

Secondary Metabolites of the Sponge-Derived Fungus Acremonium persicinum

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Supporting Information



ABSTRACT: This study reports the isolation and characterization of six new acremine metabolites, 5-chloroacremine A (4), 5chloroacremine H (5), and acremines O (6), P (7), Q (8), and R (9), together with the known acremines A (1), F (2), and N (3) from the fungus *Acremonium persicinum* cultured from the marine sponge *Anomoianthella rubra*. The relative configuration of acremine F (2) was determined by analyses of proton coupling constant values and NOESY data, and the absolute configuration confirmed as (1*S*, 4*S*, 6*R*) by X-ray crystallographic analysis of the borate ester derivative **15**. Acremines O, P, and R were each shown to be of 8*R* configuration by ¹H NMR analyses of MPA esters. The relative configurations suggested for acremines P and Q were each deduced by molecular modeling together with NOESY and coupling constant data. The ³*J*_{H-C} values in acremine P were measured using the pulse sequence EXSIDE, and the observed ³*J*_{H8-C4} of 5.4 Hz and small ³*J*_{H-C} values (<1.5 Hz) from H-8 to C-10 and C-11 were fully consistent with stereoisomer **7a**. For acremine Q, NOESY data combined with molecular modeling established the preferred diastereomer **8a**.

F ungi from the genus *Acremonium* have been reported from both terrestrial¹⁻³ and marine sources⁴⁻⁶ and produce unique and biologically active secondary metabolites, the most well known being the antibiotic cephalosporin C.⁷ Antioxidant hydroquinone derivatives⁸ and a chlorinated polyketide⁶ have been reported from an algal-derived *Acremonium* sp., while isolates derived from marine sponges have yielded alkaloids,^{4,9} peptides,^{10,11} or oxygenated metabolites.¹²

In 2005, Nasini et al. reported a series of 12 meroterpenoids, including acremines A (1), F (2), and N (3), from an endophytic strain of Acremonium byssoides isolated from sporangiospores of Plasmopara viticola in grapevine leaves.^{1,13,14} Malik et al. described the isolation of a "norterpenoid" from the plant Periploca aphylla;¹⁵ however the structure of this compound was later revised to that of acremine A (1) based on a synthetic study.¹⁶ Acremine A has also been isolated from the fungus Myceliopthora lutea by Smetanina et al. along with isoacremine D and two spiroacremines.¹⁷ Although the structure and ¹H NMR data of isoacremine D reported by the Russian group were identical to those of acremine D described by the Italian researchers,¹ the ¹³C NMR and melting point data of the two samples differed. Recently, total syntheses of acremines A, B, and I have been developed by Mehta et al.¹⁸

In this report, we describe the isolation, structure elucidation, and configurations of six new acremines (4-9) along with the known acremines A (1), F (2), and N (3). Identification of the ether product 10 as well as the known spiroacremines A (11) and B (12) during the course of the chromatographic purification highlights the sensitivity of the oxygenated cyclohexenone/cyclohexenediol ring systems to the isolation conditions used.

RESULTS AND DISCUSSION

Structural and Stereochemical Studies. The sponge *Anomoianthella rubra* was collected in December 2009 by scuba from the Gneering reef offshore from Mooloolaba in South East Queensland. Colonies of *Acremonium persicinum* were successfully cultured by streaking small pieces of sterilized sponge sample onto malt extract and potato dextrose agar media made up in artificial seawater. Identification of the isolate was undertaken by performing colony PCR of the rDNA ITS followed by DNA sequencing. Following preliminary investigation of a small-scale culture, large-scale fermentation was



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carried out in malt extract media (4 L) made up in artificial seawater. After 4 weeks, the culture broth was extracted with EtOAc followed by *n*-BuOH to afford extracts from which six new acremine metabolites (4-9) were isolated together with 9-O-methylacremine F (10) and spiroacremines A (11) and B (12) in addition to the known acremines A (1), F (2), and N (3).



