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Versicoamides F−H, Prenylated Indole Alkaloids from *Aspergillus tennesseensis*

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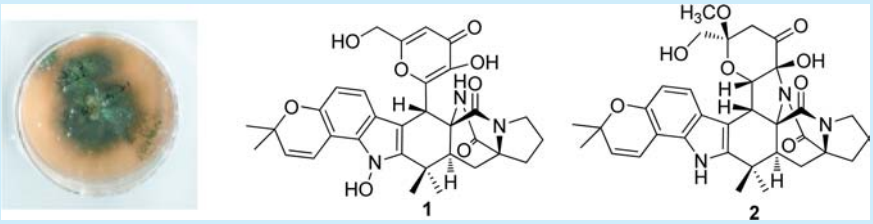
Supporting Information



ABSTRACT: Three highly modified indole alkaloids, versicoamides F−H (**1**−**3**), together with seven known alkaloids (**4**−**10**) were isolated from the fungus *Aspergillus tennesseensis*. The structures of new compounds were determined by analysis of the NMR and MS spectroscopic data. The absolute configurations of **1** and **2** were assigned by single-crystal X-ray diffraction experiments. Compounds **1** and **2** showed weak antiproliferative activity against the H460 cell line. Compounds **1**−**3** represent a new class of natural product hybrids with new chemical skeletons.

Fungi are well-known for their ability to synthesize secondary metabolites with novel skeletons and diverse biological effects.[1,2] *Aspergillus* sp. and *Penicillium* sp. comprise the most chemically investigated fungal groups with the identification of hundreds of bioactive secondary metabolites. To date, a wide structural array of prenylated indole alkaloids with interesting bioactivities have been reported from the genus of *Aspergillus* and *Penicillium*. Examples include notoamides and stephacidins from the marine derived *A. protuberus*,[3a−e] taichunamides and okaramines from *A. taichungensis*,[3f,g] spirotryprostatins from *A. fumigatus*,[3h] penioxamides from *P. oxalicum*,[3i] and mangrovamides from *Penicillium* sp.[3j]

In our search for new bioactive alkaloids from fungi, a strain of *A. tennesseensis* separated from the surface of an unidentified plant leaf was found to produce diverse alkaloids, which prompted further chemical investigation. In this work, we reported the isolation, structural determination with the absolute stereochemistry, and bioactivity evaluation of prenylated indole alkaloids from *A. tennesseensis*.

The strain of *A. tennesseensis* was fermented on a solid-substrate culture. The EtOAc extract of its solid culture was subjected to silica gel column chromatography and ODS column chromatography. The reversed-phase high performance liquid chromatography (HPLC) on the fractions containing alkaloids yields prenylated alkaloids (**1**−**10**, Figure 1). The structures of known compounds stephacidin A (**4**),[3e] notoamide C (**5**),[3c] notoamide E (**6**),[3d] notoamide O (**7**),[3d] notoamide Q (**8**),[5] dehydronotoamide E (**9**),[6] and deoxybrevianamide E (**10**)[4] were determined by NMR data analysis and comparison with the literature data.

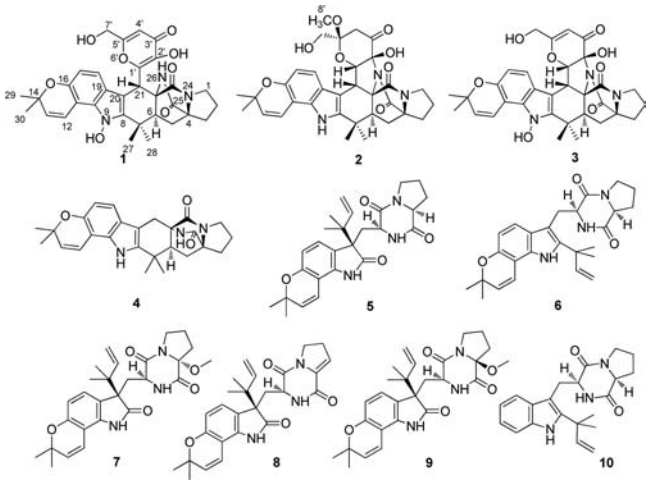


Figure 1. Structures of compounds **1**−**10**.

