Taichunamides: Prenylated Indole Alkaloids from Aspergillus taichungensis (IBT 19404)

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Abstract: Seven new prenylated indole alkaloids, taichunamides A–G, were isolated from the fungus Aspergillus taichungensis (IBT 19404). Taichunamides A and B contained an azetidine and 4-pyridone units, respectively, and are likely biosynthesized from notoamide S via (+)-6-epi-stephacidin A. Taichunamides C and D contain endoperoxide and methylsulfonyl units, respectively. This fungus produced indole alkaloids containing an anti-bicyclo[2.2.2]diazaoctane core, whereas A. protuberus and A. amoenus produced congeners with a syn-bicyclo[2.2.2]diazaoctane core. Plausible biosynthetic pathways to access these cores within the three species likely arise from an intramolecular hetero Diels–Alder reaction.

We have been studying the structures, synthesis, and biosynthesis of the notoamides and stephacidins from the fungus of the genus Aspergillus, and found that the marine-derived A. protuberus (MF297-2) produced (+)-stephacidin A [(+)-16] and (−)-notoamide B [(−)-15] as major metabolites (Scheme 1).[1] Curiously, A. amoenus (NRRL 35600) was found to produce their respective enantiomers[2] (Scheme 1). We have recently documented that both fungi produced (±)-versicolamide B [(±)-14] as a minor metabolite (Scheme 1). The putative biosynthetic precursor to (±)-14, 6-epi-stephacidin A (13), was isolated from A. amoenus as an enantiomeric mixture enriched with (−)-13[3] These stereochemical observations suggested that this fungus produced both enantiomers of 13 and contain a highly enantiodiscriminating oxidase, which converts only (+)-13 into (+)-14. The characteristic and unique bicyclo[2.2.2]diazaoctane core common to this family of prenylated indole alkaloids is most likely biosynthesized by an intramolecular hetero-Diels–Alder (IMDA) reaction. The metabolite profiles of these fungi currently indicate that the biosynthesis of these stereochemically distinct metabolites plausibly proceeds by the pathways a (main) and b (minor) in A. protuberus and pathways c (main) and b/d (minor) in A. amoenus (see Scheme 1). The recent isolation of a plausible precursor, notoamide S (22), for these metabolites from A. amoenus provided strong support for this proposal.[3] Most of the alkaloids thus far identified to contain the bicyclo[2.2.2]diazaoctane ring system, possess a relative configuration of syn at C6 (see stephacidin numbering). Distinct enantiomers of notoamide B (15) and stephacidin A (16) are among the main metabolites from A. protuberus and A. amoenus, and versicolamide B (14) was the first alkaloid within this family to be identified with the corresponding relative configuration of anti at C6. Cai et al. recently isolated natural alkaloids with the relative configuration of anti, namely, (+)-14 and (−)-13, from A. taichungensis ZHN-7-4[4] (syn and anti relationship is based on the H21 and bridging amide C18/N19). With these stereochemical differences identified, we were interested in elucidating the biosynthetic machinery of the producing organisms which give rise to the specific relative and absolute configurations observed. As part of that effort, we have carried out the isolation, structural elucidation, and stereochemical assignments of structurally unprecedented alkaloids produced by A. taichungensis (IBT 19404) and herein suggest a possible biosynthetic pathway of seven new indole alkaloids, which we have named the taichunamides A–G (1–7).

The fungus was cultured on a rice medium and extracted with nBuOH. The extract was purified to afford 1–7 and fourteen known derivatives (8–21; Figure 1).[5] Of these, the compounds 8–12 were previously obtained by the photoconversion of (+)-13[4] and herein we have isolated these compounds directly from the fungus for the first time.