The ¹H and ¹³C NMR data (Tables 1 and 2) for acremine A (1), the major component in the extract, are reported in CDCl₃, while the ¹H NMR spectrum was also measured in acetone- d_6 for direct comparison with literature data. Nasini et al. determined the relative configuration of 1 by X-ray crystallographic analysis, while the absolute configuration was deduced as (4*S*, 6*R*) following Mosher esterification.¹ The specific rotation of 1 from *A. persicinum* was measured as +13, compared to a literature value of +22.3, consistent with a (4*S*, 6*R*) configuration.

A full NMR assignment of acremine F (2) was undertaken using HSQC and HMBC experiments, as the ¹³C NMR data were not available from the literature. The signals for H-7 and H-8 were observed at $\delta_{\rm H}$ 6.19 and 6.08, respectively; on this basis the literature assignments for H-7 ($\delta_{\rm H}$ 6.09) and H-8 ($\delta_{\rm H}$ 6.17) should be reversed. The specific rotation of 2 from A. *persicinum* was +38, compared to a value of +56 reported in the literature. However in this previous work, the relative configuration at C-1 of acremine F was not defined.¹ Treatment of acremine A (1) with NaBH₄ in EtOH gave a mixture of two compounds in a 2:1 ratio that were separated by RP-HPLC using 15% MeCN/H₂O as eluent. The ¹H NMR spectrum of the major compound, which eluted second from the RP-HPLC column, was identical to that of acremine F. The minor compound shared similar ¹H NMR features to those of acremine F, except for the H-2 signal ($\delta_{\rm H}$ 5.88 (J = 4.8 Hz) vs $\delta_{\rm H}$ 5.61 (J = 2.5 Hz) in acremine F), and was assigned as the C-1 epimer of acremine F (13).

The conformational equilibria for acremine F(2) and 1-epiacremine F (13) are considered in Figure 1a. Individual conformers (2a/b, 13a/b) were modeled using ChemBio3D Ultra 12.0 (Cambridge) and by applying the MM2 force field for energy minimization to an RMS gradient of 0.100. In acremine F(2), the experimental coupling constant values for H-1/H-2, H-4/H-5a, and H-4/H-5b (2.5, 2.0, and 4.0 Hz) were in agreement with theoretical values anticipated for conformer 2a (see Table S1 in the Supporting Information) and placed H-1 and H-4 in pseudoaxial and pseudoequatorial orientations, respectively. Consistent with this interpretation, 1D-NOESY irradiation of the proton signal at $\delta_{\rm H}$ 4.00 (H-1) of 2 led to enhancement of H-2, H-5b, and Me-12 (Figure 1b). For 1-epiacremine F (13), the experimental J values of 4.8, 4.4, and 3.3 Hz for H-1/H-2, H-4/H-5a, and H-4/H-5b matched theoretical values anticipated for conformer 13a, in which both H-1 and H-4 were pseudoequatorial. 1D NOESY irradiation of the signal at $\delta_{\rm H}$ 3.92 (H-1) of 13 led to enhancement of H-2 and Me-12, but there was no enhancement of a C-5 proton. A coupling of 1.1 Hz between H-1 and H-5b confirmed the equatorial orientation of H-5b. In both acremine F and 1-epi-acremine F, conformers with a cis-1,3-pseudoaxial-axial arrangement between the C-4 and C-6 hydroxy groups were thus favored. In cyclic organic compounds with a *cis*-1,3-arrangement of hydroxy groups, the diequatorial conformer is preferred in polar solvents, but the diaxial conformer becomes an important contributor to the overall conformational equilibrium in nonpolar solvents owing to the intramolecular hydrogen bonding between the two hydroxy groups.¹⁹ In both 2a and 13a there is also the stabilizing feature of an equatorial Me group at C-6.

