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Structure revision of trichotoxin, a chlorinated polyketide isolated from a *Trichodesmium thiebautii* bloom

Matthew J. Bertin [a,*], Paul V. Zimba [b], Haiyin He [c], Peter D.R. Moeller [d]

[a] Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, United States
[b] Department of Life Sciences, Texas A&M, Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412, United States
[c] Biosortia Pharmaceuticals, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, United States
[d] Emerging Toxins Program, National Ocean Service/NOAA, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, United States

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ABSTRACT

NMR-guided fractionation of the lipophilic extract of *Trichodesmium thiebautii* filaments led to the isolation of a phenyl-containing chlorinated polyketide (**1**) and an alkyne-containing analog (**2**). Comparison of spectroscopic and spectrometric data of **1** with the data of the previously reported trichotoxin, strongly suggested that these metabolites were identical and supports a structural revision of trichotoxin and its designation as trichotoxin A. In addition, we report the isolation and characterization of the alkyne-containing analog trichotoxin B (**2**). Absolute configuration of **1** and **2** is proposed based on spectroscopic comparison to a close structural analog.

Cyanobacteria continue to be a notable microbial source for the isolation of secondary metabolites displaying diversity with respect to chemical structures and biological activities.[1,2] *Trichodesmium thiebautii* Gomont *ex* Gomont is a marine filamentous cyanobacterium ecologically relevant both for its nitrogen-fixing capability[3] and its seasonal blooms.[4] However, little is known about its secondary metabolite profile due to an inability to culture unialgal strains and difficulties in acquiring environmental material from offshore blooms. While homogenized cells, filtrates, aging cultures, and crude preparations of *Trichodesmium* filaments have shown toxicity to copepods,[5,6] there are only a few examples of biologically active pure molecules isolated and characterized from *Trichodesmium* strains.[7,8] One such molecule, trichotoxin, was isolated from an environmental collection of *T. thiebautii* from the western Gulf of Mexico collected in July and August of 2005.[9] This compound showed some cytotoxicity against both GH4C1 cells and Neuro-2A cells.[9] The present work details the isolation of a molecule (**1**) from a *T. thiebautii* collection from the western Gulf of Mexico (Padre Island) in 2014, which possessed a vinyl chloride functionality. A comparison of molecular features to the previously reported trichotoxin suggested a structural revision of the originally reported molecule. In addition, we report the characterization of an alkyne-containing analog, trichotoxin B (**2**). We propose the revision of trichotoxin based on analyses of NMR spectroscopic data and biosynthetic precedence with respect to cyanobacterial secondary metabolism. These molecules are recognizably derived from a polyketide synthase (PKS) biosynthetic system and appear to be repeatedly isolable from *T. thiebautii* blooms over time.

Results and discussion

In an effort to evaluate the secondary metabolite composition of *Trichodesmium thiebautii* blooms in the Gulf of Mexico, cyanobacterial filaments from an environmental collection were extracted and fractionated and isolation was guided by examination of ^{1}H NMR spectra, ultimately leading to the purification of an optically active colorless oil (**1**) (Fig. 1).

HRESIMS analysis of **1** gave an $[M+Na]^{+}$ of *m*/*z* 341.1647, suggesting a molecular formula of $C_{20}H_{27}ClO$ and a requirement of 7 degrees of unsaturation. Analysis of ^{1}H and ^{13}C NMR spectra of **1** showed the presence of a monosubstituted benzene ring (positions 12–17; δ_C 138.2, 129.0, 128.5, 126.5, 128.5, 129.0 and δ_H 7.15, 7.29, 7.22, 7.29, 7.15 respectively). The benzylic methylene protons (δ_H 3.35) were substantially deshielded and showed HMBC correlations to the aromatic system (C-12, δ_C 138.2; C-13/17, δ_C 129.0) as well as a moderately polarized olefin (C-10, δ_C 142.3; C-18, δ_C 113.8). The polarization of the C-10/C-18 double bond and the singlet methine proton (H-18, δ_H 5.81) was consistent with the

* Corresponding author.
E-mail address: mbertin@uri.edu (M.J. Bertin).