Versicoamide F (**1**) was determined to have a molecular formula of $C_{32}H_{33}N_3O_8$ (18 degrees of unsaturation) on the basis of HRESIMS data at *m*/*z* $[M + H]^+$ 588.2349. The ^1H and ^{13}C NMR data of **1** (Table 1) showed similarity with those

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

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	43.8	3.26 dt (12.8, 6.6) 3.35 m[b]	43.5	3.33 m	43.6	3.36 m
2	24.0	1.83 m 1.98 m	24.3	1.82 m 2.02 dt (12.4, 6.0)	24.3	1.81 m 2.02 dt (12.1, 6.1)
3	28.7	1.87 m 2.53 m	28.7	1.90 m 2.50 m	28.6	1.86 m 2.49 m
4	65.7		67.7		67.8	
5	29.4	2.08 d (7.2)	30.0	1.90 m 2.21 dd (13.2, 10.3)	29.5	1.92 m 2.10 dd (13.4, 10.3)
6	46.7	3.09 s	47.1	3.09 dd (10.3, 6.4)	48.1	2.63 dd (10.3, 6.5)
7	35.0		34.1		34.8	
8	138.3		142.6		140.5	
9(N−H)				10.58 s		
10	130.1		132.8		130.1	
11	104.5		105.0		104.6	
12	118.0	7.20 d (9.9)	118.0	6.96 d (9.8)	118.0	7.24 d (9.9)
13	128.5	5.68 d (9.9)	129.0	5.75 d (9.8)	128.6	5.71 d (9.9)
14	74.9		75.1		74.9	
16	148.7		147.5		148.7	
17	109.5	6.43 d (8.4)	109.0	6.47 d (8.3)	109.5	6.53 d (8.4)
18	117.9	7.10 d (8.4)	117.4	7.21 d (8.3)	118.5	7.33 d (8.4)
19	116.1		121.6		116.8	
20	101.3		102.1		97.9	
21	30.9	5.21 s	36.2	4.23 d (4.1)	36.4	4.34 d (3.9)
22	61.3		68.7		67.8	
23	167.1		167.8		167.2	
25	173.3		168.4		167.3	
26(N−H)		8.02 s				
27	19.8	1.12 s	20.9	1.03 s	19.1	1.10 s
28	27.5	1.46 s	27.4	1.29 s	26.4	1.40 s
29	26.9	1.35 s	27.0	1.37 s	26.8	1.40 s
30	27.1	1.37 s	27.6	1.40 s	27.1	1.40 s
1′	146.5		81.5	4.36 d (4.1)	85.9	5.12 d (3.9)
2′	143.4		87.3		83.5	
3′	173.8		198.7		185.5	
4′	108.4	6.18 s	44.6	2.47 d (13.4) 2.83 d (13.4)	97.6	5.49 s
5′	167.4		104.0		174.5	
7′	59.4	4.06 d (16.1) 4.17 d (16.1)	61.1	3.21 m	59.9	3.69 dd (16.6,6.0) 3.80 dd (16.6,6.0)
8′			47.9	2.92 s		
9-OH		10.87 s				10.91 s
2′-OH		9.14 s		6.90 s		7.12 s
7′-OH		5.53 m		4.85 t (5.8)		5.45 t (6.0)

[a]Recorded for ^{1}H NMR and 125 MHz for ^{13}C NMR at 500 MHz in DMSO-d_6, δ_H in ppm, J in Hz. [b]"m" means multiplet or overlapped with other signals.