The relative and absolute configurational features of 2 were confirmed by an X-ray crystallographic study conducted on a derivative prepared from 9-O-methylacremine F(10). For ether 10, a sodiated ion peak at m/z 265.1422 provided the molecular formula $C_{13}H_{22}O_4$. The ¹H NMR data were very similar to those of **2**, except for a singlet at $\delta_{\rm H}$ 3.16 (3H), which gave HMBC correlations to the methyl groups at C-10 and C-11, as well as to C-9, and so revealed that a methoxy group had replaced the hydroxy group at C-9. On exposure to mildly acidic conditions during isolation, acremine F(2) may form a stable tertiary allylic carbocation at C-9, which then reacts with MeOH to form 10. Treatment of 10 with p-bromophenylboronic acid (1.1 equiv) in CH₂Cl₂ for 24 h gave a mixture of products, including the desired *p*-bromophenylboronate ester 14. Recrystallization of the ester mixture from EtOAc gave single crystals highly suitable for X-ray analysis, except that these corresponded to the borate ester 15 rather than 14. In its ¹H NMR spectrum, **15** lacked signals associated with a phenyl moiety, while the mass spectrum exhibited an adduct ion at m/z 291.1350 [M + Na]⁺ for the molecular formula C13H21BO5. Boric acid is commonly found as a contaminant in commercial samples of boronic acid and related substances.²⁰

Table 1.	¹ H NMR Assignment	ts for Acremines 1, 2	l, and 4–9 ^{<i>a,b</i>}					
position	1	7	4	S	6	7	8	6
1		4.00, dd (8.5, 2.5)						
2	6.00, br s	5.61, d (2.5)	6.18, d (1.8)	6.13, t (1.5, 1.0)	a 3.26, d (16.1)	5.20, s	a 2.90, dd (16.0, 7.5)	6.49, s
					b 3.02, d (16.1)		b 1.98, dd (16.0, 6.0)	
ю							2.73, m	
4	4.63, m	4.43, m	4.60, m	4.78, m				
S	a 2.36, dd (13.0, 5.1)	a 2.24, dd (14.5, 2.0)	4.23, d (8.2)	4.21, d (5.7)		3.57, s	3.51, s	6.61, s
	b 2.10, dd (13.0, 8.8)	b 1.80, dd (14.5, 4.0)						
7	6.44, d (16.0)	6.19, d (16.0)	6.49, d (16.0)	3.94, ddd (2.0, 1.0, 0.5)	a 3.01, dd (13.7, 5.8)	5.83, br d (8.8)	a 2.34, ddd (13.1, 7.4, 7.0)	a 2.99, dd (16.8, 5.0)
					b 1.84, dd (13.7, 4.0)		b 1.54 ^c	b 2.69, dd (16.8, 5.0)
8	6.67, d (16.0)	6.08, d (16.0)	6.70, d (16.0)	2.99, d (2.0)	4.15, dt (5.8, 4.0)	4.15, s	3.98, t (7.0)	3.73, dt (8.0, 5.0)
10	1.35, s	1.36, s	1.404, s	1.36, s	1.27, s	1.47, s	1.32, s	1.34, s
11	1.35, s	1.35, s	1.400, s	1.31, s	1.16, s	1.43, s	1.14, s	1.27, s
12	1.30, s	1.32, s	1.39, s	1.58, s	2.16, s	1.51, s	1.44, s	2.17, s
1-OH		2.65, d (8.5)						4.33, s
4-OH	2.96, br d (6.5)	3.42, d (7.0)	2.90, d (4.8)	2.79, d (4.9)				
HO-9	3.41, s	3.44, br s						
HO-7						3.17, d (8.8)		
HO-8								1.75, d (8.0)
^a Chamica	1 shifts (nnm) noferenced			since the H O mark action	NODECOSV			

^aChemical shifts (ppm) referenced to CHCl₃ ($\delta_{\rm H}$ 7.26). ^bAt 500 MHz. ^cObscured by H₂O peak, assigned by DQFCOSY.

