV (MeOH) $\lambda_{\rm max}$ (log ε) 210 (4.38), 252 (3.99); IR (film) $\nu_{\rm max}$ 3340, 2984, 2933, 1706, 1483, 1387, 1293, 1242, 1183, 1155, 1115, 1078, 1015, 980, 755 cm⁻¹; ¹³C and ¹H NMR data, see Tables 1 and 2; HRESIMS m/z563.3117, [M + H]⁺ (calcd for C₃₃H₄₃N₂O₆, 563.3121). (10) Liu, J.-Q.; Wang, C.-F.; Li, Y.; Chen, J.-C.; Zhou, L.; Qiu, M = *Device homistry* 2012, 76, 141–140

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	fable 2. ¹ H NMR Data ((500 MHz) of Teras	spiridoles A–D	(2-5) in CD ₃ OD
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	2	3	4	5
position	$\delta_{ m H}$, mult (J in Hz)	$\delta_{\mathrm{H}}, \mathrm{mult}\left(J \mathrm{~in~Hz}\right)$	$\delta_{\rm H}$, mult (J in Hz)	$\delta_{\rm H}$, mult (J in Hz)
1	6.77 d (12.1)	6.76 d (12.1)	1.85 m; 1.70 m	1.82 m; 1.70 m
2	5.92 d (12.1)	5.91 d (12.1)	2.80 ddd (4.8, 13.2, 14.0)	2.79 m
			2.54 ddd (4.2, 4.3, 14.0)	2.54 ddd (3.9, 4.4, 14.7)
5	2.36 dd (3.5, 13.3)	2.33 dd (3.6, 13.3)	2.23 dd (2.6, 12.7)	2.21 dd (2.7, 13.0)
6	1.75 m; 1.89 m	1.75 m; 1.86 m	1.85 m; 1.66 m	1.85 m; 1.70 m
7	4.60 dd (4.3, 11.2)	4.55 dd (4.4, 11.4)	4.60 dd (4.0,11.5)	4.56 dd (3.9, 11.2)
9	1.52 br d (11.2)	1.50 br d (11.4)	1.35 br d (10.0)	1.34 m
11	1.95 m; 1.82 m	1.94 m; 1.75 m	1.85 m; 1.66 m	1.85 m; 1.70 m
12	1.75 m; 2.04 br d (11.4)	1.75 m; 2.04 m	2.00 m; 1.66 m	2.00 m; 1.70 m
14	2.08 dd (6.6, 13.5)	2.04 m	2.00 m	2.00 m
15	2.99 dd (12.7, 13.5)	2.80 dd (11.9, 13.6)	2.99 dd (12.6, 13.1)	2.80 dd (13.0, 13.0)
	1.18 dd (6.6, 12.7)	1.19 m	1.19 dd (6.5, 12.6)	1.19 m
17	1.29 s	$1.33 \mathrm{~s}$	$1.27 \mathrm{~s}$	$1.31 \mathrm{s}$
18	$1.27~\mathrm{s}$	1.26 s	1.11 s	$1.10 \mathrm{~s}$
19	1.14 s	$1.12~\mathrm{s}$	1.11 s	$1.10 \mathrm{~s}$
20	1.46 s	$1.44 \mathrm{~s}$	$1.42 \mathrm{~s}$	$1.41 \mathrm{s}$
21	1.43 s	$1.41 \mathrm{s}$	$1.51~\mathrm{s}$	$1.50 \mathrm{~s}$
22	5.43 s	5.49 s	$5.43~\mathrm{s}$	$5.49~\mathrm{s}$
23	4.24 br q (6.8)		4.24 q (6.8)	
26	7.43 br d (7.9)	7.35 br d (7.9)	7.44 d (7.8)	7.36 br d (7.8)
27	7.31 br dd (7.6, 7.9)	7.31 br dd (7.5, 7.9)	7.32 dd (7.6, 7.8)	7.33 br dd (7.2, 7.8)
28	7.17 br dd (7.6, 8.0)	7.21 ddd (7.4, 7.5, 1.5)	7.18 dd (7.5, 7.6)	7.22 br dd (7.2, 7.6)
29	7.41 br d (8.0)	7.39 br d (7.4)	7.39 d (7.5)	7.38 br d (7.6)
31	1.32 d (6.8)	1.31 m; 1.17 m	1.32 d (6.8)	1.34 m; 1.19 m
31a		1.13 m; 0.87 m		1.14 m; 0.88 m
33	1.89 s	1.87 s	1.89 s	1.87 s