of stephacidin A (**4**), except for the presence of a hydroxylmethyl group [δ_H 4.06 (d, J = 16.1 Hz, H_2-7′), 4.17 (d, J = 16.1 Hz, H_2-7′); δ_C 59.4 (C-7′)], three olefinic quaternary carbons at δ_C 146.5 (C-1′), 143.4 (C-2′), and 167.4 (C-5′), one olefinic methine [δ_H 6.18 (s, H-4′), δ_C 108.4 (C-4′)], and an extra ketone at δ_C 173.8 (C-3′) in **1**. Considering the chemical shifts of olefinic quaternary carbons, as well as the requirement of unsaturation, combined with the $^{1}H-^{1}H$ COSY correlation between HO-7′ (δ_H 5.53) and H_2-7′, the HMBC correlations of HO-7′ with C-5′ and H-4′ with C-2′, C-3′, C-5′, and C-7′, the substructure moiety of the 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one was assigned temporarily (Figure S1). The connection of this substructure with C-21 was confirmed by the HMBC correlation of H-21 (δ_H 5.21) with C-1′ (δ_C 146.5). Besides the signal of HO-7′, three exchangeable hydrogen signals were observed in the ^{1}H NMR spectrum of **1**. The exchangeable hydrogen signal at δ_H 8.02 (s) was assigned for HN-26 by its HMBC correlations with C-4, C-5, C-22, C-23, and C-25. The signal at δ_H 10.87 was deduced to be HO-N-9 by comparing the NMR data between **1** and *N*-hydroxy-6-*epi*-stephacidin A.[7] The left exchangeable proton signal (δ_H 9.14) was attached at C-2′ to satisfy the chemical shift of C-2′. The NOE correlation of H-6 (δ_H 3.09) with H_3-28 (δ_H 1.46) placed them on the same side of the rings. The NOE

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correlation of H-21 (δ_H 5.21) with H_3-27 (δ_H 1.12) indicated that H-21 and H_3-27 were on the opposite side (Figure S2). The planar structure and the absolute configuration of **1** were finally confirmed by a single-crystal X-ray crystallography analysis (Figure 2). Thus, the absolute configuration of 4*S*, 6*S*, 21*S*, 22*R* was determined on the basis of the Flack parameter [0.1 (3)] obtained by Cu Kα radiation.

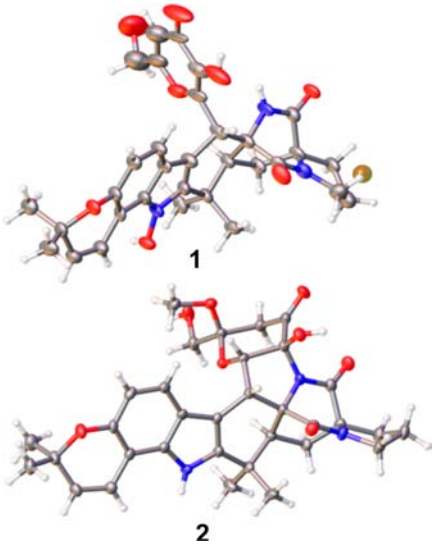
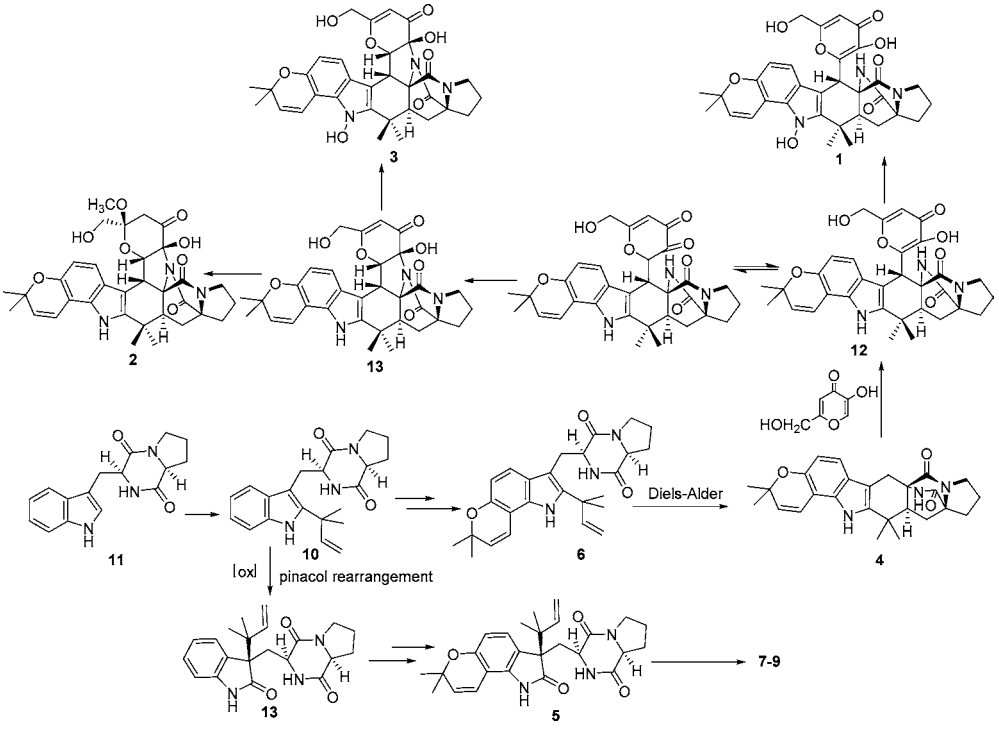