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Table 2.	¹³ C NMR	Assignments	for	Acremines	1,	2,	and	4–9 ^{<i>a,b</i>}

position	1	2	4	5	6	7	8	9
1	200.1	73.0	198.3	195.5	192.6	192.3	205.3	147.8
2	123.1	130.2	121.5	122.2	51.3	102.4	38.9	115.6
3	157.9	137.7	156.2	155.7	83.4	162.5	43.7	116.8
4	65.6	64.1	72.2	70.5	189.1	99.0	102.7	146.3
5	43.9	40.5	70.4	69.6	144.8	59.1	66.0	119.2
6	73.1	71.1	75.9	75.2	147.8	57.4	60.7	123.7
7	124.3	126.2	122.7	53.0	40.1	95.0	31.7	31.3
8	147.1	138.4	149.2	67.9	78.0	86.2	86.2	69.8
9	71.4	71.1	71.3	67.9	86.6	78.2	71.3	76.3
10	29.8	30.1	29.6	27.5	22.8	25.8	27.7	22.6
11	29.8	29.7	29.6	25.0	26.8	23.4	25.3	24.5
12	24.1	26.8	21.0	23.7	14.8	14.5	14.7	15.7

^aChemical shifts (ppm) taken from 2D spectra referenced to CDCl₃ ($\delta_{\rm C}$ 77.16). ^bAt 500 MHz.



Figure 1. (a) Cyclohexene ring conformers for acremine F (2) and 1-*epi*-acremine F (13) (R = side chain). (b) Energy-minimized model structures of 2a and 13a showing NOESY correlations observed for 2 and 13.

The absolute configuration of **15** was determined by the anomalous dispersion method on a highly redundant data set collected with Cu K α radiation and confirmed a (1*S*, 4*S*, 6*R*) configuration (Figure 2).



Figure 2. ORTEP view of borate ester 15 showing absolute and relative configurations.

5-Chloroacremine A (4) and 5-chloroacremine H (5) were obtained from RP-HPLC as a mixture in a 1:1 ratio; ESIMS suggested a mixture of chlorinated compounds from the ion clusters at m/z 283/285 and at m/z 299/301, each with an intensity ratio of 3:1. HRMS measurements provided the respective molecular formulas as $C_{12}H_{17}ClO_4$ and $C_{12}H_{17}ClO_5$, respectively. The ¹H and ¹³C NMR data of 5-chloroacremine A suggested a substituted cyclohexenone ring [$\delta_{\rm H}$ 6.18 (1H), 4.60 (1H), 4.23 (1H); $\delta_{\rm C}$ 198.3 (s), 121.5 (d), 156.2 (s), 72.2 (d), 70.4 (d), and 75.9 (s)] with three methyl groups [$\delta_{\rm H}$ 1.404,

1.400, 1.39; $\delta_{\rm C}$ 29.6, 29.6, 21.0], closely resembling those of 1¹ except that the methylene group at C-5 was replaced by a methine ($\delta_{\rm H}$ 4.23, $\delta_{\rm C}$ 70.4). The chlorine substituent was placed at C-5 based on HMBC correlations from H-5 to both C-4 ($\delta_{\rm C}$ 72.2) and C-6 ($\delta_{\rm C}$ 75.9). The 8.2 Hz coupling between H-4 and H-5 established the axial orientation of H-5. In the preferred conformation, the C-6 hydroxy group is close to coplanar with the adjacent carbonyl group, resulting in hydrogen bonding (note that this is also the case for acremine A). In the side chain of 4, signals at $\delta_{\rm H}$ 6.49 and 6.70, each a doublet with a *J* of 16 Hz and linked to signals at $\delta_{\rm C}$ 122.7 and 149.2 by HSQC, respectively, confirmed a *trans* double bond; HMBC correlations from the alkene proton at $\delta_{\rm H}$ 6.70 to C-9, Me-10, and to Me-11 positioned the alkene between C-7 and C-8.