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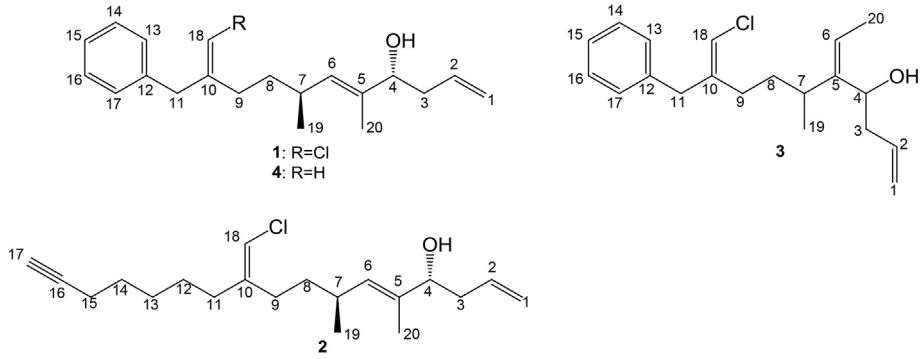

Fig. 1. Structures of compound **1**, **2**, the original trichotoxin[9] and phenyl alkene[10] compound.

presence of a vinyl chloride functionality. Moderately deshielded allylic methylene protons (H_2-9, δ_H 2.06) showed HMBC correlations to the vinyl chloride moiety and COSY correlations to diastereotopic methylene protons (H-8a, δ_H 1.42; H-8b, δ_H 1.25). The H-8 methylene protons showed a COSY correlation to a methine proton (H-7, δ_H 2.33). The H-7 methine showed COSY correlations to an olefinic proton (H-6, δ_H 5.14) and a methyl group (H-19, δ_H 0.94). The H-6 olefin and an olefinic methyl (H-20, δ_H 11.7) both showed HMBC correlations to a quaternary carbon (C-5, δ_C 135.7). An oxymethine proton (H-4, δ_H 4.01) showed HMBC correlations to C-5, C-20 and a methylene carbon (C-3, δ_C 40.0). The H_2-3 protons (δ_H 2.27) showed HMBC correlations to a second polarized olefin (C-2, δ_C 135.0; C-1, δ_C 117.5) supporting a terminal alkene functionality and satisfying the final degree of unsaturation. Thus, the planar structure of **1** was a linear polyketide hallmarked by a vinyl chloride functionality and terminal alkene. Key HMBC and COSY correlations are shown in Fig. 2.

An NOE correlation between the H-18 methine (δ_H 5.80) and H-13 (δ_H 7.15) of the benzene ring supported a *Z* configuration of the vinyl chloride (Fig. 2). An *E* configuration of the C-5/C-6 olefin was supported by examining the chemical shift of the olefinic methyl (H-20, δ_H 11.7) and the NOE correlation between H-7 and H-20.

This revised structure of trichotoxin (**1**) is a chlorinated analog of a phenyl alkene compound isolated from a mixture of three Florida sponges (Fig. 1).[10] While the authors note that this linear phenyl alkene molecule is an unusual find in sponges, unlike more prevalently isolated terpenoids and amino acid derivatives,[10] this phenyl alkene does have structural features consistent with an underlying cyanobacterial biosynthetic architecture such as the vinyl group at C-10,[11] and the terminal alkene.[12] Metagenomic and other genetic methods have provided strong support for the microbial production of onnamide,[13] swinholide A[14] and chlorinated peptides[15] in sponge-microbe assemblages.

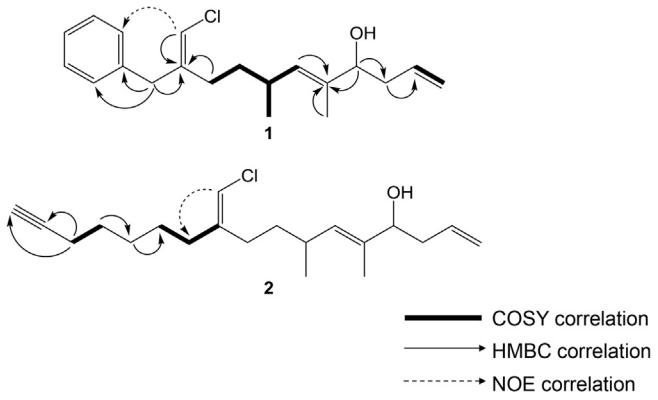

Fig. 2. Selected HMBC, COSY and NOE correlations for trichotoxin A (**1**) and B (**2**).