Taichunamide A (1) has the molecular formula C_{29}H_{32}N_{2}O_4, which was established by HRESIMS. The $^1$H and $^{13}$C NMR spectra in [D$_6$]DMSO (see Table S1 in the Supporting Information) were similar to those of versicolamide B (14) and indicated the presence of a bicyclo[2.2.2]diazaoctane core comprising proline (A in Figure 2a) and a 5,6-disubstituted 2,2-dimethyl-2H-chromene (B), which is also present in 14. The presence of a 2,2-dimethylecyclo-
hexanone ring fused with a A unit was indicated by the presence of a ketone carbon atom \( \delta_C = 190.7 \text{ ppm (C2)} \) and two methyl groups \( \delta_C = 1.35 \text{ ppm, } \delta_C = 19.7 \text{ ppm (C23); } \delta_H = 1.21 \text{ ppm, } \delta_C = 27.2 \text{ ppm (C24)} \), along with the HMBC correlations \( H_23/C2, C21, C2, \) and \( C24, H_10/C2, C3, C11, C12, \) and \( C21, \) and \( H21/C22. \) The direct connection between \( C9 \) in the B unit and the quaternary C3 was shown by the HMBC correlations \( H4/C3 \) and \( H10/C9. \) The chemical shifts of \( C3 (\delta_C = 81.3 \text{ ppm}) \) and \( C8 (\delta_C = 147.9 \text{ ppm}) \) revealed that the two carbon atoms were linked through the remaining portion NH, and resulted in the formation of an azetidine ring. Two exchangeable hydrogen signals were observed at \( \delta = 6.29 (s) \) and \( 7.54 \text{ ppm (s).} \) The latter signal showed HMBC correlations with \( C11, C12, \) and \( C17, \) thus indicating that the signal was \( H19, \) and the former signal was that of \( H1. \) The relative configuration of 1 was established by the NOE correlations \( H1/H10, \delta = 1.35 \text{ ppm), } H23/H19, \) and \( H21/ \) \( H10 (\delta = 1.71 \text{ ppm),} \) which showed that \( H1, H10 (\delta = 2.63 \text{ ppm), } H19, \) and \( H23 (\delta = 1.35 \text{ ppm) were on the same side and } H21 \) \( \) and \( H10 (\delta = 1.71 \text{ ppm) were located on the opposite side (see Figure 2b and Figure S1). \) The Cotton effect at \( \lambda = 225–250 \text{ nm arises from an } n \rightarrow \pi^* \text{ transition of the dioxopiperazine moiety, and is diagnostic of the bicyclo-} \) \( [2.2.2] \text{diazaoctane moiety as observed in 1. \) The HMBC correlations \( H4 (\delta_H = 7.92 \text{ ppm)}/C3 (\delta_C = 172.8 \text{ ppm), } H1 \) \( (\delta_H = 10.42 \text{ ppm)/C8 (\delta_C = 153.7 \text{ ppm), } C9 (\delta_C = 121.1 \text{ ppm), } C10 \) \( (\delta_C = 119.9 \text{ ppm), and } C22 (\delta_C = 43.7 \text{ ppm), } H24 (\delta_H = 1.41 \text{ ppm)/C2 (\delta_C = 164.1 \text{ ppm) and } C21 (\delta_C = 53.5 \text{ ppm), and } \) \( H19 (\delta_H = 8.47 \text{ ppm)/C10, revealed that the major tautomer of 2 comprised a 4-pyridine ring (Figure 3). In contrast, the HMBC correlations \( 3OH (\delta_H = 1.12 \text{ ppm)/C3 (\delta_C = 157.3 \text{ ppm), } C9 (\delta_C = 114.4 \text{ ppm), and } C10 (\delta_C = 107.4 \text{ ppm), } H4 (\delta_H = 7.96 \text{ ppm)/C3, and } H19 (\delta_H = 8.93 \text{ ppm)/C10, } H24 (\delta_H = 1.30 \text{ ppm) and } H21 (\delta_H = 2.15 \text{ ppm)/C2 (\delta_C = 174.2 \text{ ppm), secured the presence of a 4-pyridyl ring as the minor tautomer (Figure 3). Thus, 2 exists as an equilibrium mixture of keto–enol tautomers in [D$_6$]DMSO. \) Curiously, the ratio of the keto and enol forms is highly solvent-dependent. A single keto form is evident in CD$_2$OD and a single enol form is apparent in [D$_6$]acetone (see Table S3). The NOE correlations \( H21/H24 \) and \( H19/H23 \) in the keto form (Figure 3, see Figure S1), and the ECD spectrum established the 11R,17S,21R configuration.