that C-16 served as a spiro junction between fragments A and B.

The relative configuration of **2** was determined by ROESY experiment (Figure 3). Correlations between H-20 and H-18, H-18 and H-19, and H-19 and H-17 indicated that these four methyl groups were on the same face of the diterpene portion of **2**. Similarly, correlations between H-21 and H-5, H-5 and H-9, H-9 and H-7, and H-9 and H-14 revealed that these protons were on the opposite face of the molecule. Additional correlations between H-14 and H-29, H-17 and H-22, and H-22 and H-23 were essential for assigning the relative configurations of C-16, C-22, and C-23 as S^*, S^*, S^* , respectively.

The absolute configuration of **2** was established using a combination of Marfey's method¹¹ and comparisons of ECD and VCD experimental data with theoretical spectra. Acid hydrolysis followed by workup with L-FDAA provided for the identification of L-Ala in **2**, which substantiated an *S* configuration for C-23 (SI, Figure S2). In addition, *ab initio* ECD and VCD calculations were carried out to generate theoretical spectra for comparisons with experimentally derived data. Density functional theory (DFT) calculations performed at the B3LYP/6-31+G** level provided ECD and VCD spectra for the two lowest-energy conformers of **2**. The ECD and VCD spectra of the two conformers were averaged using Boltzmann statistical weighing to obtain the theoretical spectra (Figure 4A and C). Both of the predicted



Figure 3. Key COSY and HMBC correlations for partial structures A and B, and ROESY correlations for 2.

ECD and VCD spectra matched well with the experimental data enabling us to assign the absolute configuration of **2** as *5R*,*7S*,*8R*,*9R*,10*R*,13*S*,14*S*,16*S*,22*S*,23*S*.

A molecular formula of $C_{34}H_{42}N_2O_6$ was established for teraspiridole B (3)¹² based on HRESIMS. This required one additional degree of unsaturation compared to 2. The ¹³C NMR and HSQC data provided evidence for two new methylenes (δ_C 11.4 and 16.2) and one new quaternary carbon (δ_C 45.4) in 3 verses 2 (Table 1). In addition, we noted that one methyl and one methine present in 2 were absent in compound 3. All the other carbon and proton spins (Tables 1 and 2) remained relatively unchanged.

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⁽¹²⁾ Teraspiridole B (3): pale yellow solid; $[\alpha]^{24}{}_{\rm D}$ +28.0 (*c* 0.2, CHCl₃); UV (MeOH) $\lambda_{\rm max}$ (log ε) 208 (4.54), 252 (3.92); IR (film) $\nu_{\rm max}$ 3327, 2985, 2944, 1713, 1478, 1386, 1292, 1243, 1186, 1155, 1115, 1026, 1016, 981, 756 cm⁻¹; ¹³C and ¹H NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 575.3116, [M + H]⁺ (calcd for C₃₄H₄₃N₂O₆, 575.312).

In light of the correlation data from COSY (between H-31 and H-31a) and HMBC (H-31/31a to C-23/24, and from N*H* to C-23) experiments (SI, Figure S3), we deduced that the L-Ala methyl group in **2** was replaced by a cyclopropane in **3**. ROESY correlations confirmed that **2** and **3** shared the same relative configuration (SI, Figure S3). Comparisons of the ECD and VCD data of **3** with the calculated spectra (Figure 4, B and D) provided the absolute configuration of **3** (5R,7S,8R,9R,10R,13S,14S,16S,22S).