The absolute configuration of **1** is proposed to be identical to that of phenyl alkene based on a comparison of optical rotation values, relative stereochemistry and ^{13}C NMR chemical shift identity of the stereogenic centers (Tables S2 and S3). The absolute configuration of the phenyl alkene compound was determined using a modified Mosher's protocol and *J*-coupling analysis.[10]

HRESIMS analysis of **2** gave an $[M+Na]^+$ of *m*/*z* 345.1960, suggesting a molecular formula of $C_{20}H_{31}ClO$ and a requirement of 5 degrees of unsaturation. Analysis of 1D and 2D NMR spectra clearly showed **2** was an analog of **1** and the planar structure was identical from C-1 to C-10 of the carbon backbone, including branched methylation, the presence of the vinyl chloride and the relative double bond configuration (cf. Tables 1 and 2). Moderately deshielded allylic methylene protons (H-11, δ_H 2.05) showed HMBC correlations to C-10 (δ_C 142.7). Bidirectional HMBC and COSY correlations showed the presence of an aliphatic chain consisting of 5 methylene groups (positions 11–15) between the C-10 quaternary carbon and the C-16 quaternary carbon (δ_C 84.0) (Fig. 2). A COSY correlation between H-17 (δ_H 1.95) and H-15 (δ_H 2.19) and the chemical shift of C-17 (δ_C 68.2) strongly supported an alkyne functionality, satisfied the final two degrees of unsaturation, and completed the planar structure of **2** (Fig. 1). We propose the absolute configuration of **2** by analogy to **1** and phenyl alkene compound based on ^{13}C NMR data and identical relative configuration (cf. Tables 2 and S2 and S3).

Support for revised structure – NMR analysis

The significant structural differences between **1** and the original trichotoxin structure center on positions 4–6 and the olefinic methyl at position 20. The cis orientation of the vinyl methyl functionality and the secondary alcohol in the original trichotoxin structure should result in an upfield shift of C-4 (δ_C 65–70),[11,16] The originally reported chemical shift of C-4 was considerably downfield (δ_C 76.7). Additionally, comparing ^1H NMR splitting patterns of the vinyl methyl of the original trichotoxin structure to the exocyclic vinyl methyl present in the jasnudiflosides,[17] we would predict that the H-6 splitting pattern would be a quartet, while the splitting pattern of H-20 would be a doublet or doublet of doublets. However, examining the ^1H NMR splitting patterns of the original trichotoxin structure, H-6 is a doublet, suggesting it is adjacent to a methine proton and H_3-20 is a singlet,[9] indicating it is adjacent to a quaternary carbon, and strongly supporting that H-6 and H_3-20 are not coupled. In the original structure elucidation of trichotoxin, Schock et al., reported HMBC correlations from H_3-20 to C-4 and C-6. Examining the HMBC data in the present work, H_3-20 showed strong correlations to C-4, the quaternary carbon C-5, and C-6. The original work reported a COSY correlation between H_3-20 and H-6. This correlation may have been assumed to indicate a vicinal relationship between the H-20 methyl and H-6

Table 1
NMR data for trichotoxin A (**1**)[a] (800 MHz, $CDCl_3$).

Position	δ_C, type	δ_H (J in Hz)	HMBC	COSY
1a	117.5, CH_2	5.10, dd (17.0, 1.9)	C-2, C-3	H-2
1b		5.05, dd (10.0, 1.9)	C-3	H-2
2	134.9, CH	5.73, m	C-3, C-4	H-1, H-3
3	40.0, CH_2	2.27, m	C-1, C-2, C-4, C-5	H-2, H-4
4	76.7, CH	4.01, t (6.7)	C-2, C-3, C-5, C-6, C-20	H-3
5	135.7, qC			
6	132.7, CH	5.14, d (9.4)	C-4, C-7, C-8, C-19, C-20	H-7, H-20
7	32.2, CH	2.33, m	C-5, C-6, C-8, C-9, C-19	H-6, H-8a, H-8b H-19
8a	34.6, CH_2	1.42, m	C-6, C-7, C-9, C-10 C-20	H-7, H-8b, H-9
8b		1.25, m	C-6, C-7, C-9, C-10 C-20	H-7, H-8a, H-9
9	28.2, CH_2	2.06, t (8.6)	C-7, C-8, C-11, C-10, C-18	H-8a, H-8b
10	142.3, qC			
11	41.2, CH_2	3.35, s	C-9, C-10, C-12, C-13, C-17, C-18	H-18
12	138.2, qC			
13/17	129.0, CH	7.15, d (7.6)	C-11, C-15	H-14, H-16
14/16	128.5, CH	7.29, t (7.6)	C-12	H-13, H-15, H-17
15	126.5, CH	7.22, t (7.6)	C-13, C-17	H-14, H-16
18	113.8, CH	5.81, s	C-9, C-10, C-11	H-11
19	20.9, CH_3	0.94, d (6.6)	C-6, C-7, C-8	H-7
20	11.7, CH_3	1.59, s	C-4, C-5, C-6, C-7, C-8, C-19	H-6

[a] *Trichotoxin A* (**1**): colorless oil; α_D^{22} −2.5 (MeOH, *c* 0.20); UV (MeOH) λ_{max} (log ε) 209 nm (5.5); (^{1}H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR (800 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 341.1647 $[M+Na]^+$ (calcd for $C_{20}H_{27}ClONa$, 341.1639).