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On the basis of comparison of its ¹H and ¹³C NMR data with those of 4, 5-chloroacremine H (5) was likewise a chlorinated cyclohexenone; however the prenyl side chain contained an epoxy group ($\delta_{\rm H}$ 3.94, 2.99; $\delta_{\rm C}$ 53.0, 67.9) whose position was determined by HMBC correlations from both Me-10 and Me-11 to the epoxy carbon at $\delta_{\rm C}$ 67.9, as well as from the epoxy proton ($\delta_{\rm H}$ 3.94) to the olefinic carbons (C-2 and C-3). HMBC correlations from the methine proton ($\delta_{\rm H}$ 4.21) to C-1 ($\delta_{\rm C}$ 195.5), C-3 ($\delta_{\rm C}$ 155.7), C-4 ($\delta_{\rm C}$ 70.5), and C-6 ($\delta_{\rm C}$ 75.2) confirmed chlorine substitution at C-5. A 5.7 Hz coupling between H-4 and H-5 established the equatorial orientation of H-5. The epoxy protons in 5-chloroacremine H shared a 2.0 Hz coupling, closely identical to the value in each of acremines H and L (J = 2.1 Hz),¹⁴ which supported a *trans* configuration for the epoxy group. The orientation of the epoxy group relative to the stereogenic centers of the cyclohexenone core could not be conclusively determined owing to the small amount of material available; the similarity in the chemical shift of H-7 ($\delta_{\rm H}$ 3.94) to the value of H-7 reported for acremine H ($\delta_{\rm H}$ 3.93)¹⁴ may suggest the same diastereomer.

During NMR analysis of the mixture of 4/5 in CDCl₃, it was noticed that the signal intensity corresponding to 5-chloroacremine A decreased over time, while signals for a *cis* double bond ($\delta_{\rm H}$ 5.96 and 5.60, d 5.9 Hz), two methines ($\delta_{\rm H}$ 4.18 and 3.84), and an AB methylene ($\delta_{\rm H}$ 2.94 and 2.60, d 14.4 Hz) appeared. By comparison with data for spiroacremines A (11) and B (12), both of which have been identified as artifacts,¹⁷ the new product was identified as the spiro compound 16. The relative configuration at the spiro center was not determined due to the small amount of material available.

The final chlorinated compound isolated from the A. persicinum extract was named acremine O (6). The mass spectrum of this compound exhibited a typical ion cluster for a chlorinated molecule at m/z 281/283 [M + Na]⁺ in a ratio of 3:1; a molecular formula of C₁₂H₁₅ClO₄ was determined by HRESIMS. The ¹H NMR spectrum showed signals for one oxymethine ($\delta_{\rm H}$ 4.15), two methylene groups ($\delta_{\rm H}$ 3.26, 3.02 and $\delta_{\rm H}$ 3.01, 1.84), and three methyl singlets ($\delta_{\rm H}$ 2.16, 1.27, 1.16). In the ¹³C NMR spectrum, there were two carbonyl groups ($\delta_{\rm C}$ 189.1, 192.6), while the downfield chemical shift of C-3 ($\delta_{\rm C}$ 83.4) suggested a spiro ring.¹⁷ The carbonyl at $\delta_{\rm C}$ 192.6 was placed at C-1 based on an HMBC correlation to Me-12 ($\delta_{\rm H}$ 2.16), while the carbonyl group at $\delta_{\rm C}$ 189.1 was assigned to C-4 based on HMBC correlations to the methylene protons of C-7. In the side chain, a hydroxy group was placed at C-8 based on HMBC correlations from the methyl groups ($\delta_{\rm H}$ 1.27, 1.16) to C-8 ($\delta_{\rm C}$ 78.0). As in 4 and 5, the chlorine substituent was therefore placed at C-5. NOESY correlations between Me-11/H-8 and Me-11/H-7a indicated that these protons were all on the same face of the tetrahydrofuran ring. The NOESY correlation between H-2a and H-7b then established the relative configuration at the spiro centre. When 6 was esterified at C-8 to its (R)- and (S)-O-methylmandelate (MPA) esters 17a and 17b, the $\Delta \delta^{RS}$ values (where $\Delta \delta^{RS} = \delta R - \delta S$) were positive for H₂-2 and H₂-7, while negative for Me-10 and Me-11, which was consistent with an 8R configuration. The absolute configuration of acremine O is thus (3R, 8R). The natural product status of acremine O is unclear.