The molecular formula of taichunamide C (3) was established by HR-ESI-MS to be C$_{23}$H$_{28}$N$_{3}$O$_{6}$, thus indicating one more CH$_2$O unit more than that of 13. Analysis of the 2D NMR spectra (see Table S4) indicated that the structure of 3 was similar to that of 13. Carbon chemical shifts of C2 (\( \delta = 107.2 \text{ ppm) and } C3 (\delta = 73.2 \text{ ppm) suggested that the olefinic carbon atoms, C2 and C3, in 13 were replaced with oxygen-bearing carbon atoms in 3. The presence of a hydroxy group at

Scheme 1. Proposed facial specificities of IMDA reactions for metabolites in A. protuberus (circles), A. amoena (triangles), and A. taichungensis (squares). Major and minor metabolites in each fungus are represented with large and small symbols, respectively.
C3 was determined by HMBC correlations from 3OH \( (\delta_H = 5.75 \text{ ppm}) \) to C9 \( (\delta_C = 130.1 \text{ ppm}) \) and C10 \( (\delta_C = 39.1 \text{ ppm}; \text{Figure 4a}) \). Geminal hydrogen atoms \( (\delta_H = 4.65 \text{ and } 4.57 \text{ ppm}, H_230) \) of the isolated methylene unit \( (\delta_C = 88.4 \text{ ppm}, C30) \) showed HMBC correlations with C2 \( (\delta_C = 107.2 \text{ ppm}) \) and C8 \( (\delta_C = 139.8 \text{ ppm}) \), which clearly indicated that the methylene unit was directly attached to N1. Since C30 was observed in low field, it may be attached to an oxygen atom. Considering the remaining two oxygen atoms in the molecular formula, there were two plausible possibilities for the structure of 3, namely the peroxide 3a or N-oxide 3b (Figure 3a). The NOE correlations H19/H323 \( (\delta = 1.35 \text{ ppm}) \) and H21/H324 \( (\delta = 0.87 \text{ ppm}; \text{Figure 4a}; \text{see Figure S1}) \), and the positive Cotton effect at 225 nm permits the stereochemical assignment as 11S,17S,21R. To establish the stereochemistry of C2 and C3, a low-energy conformational search was quantum mechanically conducted at the DFT level of theory, in Spartan14, using four possible configurations, 2R,3R, 2R,3S, 2S,3R, and 2S,3S, for 3a and 3b (see Table S9). Although the NOE correlation was observed for H25/H30 \( (\delta = 4.57 \text{ ppm}) \), its calculated distance in 3b was over 3 Å in every configuration, and therefore 3b was excluded. Since the calculated distances are all sufficiently proximal in every configuration of 3a, computer simulation of ECD spectra were performed. As shown in Figure 4b, the calculated spectrum of \((2R,3R)-3a\) matched the experimental

Figure 1. Structures of prenylated indole alkaloids from Aspergillus taichungensis (IBT 19404).

Figure 2. a) COSY and key HMBC correlations and b) key NOE correlations for 1.

Figure 3. Key HMBC and NOE correlations for 2.

Figure 4. a) Key HMBC and NOE correlations for two possible structures of 3. b) Experimental ECD spectrum of 3 along with calculated ECD spectra of 2R,3R, 2R,3S, 2S,3R, and 2S,3S-3a after optimization at the B3LYP/6-31G* level of theory.
spectrum, and consequently the structure of 3 was determined to be (2R,3R,11S,17S,21R)-3a.

Taichunamide D (4) has a molecular formula of C₃₂H₃₄N₂O₇S, which was established by HREIMS. Although the ¹H and ¹³C NMR spectra of 4 (see Table S5) were almost superimposable with those of 13, the presence of a single methyl group (δ_H = 2.55 ppm and δ_C = 33.8 ppm; C30), which showed no HMBC correlation, and the absence of an exchangeable hydrogen atom (δ_H = 10.47, br s, NH) were evident. HREIMS suggested the presence of an additional SO₂ in 4 and ¹H and ¹³C chemical shifts of the methyl group indicated that 4 was the corresponding N-methylsulfonyl derivative of (+)-13, and was supported by the absorption bands at 1361 and 1179 cm⁻¹ arising from asymmetric and symmetric SO₂ stretching, respectively, in the IR spectrum along with the ECD and NOE spectra (see Figure S1).