Table 2
NMR data for trichotoxin B (**2**)[a] (800 MHz, $CDCl_3$).

Position	δ_C, type	δ_H (J in Hz)	HMBC	COSY
1a	117.6, CH_2	5.13, dd (17.0, 1.9)	C-2, C-3	H-2
1b		5.08, dd (10.0, 1.9)	C-3	H-2
2	134.8, CH	5.78, m	C-3, C-4	H-1a, H-1b, H-3
3	40.0, CH_2	2.32, m	C-1, C-2, C-4, C-5	H-2, H-4
4	76.7, CH	4.06, t (6.7)	C-2, C-3, C-5, C-6, C-20	H-3
5	135.7, qC			
6	132.8, CH	5.23, d (9.4)	C-7, C-8, C-19, C-20	H-7
7	32.3, CH	2.38, m	C-8, C-19	H-6, H-19
8a	34.8, CH_2	1.43, m	C-6, C-7, C-9, C-10, C-19	H-7, H-8b, H-9
8b		1.28, ovlp	C-6, C-7, C-9, C-10, C-19	H-7, H-8a, H-9
9	28.3, CH_2	2.11, m	C-7, C-8, C-10, C-18	H-8a, H-8b
10	142.7, qC			
11	34.7, CH_2	2.05, t (7.1)	C-10, C-12, C-13, C-18	H-12
12	27.1, CH_2	1.41, ovlp	C-11, C-13	H-11
13a	28.3, CH_2	1.52, ovlp	C-12, C-15, C-16	H-13b
13b		1.40, ovlp	C-12	H-13a
14a	28.2, CH_2	1.53, ovlp	C-12, C-13, C-15, C-16	H-14b, H-15
14b		1.40, ovlp	C-12, C-13	H-14a
15	18.3, CH_2	2.19, td (7.1, 2.7)	C-14, C-16, C-17	H-14a
16	84.0, qC			
17	68.2, CH	1.95, t (2.7)		H-15
18	112.0, CH	5.75, s	C-9, C-10, C-11	
19	20.9, CH_3	0.98, d (6.6)	C-6, C-7, C-8	H-7, H-8a
20	11.5, CH_3	1.64, s	C-4, C-5, C-6	H-6

[a] *Trichotoxin B* (**2**): colorless oil; α_D^{22} −9.2 (MeOH, *c* 0.10); UV (MeOH) λ_{max} (log ε) 204 nm (3.5); ^{1}H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR (800 MHz, $CDCl_3$), see Table 2; HRESIMS *m/z* 345.1960 $[M+Na]^+$ (calcd for $C_{20}H_{31}ClONa$, 345.1956).

methine. We did observe a weak COSY correlation between H_3-20 and H-6. However, we considered this correlation to represent an allylic correlation as is often observed in sesquiterpenoid molecules.[18] The revised trichotoxin A structure (**1**) shows consistency with respect to predicted ^{13}C chemical shifts, ^{1}H NMR splitting patterns, and vicinal ^{1}H–^{1}H coupling (Table 1). An authentic sample of the original trichotoxin no longer exists, thus trichotoxin A and the orginal trichotoxin were not able to be analyzed by a chromatographic comparison. An optical rotation was not recorded for the original trichotoxin. However, both compounds were isolated from *T. thiebautii* blooms. Additionally, a comparison of ^{1}H and ^{13}C NMR signals provides strong support for these structures as identical chemical entities (Table S1).

Support for revised structure – polyketide biosynthesis

We predict that the revised trichotoxin structure (**1**) would be generated from a PKS biosynthetic pathway extending acetate units from a proposed phenyl acetic acid starter unit in **1** and a 7-octynoate unit in **2**. The carbonyl of the starter unit would be modified into a vinyl functionality by the action of an HMG-CoA synthase cassette[19] followed by chlorination by a halogenase in a similar fashion to that observed in the biosynthesis of jamaicamide.[20] This would be followed by the incorporation of four acetate units. The first fully reduced; the second subjected to ketoreduction and dehydration; the third subjected to ketoreduction and fourth fully reduced. Methylation at C-19 and C-20 would

likely originate from S-adenosyl methionine (SAM) catalyzed by methyltransferases or they could come from the incorporation of proprionate units into the growing polyketide chain instead of acetate units. We predict that the terminal alkene results from the action of a decarboxylating thioesterase as has been observed in the curacin A pathway.[21,22] A cultured *T. thiebautii* strain originally isolated from the Sargasso Sea did show the presence of PKS genes[23] and further analysis of *T. thiebautii* bloom metagenomes may yield important information with respect to the biosynthetic capacity for polyketide production from this cyanobacterium.