A molecular formula of C12H14O6 was determined for acremine P (7) from the adduct ion at m/z 277.0673 [M + Na]⁺. The ¹H and ¹³C NMR spectra supported the presence of a substituted cyclohexenone ring [$\delta_{\rm H}$ 5.20 (1H); $\delta_{\rm C}$ 192.3 (s), 162.5 (s), 102.4] containing epoxy [$\delta_{\rm H}$ 3.57 (1H); 59.1 (d), 57.4 (s)] and acetal ($\delta_{\rm C}$ 99.0) groups. Three methyl groups observed at $\delta_{\rm H}$ 1.51, 1.47, and 1.43 were linked to signals at $\delta_{\rm C}$ 14.5, 25.8, and 23.4 by HSQC, respectively, and there were two oxymethines ($\delta_{
m H}$ 5.83, 4.15; $\delta_{
m C}$ 95.0, 86.2) and one other oxygenated carbon ($\delta_{\rm C}$ 78.2). HMBC correlations from the epoxy proton to C-3 ($\delta_{\rm C}$ 162.5), C-4 ($\delta_{\rm C}$ 99.0), C-6 ($\delta_{\rm C}$ 57.4), and Me-12 ($\delta_{\rm C}$ 14.5) secured the position of the epoxy ring between C-5 and C-6. Three other signals (H-2, H-7, H-8), as well as Me-12, all showed HMBC correlations to the acetal signal at $\delta_{\rm C}$ 99.0, which was therefore assigned to C-4. A hydroxy group ($\delta_{\rm H}$ 3.17) was located at C-7 from its coupling to H-7 and by HMBC correlations to C-7 ($\delta_{\rm C}$ 95.0) and C-8 ($\delta_{\rm C}$ 86.2); therefore an ether linkage between C-4 and C-8 accounted for the HMBC correlation between H-8 and C-4. On the basis of its molecular formula, acremine P had six degrees of unsaturation, of which five had been identified; the oxygen substituents at C-4 and C-9 were therefore connected to form a peroxy ring, as shown in 7.

Reduction of 7 under mild conditions (H₂, Pd/C, EtOAc, 24 h) gave acremine A (1) as the sole product. The specific rotation of the synthetic sample was +25, comparable to the value of +13 for the natural isolate from *A. persicinum*. The absolute configuration at C-7 was pursued by preparation of the (*R*)- and (*S*)-*O*-methylmandelate (MPA) esters **18a**/**18b**, respectively. The $\Delta \delta^{RS}$ values of the MPA esters (where $\Delta \delta^{RS}$ = $\delta R - \delta S$) were positive for H-2 and Me-12 and negative for H-8, Me-10, and Me-11 and established a 7*R* configuration. With this information in hand, and recognizing the stereochemical constraint imposed by the 2,3,8-trioxabicyclo[3.2.1]octane ring system, two candidate diastereomers, **7a** and **7b** (Figure 3a), were next considered. In the ¹H NMR spectrum of



Figure 3. (a) Structures of candidate diastereomers 7a and 7b for acremine P. (b) Energy-minimized model structures of diastereomers of acremine P showing NOESY correlations for preferred conformer 7a.

7, the signals for H-2 and H-8 were noticeably sharp. The zero coupling between H-7 and H-8 indicated a dihedral angle of approximately 90° and so revealed that these two protons were on opposite faces of the ether ring, as in 7a rather than 7b. Other pieces of spectroscopic information that pointed to 7a included (i) NOESY correlations from H-8 to H-7, Me-10, and Me-11 and (ii) the absence of coupling between H-2 and H-7. Also there was no evidence of NOESY correlations between H-2 and H-7, although such information must be interpreted with caution. Structure 7a was modeled using ChemBio3D Ultra with MM2 software for energy minimization to an RMS gradient of 0.100 (Figure 3b). On the basis of the measured dihedral angles, diagnostic ${}^{3}J_{H-C}$ values were predicted by application of the Karplus equation and revealed that stereoisomer 7a should give a medium ${}^{3}J_{H8-C4}$ value (4–5 Hz) and small couplings (0-3 Hz) from H-8 to Me-10 and to



Figure 4. (a) Candidate diastereomers of acremine Q. (b) Energy-minimized model structures of the preferred diastereomer of acremine $Q_{(8a)}$ showing observed NOESY correlations and hydrogen bonding between the C-4 and C-9 hydroxy groups (dashed line).