Figure 4. Calculated and experimental ECD data for **2**(A) and **3** (B) and VCD data for **2**(C) and **3** (D).

Two structurally related metabolites, teraspiridoles C and D (4 and 5, respectively),¹³ were also obtained from the *A. terreus* extract. Compounds 4 and 5 were determined to have molecular formulas consisting of $C_{33}H_{44}N_2O_6$ and

C₃₄H₄₄N₂O₆, respectively, based on HRESIMS. Both compounds exhibited ¹³C NMR resonances similar to 2 and 3 (Table 1) with the exceptions being that, in each instance, the C-1 and C-2 olefinic methines were replaced by methylenes. Further evidence for the increased saturation at these positions was afforded by ¹H NMR, which revealed new proton resonances at δ 1.70, 1.85, 2.54, and 2.80, as well as δ 1.70, 1.82, 2.54, and 2.79 for **4** and **5**, respectively. Confirmation of the planar structures of 4 and 5 was provided by analysis of COSY and HMBC correlation data (SI, Figure S3) that demonstrated these compounds shared the same atom arrangements as their unsaturated analogs. Additionally, ROESY correlation data (SI, Figure S3) indicated that 4 and 5 possessed the same relative configurations as 2 and 3, respectively. Finally, quantum chemical ECD calculations (SI, Figure S4) enabled us to independently establish the absolute configurations of 4 (5R.7S,8R.9R,10R,13S,14S, 16*S*,22*S*,23*S*) and **5** (5*R*,7*S*,8*R*,9*R*,10*R*,13*S*,14*S*,16*S*,22*S*).

Compounds 2–5 were evaluated in a panel of assays (cancer cell cytotoxicity,¹⁴ antibiosis against pathogenic bacteria and fungi,¹⁵ disruption of *Candida* biofilm formation,¹⁶ and inhibition of planarian regeneration and survival¹⁷). Only compound **3** exhibited weak activity in the planaria assay (SI, Figure S5). At a concentration of 75 μ M, ~80% of the planarian segments did not regenerate, but instead disintegrated within 48 h. At 50 and 25 μ M, no degeneration of the planarian fragments occurred; however, the surviving body segments appeared to regenerate more slowly, with the planaria exhibiting sluggish swimming motions.

There are relatively few examples of the incorporation of 1 into natural products with 2-5 representing the first examples of this scaffold being linked to a terpenoid backbone. Although the compounds exhibited limited biological activities, we find their novel structural features highly valuable for adding a new element of chemical diversification to our pure natural product library.

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Supporting Information Available. NMR (¹H and ¹³C NMR, HSQC, COSY, HMBC, and ROESY) and HRE-SIMS data for compounds **2–5**. Fungal identification and associated data. This information is available free of charge via the Internet at http://pubs.acs.org.

⁽¹³⁾ Teraspiridole C (4): pale yellow solid; $[\alpha]^{24}_{D} + 62.8$ (*c* 0.175, CHCl₃); UV (MeOH) λ_{max} (log ε) 210 (4.34), 252 (4.01); IR (film) ν_{max} 3346, 2978, 2929, 1710, 1604, 1482, 1391, 1293, 1244, 1183, 1145, 1064, 1014, 979, 756 cm⁻¹; ¹³C and ¹H NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 565.3278). Teraspiridole D (5): pale yellow solid; $[\alpha]^{24}_{D} + 14.4$ (*c* 0.125, CHCl₃); UV (MeOH) λ_{max} (log ε) 210 (4.34), 252 (3.97); IR (film) ν_{max} 3345, 2982, 2926, 1722, 1605, 1477, 1373, 1305, 1293, 1245, 1155, 1145, 1065, 979, 756 cm⁻¹; ¹³C and ¹H NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 577.3272, [M + H]⁺ (calcd for C₃₄H₄₅N₂O₆, 577.3278).

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