Figure 2. X-ray crystallographic structures of **1** and **2**.

The molecular formula of compound versicoamide G (**2**) was established as $C_{33}H_{37}N_3O_8$ with 17 degrees of unsaturation on the basis of HRESIMS and NMR data. The ^{1}H and ^{13}C NMR of **2** resembled with those of **1**, except for the absence of two pairs of olefinic carbons at δ_C 146.5 (C-1′) and 143.4 (C-2′), 108.4 (C-4′), and 167.4 (C-5′) in **1** and the presence of an additional methoxy group [δ_H 2.92 (s, H_3-8′); δ_C 47.9 (C-8′)], an extra methylene [δ_H 2.47 (d, J = 13.4 Hz, H_2-4′), 2.83 (d, J = 13.4 Hz, H_2-4′); δ_C 44.6 (C-4′)], an oxygenated methine [(δ_H 4.36 (d, J = 4.1 Hz, H-1′); δ_C 81.5 (C-1′)], and two more quaternary carbon at δ_C 87.3 (C-2′) and 104.0 (C-5′) in **2**. HMBC correlations from H-1′ to C-21, C-22, and C-2′; HO-2′ to C-1′, C-2′, and C-3′; H_2-4′ to C-2′, C-3′, and C-5′; H_2-7′ to C-4′ and C-5′; HO-7′ to C-5′ and C-7′; and H_3-8′ to C-5′ confirmed the structure changes at the kojic acid moiety (Figure S1). The exchangeable hydrogen signal at δ_H 10.58 showed HMBC correlations with C-8, C-10, C-19, and C-20, which confirmed the presence of the HN-9 group. Due to the absence of the exchangeable proton signal at δ_H 8.02 (HN-26) in **2**, a C−N bond between C-2′ and N-26 was temporarily deduced to satisfy the requirement of unsaturation degrees. The NOE correlations of HO-2′ with H-21 and H-1′ as well as H_3-8′ with H-1′ and H-4′β (δ_H 2.83) were observed in its ROESY spectrum. Thus, H-21, HO-2′, H-1′, H-8′, and H-4′β were located on the same side (Figure S2). The ^{1}H and ^{13}C NMR data of **2** were fully assigned by detailed interpretation of ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Table 1). The single-crystal X-ray diffraction experiment confirmed the structure of **2** (Figure 2). The absolute configuration of **2** was determined to be 4*S*, 6*S*, 21*S*, 22*R*, 1′*S*, 2′*S*, 5′*S* on the basis of the Flack parameter [−0.04 (12)].