Me-11.²¹ The ${}^{3}J_{H-C}$ values in acremine P were measured using the pulse sequence EXSIDE;²² the observed ${}^{3}J_{H8-C4}$ of 5.4 Hz and small ${}^{3}J_{H-C}$ values (<1.5 Hz) from H-8 to C-10 and C-11 were fully consistent with stereoisomer 7a.

Also isolated from the large-scale extract of A. persicinum was acremine Q (8), with a molecular formula of $C_{12}H_{18}O_5$ indicated by HRESIMS. The ¹H and ¹³C NMR spectra revealed a saturated ketone ($\delta_{\rm C}$ 205.3 (s)), an acetal ($\delta_{\rm C}$ 102.7 (s)), an epoxy group [$\delta_{\rm H}$ 3.51 (1H); 66.0 (d), 60.7 (s)], an oxymethine ($\delta_{\rm H}$ 3.98; $\delta_{\rm C}$ 86.2), and one other oxygenated carbon ($\delta_{\rm C}$ 71.3). Three methyl groups at $\delta_{\rm H}$ 1.44, 1.14, and 1.32 were linked to signals at $\delta_{\rm C}$ 14.7, 25.3, and 27.7 by HSQC, respectively. Because the molecular formula implied two rings, a comparison of HMBC data with those of 7 was undertaken, leading to the planar structure suggested. Key HMBC correlations included those from the epoxy proton (H-5) to C-3, C-4, C-6, and Me-12, from H-8 to C-3 and C-4, and from H-7 to C-4 and C-9. The signal for H-7b, which was obscured by the H₂O peak in the ¹H NMR spectra recorded in CDCl₃, was detected when the spectrum was rerun in acetone d_6 or in benzene- d_6 . In acetone- d_6 , signals for the hydroxy groups at C-4 and C-9 were observed at $\delta_{\rm H}$ 5.73 and 3.87, respectively.

The relative configuration was suggested from a combination of NOESY data, molecular modeling, and coupling constant values. Only diastereomers with a (5S, 6R) configuration were considered on the reasonable expectation that 8 would share the same configuration at C-6 as other acremines. In CDCl₃, H-3 showed couplings of 7.5, 6.0, 7.4, and 4.4 Hz to H-2a, H-2b, H-7a, and H-7b, respectively, which suggested an equatorial rather than an axial orientation for H-3. On stereoelectronic grounds, the C-4 OH was anticipated to be axial. As a consequence of the flexible nature of the *cis*-fused ring junction, the coupling constant values for H-3 represented conformationally averaged values. A key piece of information was a pronounced NOESY correlation between H-2b and H-8. Four possible diastereomers, 8a-8d, with a cis ring junction were considered (Figure 4a), and minimum energy conformations calculated using ChemBio3D Ultra with MM2 software for energy minimization to an RMS gradient of 0.100. Stereoisomers 8a and 8d had interproton distances of 2.4 and 2.7 Å between H-2b and H-8, respectively; for each of the two diastereomers 8b and 8c, the interproton distance was >4 Å. NOESY correlations between H-2b/H-7b, H-7a/Me-11, and H-7b/Me-11 were in complete agreement with either 8a or 8d. Candidate diastereomers 8a and 8d were modeled using Macromodel version 9.9 (Schrödinger LLC),²³ and the structures further optimized with Gaussian version 09,24 resulting in twelve and eight conformations within 3 kcal/mol

of the global energy minimum for **8a** and **8d**, respectively. The Boltzmann-weighted chemical shifts were calculated, but the values for **8a** and **8d** were similar and neither set could be conclusively assigned to acremine Q. However the Boltzmann-weighted proton NMR coupling constants calculated under vacuum using the method of Jain et al.²⁵ gave values that supported **8a** as the preferred diastereomer (see Table S3 in the Supporting Information). The noticeable difference in chemical shift values for the Me-10 and Me-11 signals ($\delta_{\rm H}$ 1.32 and 1.14 in CDCl₃) may be a consequence of the hydrogen bonding between the C-4 and C-9 hydroxy groups. The absolute configuration of acremine Q was assigned (3*R*, 4*R*, 5*S*, 6*R*, 8*R*) as shown in **8**. Attempted reduction of acremine Q using Pd/C in EtOAc led to a mixture of products in insufficient quantity for individual identification.