The biosynthesis of the original trichotoxin structure would follow a similar pattern for the starter unit and the first acetate extension. The following acetate extension could be modified by the HCS cassette to generate the vinyl functionality followed by methylation, and the reforming of the double bond. If the terminal alkene is created by the action of a decarboxylating thioesterase then the penultimate extender unit in the biosynthesis of the original trichotoxin structure would need to possess three carbon atoms. There are examples of compounds in which three carbon units, derived from the pool of glycolytic intermediates, are incorporated into growing polyketide chains.[24] However, these three carbon units are generally incorporated via an ester linkage, which is not present in this original structure.

While the revised structure (**1**) in this report is more consistent with conventional polyketide biosynthesis, an argument from biosynthesis alone cannot provide enough evidence for structural revision without analyzing a pure strain via stable isotope feeding studies or garnering genetic information from the biosynthetic gene cluster. However, when the NMR data and biosynthetic precedence are used in an orthogonal manner, the argument for revision is strongly supported. Interestingly, we have isolated the same metabolite from *T. thiebautii* blooms that occurred years apart from each other, making spatial and temporal secondary metabolomics studies of these blooms an intriguing prospect.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.11.062.

References

1. Tan LT. *J Appl Phycol*. 2010;22:659–676.
2. Nunnery JK, Mevers E, Gerwick WH. *Curr Opin Biotechnol*. 2010;21:787–793.
3. Bergman B, Sandh G, Lin S, Larsson J, Carpenter EJ. *FEMS Microbiol Rev*. 2013;3:286–302.
4. Westberry TK, Siegel DA. *Global Biogeochem Cycles*. 2006;20:6. http://dx.doi.org/10.1029/2005GB002673.
5. Hawser SP, O'Neil JM, Roman MR, Codd GA. *J Appl Phycol*. 1992;4:79–86.
6. Guo C, Tester PA. *Nat Toxins*. 1994;2:222–227.
7. Sudek S, Haygood MG, Youssef DTA, Schmidt EW. *Appl Environ Microbiol*. 2006;72:4382–4387.
8. Malloy KL, Suyama TL, Engene N, et al. *J Nat Prod*. 2012;75:60–66.
9. Schock TB, Huncik K, Beauchesne KR, Villareal TA, Moeller PDR. *Environ Sci Technol*. 2011;45:7503–7509.
10. Hwang H, Oh J, Kochanowska-Karamyan A, Doerksen RJ, Na M, Hamann MT. *Tetrahedron Lett*. 2013;54:3872–3876.
11. Williamson RT, Boulanger A, Vulpanovici A, Roberts MA, Gerwick WH. *J Org Chem*. 2002;67:7927–7936.
12. Gerwick WH, Proteau PJ, Nagle DG, Hamel E, Blokhin A, Slate DL. *J Org Chem*. 1994;59:1243–1245.
13. Piel J, Hui D, Wen G, et al. *Proc Natl Acad Sci U S A*. 2004;101:16222–16227.
14. Bewley CA, Holland ND, Faulkner DJ. *Experientia*. 1996;52:716–722.
15. Simmons TL, Coates RC, Clark BR, et al. *Proc Natl Acad Sci U S A*. 2008;105:4587–4594.
16. Graber MA, Gerwick WH. *J Nat Prod*. 1998;61:677–680.
17. Takenaka Y, Tanahashi T, Taguchi H, Nagakura N, Nishi T. *Chem Pharm Bull*. 2002;50:384–389.
18. Prakash O, Roy R, Kulshreshta DK. *Magn Reson Chem*. 1988;26:47–50.
19. Calderone CT. *Nat Prod Rep*. 2008;25:845–853.
20. Edwards DJ, Marquez BL, Nogle LM, et al. *Chem Biol*. 2004;11:817–833.
21. Chang Z, Sitachitta N, Rossi JV, et al. *J Nat Prod*. 2004;67:1356–1367.
22. Gehret JJ, Gu L, Gerwick WH, Wipf P, Sherman DH, Smith JL. *J Biol Chem*. 2011;286:14445–14454.
23. Ehrenreich IM, Waterbury JB, Webb EA. *Appl Environ Microbiol*. 2005;71:7401–7413.
24. Chan YA, Podevels AM, Kevany BM, Thomas MG. *Nat Prod Rep*. 2009;26:90–114.