Versicoamide H (**3**) possessed the molecular formula of $C_{32}H_{33}N_3O_8$ (18 degrees of unsaturation), as determined by HRESIMS at m/z [M + H]$^+$ 588.2345. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **3** were very similar to those of **2** except for the loss of the methoxyl group [δ_H 2.92 (s, H_3-8′); δ_C 47.9 (C-8′)] and the presence of a double bond between C-4′ and C-5′, which was further confirmed by HMBC correlations from H-1′ to C-22; HO-2′ to C-1′, C-2′, and C-3′; H-4′ to C-2′, C-5′, and C-7′; H-7′ to C-4′ and C-5′; and

Scheme 1. Plausible Biosynthetic Pathway of Compounds 1−10



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HO-7′ to C-5′ and C-7′ (Figure S1). The exchangeable hydrogen signal at δ_H10.92 was assigned for HO-N-9 by considering the molecular formula and referring to the similar feature of **1**. The NOE correlations of HO-2′ (δ_H 7.12) with H-21 (δ_H 4.34) and H-1′ (δ_H 5.12) assigned β configurations of H-21, H-1′, and HO-2′ (Figure S2). The CD spectra of **3** showed similar positive Cotton effects at 225 nm[3f,8] as observed in **1**, **2**, and **4**, which together with the same biogenetic origin helped us deduce the absolute configuration of **3** as 4*S*, 6*S*, 21*S*, 22*R*, 1′*S*, 2′*S*.

This is the first report of secondary metabolites from *A. tennesseensis*. A proposed biogenetic pathway for the assembly of **1**−**10** in this fungus is shown in Scheme 1. Compound **11** derived from the condensation of γ-methyl proline and L-tryptophan was converted into **10** by reverse prenylation. Subsequent oxidiation and pinacol-like rearrangement transformed **10** into **13**. Compound **5** was biosynthesized from **13** by oxidation and prenylation. Compounds **7**−**9** were formed from **5** by oxidation at C-4 or dehydration at C-3 and C-4. Compound **6** derived from **10** was further converted into **4** by the intra-Diels−Alder addition reaction. The key intermidate (**12**) was derived from **4** by the reaction with a kojic acid unit. Compound **12** was transformed into **1** by hydroxylation at N-9. The keto tautomeric isomer of **12** was followed by the hemiaminal reaction to form the N−C bond between NH-26 and C-2′ of the kojic acid unit. Compounds **2** and **3** were synthesized from **13** by the hydroxylation at N-9 and the addition with methanol at C-4′ and C-5′, respectively.

Prenylated indole alkaloids have been demonstrated to possess diverse biological activities, such as antinematodal, antitumor, anthelmintic, calmodulin inhibitory, and antibacterial bioactivities.[9] In this work, all of the isolates were evaluated for their cytotoxic activities against a small panel of tumor cell lines including A549, K562, ASPC, and H460 using the CCK8 method. Compounds **1** and **2** exhibited weak cytotoxicity against H460 cells with an IC_{50} of 83.4 and 95.5 μM, respectively. None of them showed cytotoxicities against A549, K562, and ASPC at the concentration of 200 μM.

In conclusion, three highly modified prenylated indole alkaloids versicoamides F−H (**1**−**3**) were isolated from *A. tennes seensis*, a strain separated from an unidentified plant leaf. Compounds **1**−**3** are new natural products hybrids incorporating a kojic acid moiety and a prenylated indole alkaloid with the bicyclo[2.2.2]diazaoctane core. Compound **1** is a new prenylated indole alkaloid with a kojic acid unit attached at C-21 of stephacidin A. Compounds **2** and **3** possess an unusual ring system of methanoindolizino[6,7-*h*]pyrano[3,2-*a*]pyrano-[2′,3′:4,5]pyrrolo[3,2-*g*]carbazol. The structures of **1**−**3** represent an unusual branch point for the modification of other members in the family of prenylated indole alkaloids in the biogenetic pathway. The discovery of new prenylated indole alkaloids **1**−**3** provides a new strategy for organic chemists in the chemical modification of this unique group of alkaloids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00145.

Crystallographic data for **1** and **2** (CIF)

Full details of experiments, the ^{1}H and ^{13}C NMR spectra of **1**−**3** (PDF)

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Notes

The authors declare no competing financial interest.

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