¹H and ¹³C NMR spectra of taichunamide F (6) were similar to those of notoamide U[9] (8) and showed the presence of a methoxy residue (δ_H = 3.02 ppm, δ_C = 61.6 ppm; Table S7). HMBC correlations from the hydrogen to C10 (δ_C = 76.4 ppm) indicated that the methoxy group was attached to C10. Although the NOE correlations, H19/H23 (δ = 1.29 ppm) and H21/H24 (δ = 1.30 ppm; see Figure 5a) and Figure S1), and the positive Cotton effect at 225 nm indicated a 11R,17S,21R configuration, the configurations of C3 and C10 could not be determined by spectroscopic data. A low-energy conformational search was then conducted at the DFT level in Spartan14 using the four possible isomers, 3R,10R-, 3R,10S-, 3S,10R-, and 3S,10S-6. Although a NOE crosspeak was observed for H4/H10, its calculated distances in 3S,10S- and 3S,10R-6 were beyond 3 Å (see Table S10), which indicated that these configurations were excluded. Computer simulation of the ECD spectra for 3S,10R- and 3S,10S-6 were performed and the spectra for 3S,10R-6 matched well with the experimental spectrum (Figure 5b). Therefore, the structure of 6 was assigned to be the 3-epi-10R-methoxy derivative of 8.

The structure determination of the other new compounds, taichunamides E (8) and G (7), is described in the Supporting Information. Regarding preliminary biological activity, taichunamide F (6) and 6-epi-avainvillamide (21) were found to inhibit the chymotrypsin-like activity of the proteasome by 81 and 95%, respectively, at a concentration of 10 µM. However, other compounds were inactive at this concentration.

The new alkaloids described herein, include unprecedented structures, in particular, taichunamides A, B, and C (1-3) and all constitute hitherto unknown systems derived from tailoring of the tryptophan moiety. Thus, the biosynthesis of these novel compounds constitutes a series of fascinating bond constructions. We suggest plausible biosynthetic pathways for the construction of these natural compounds (Scheme 2). The most plausible biosynthetic precursor is (+)-13, a main metabolite in this fungus, which would afford 1 and (+)-14/(-)-12 as minor and major metabolites, respectively, through β-face oxidation followed by distinct pinacol rearrangements. This hypothesis is consistent with the stereochemical configuration at C3 of 1, and was determined by NOE correlations and ECD spectra. In contrast, taichunamide E (5) plausibly arises by α-face oxidation followed by a pinacol rearrangement. Compared with β-face oxidation, α-face oxidation is more sterically demanding with N19, as reflected by the metabolite ratios of (+)-14 (15.9 mg) / (-)-12 (240 mg) versus 5 (0.43 mg). Although 5 was identified as a minor product along with 12[7] this is the first report of its isolation from the fungal culture. The 4-pyridone unit in 2 would reasonably arise by singlet oxygen reaction at the indole 2,3-position of (+)-13, followed by cyclization (Scheme 2). A wide structural array of prenylated indole alkaloids have been isolated from the genera of *Aspergillus* and *Penicillium* to date[1,2,8,9] yet their respective carbon frameworks are very unique. Relatively few natural products containing an azetidine ring are known,[9] and to our knowledge, taichunamide A (1) is the first naturally occurring spiroindole-derived metabolite bearing an azetidine moiety. Taichunamide D (4) was found to contain a 1-methylsulfonyl group. To the best of our knowledge, this is the first report of the isolation of 1-methylsulfonylindole alkaloid from natural sources. In this study, we isolated (+)-versicoloramide C [(+)-12, 240 mg] and (+)-6-epi-stephacidin A [(+)-13, 160 mg] as major metabolites and (+)-notoamide B [(+)-15, 0.77 mg] and (-)-stephacidin A [(−)-16, 0.82 mg] as minor metabolites from *A. taichungensis* (IBT 19404), and they are most plausibly biosynthesized by pathways b and c (see Scheme 1). Previously, we reported that *A. protuberus* (MF297-2) produced (+)-16 and (-)-15 as major metabolites[1] and *A. antonius* (NRRL 35600) produced their respective enantiomers.[2] Comparing the metabolite profiles of these three ancestrally related (orthologous) species clearly reveals the distinct yet subtle stereochemical diversity within this genus and it involves both enantiodivergent and diasterodiester divergent biosynthetic constructions. We have previously reported the high sequence homology between the genes encoding the biosynthetic enzymes in this genus. However, the underlying biochemical basis for the stereochemical divergence observed remains to be elucidated at high resolution.[10] The structurally new and unique metabolites recorded here are likely just a tantalizing microcosm of secondary metabolite tailoring leading to unprecedented structural diversity from a relatively small pool of primary metabolite building blocks. Future efforts of our laboratories.
are directed at further elucidating the breadth of structural diversity extant within the *Aspergillus* genus, and that of closely related marine and terrestrial fungi.

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