Acremine R (9) was isolated as a colorless oil from RP-HPLC and had the same molecular formula $(C_{12}H_{16}O_3)$ as that for acremine N (3) from HRESIMS measurements. The ${}^{1}H$ and ¹³C NMR spectra of this compound also revealed a close relationship to acremine N, notably the signals for a tetrasubstituted benzene ring [$\delta_{
m H}$ 6.61 (1H), 6.49 (1H); $\delta_{
m C}$ 147.8 (s), 146.3 (s), 123.7 (s), 119.2 (d), 116.8 (s), 115.6 (d)], a quaternary carbon ($\delta_{\rm C}$ 76.3), one oxymethine ($\delta_{\rm H}$ 3.73, $\delta_{\rm C}$ 69.8), one methylene group ($\delta_{\rm H}$ 2.99, 2.69; $\delta_{\rm C}$ 31.3), and three methyl singlets ($\delta_{\rm H}$ 2.17, 1.34, 1.27; $\delta_{\rm C}$ 15.7, 22.6, 24.5). Two methyl groups ($\delta_{
m H}$ 1.34, 1.27) and the oxymethine showed HMBC correlations to the quaternary carbon at $\delta_{\rm C}$ 76.3, assigned as C-9. The downfield chemical shift of C-9 suggested a chromane ring.²⁶ The signal at $\delta_{\rm C}$ 69.8 was assigned as C-8 based on HMBC correlations to Me-10 ($\delta_{\rm H}$ 1.34), Me-11 ($\delta_{\rm H}$ 1.27), and the methylene group ($\delta_{\rm H}$ 2.99, 2.67). A DQFCOSY experiment located the hydroxy group ($\delta_{\rm H}$ 1.75 d, 8.0) at C-8. When 9 was converted to its (R)- and (S)-O-methylmandelate (MPA) esters 19a and 19b, the $\Delta \delta^{RS}$ values (where $\Delta \delta^{RS} = \delta R$ $-\delta S$) were positive for H-2 and H₂-7 and negative for H-5, Me-10, Me-11, and Me-12. These data supported an 8R configuration. Also isolated from the fungal extract was acremine N (3), with ¹H and ¹³C NMR data identical to those reported by Nasini and co-workers. The $[\alpha]_{D}$ was measured as +15, in reasonable agreement with the literature value ($[\alpha]_D$ +35). On the basis of a comparison of its ECD data with those of other 2,3-dihydrobenzofurans, Nasini et al. proposed that (+)-acremine N has an 8S configuration.¹⁴

CONCLUSIONS

This study reported six new acremine metabolites, 5chloroacremine A (4), 5-chloroacremine H (5), and acremines O (6), P (7), Q (8), and R (9), together with the known acremines A (1), F (2), and N (3). The relative configuration of acremine F (2) was determined by analyses of proton coupling constant values and NOESY data, and the absolute configuration was confirmed as (1S, 4S, 6R) by X-ray crystallographic analysis of borate ester **15**. Acremines O, P, and R were all shown to have an 8*R* configuration by MPA ester determination. The relative configurations of acremines P and Q were each investigated by molecular modeling together with NOESY and coupling constant data.

EXPERIMENTAL SECTION

General Experimental Procedures. These have been reported previously.²⁷ NMR spectra were recorded at ambient probe temperature on a Bruker Avance 500 spectrometer unless otherwise stated. EXSIDE²² data were acquired on a 750 MHz Bruker Avance NMR spectrometer. In this two-dimensional experiment, a selective pulse was centered on the single proton of interest (H-8 of 7) such that proton-coupled peaks were not excited. Cross-peaks appeared as doublets at the frequency of the coupled carbon resonances in F1. A *J*-scaling factor of 15 was applied to increase the magnitude of the splitting in F1, and this enabled couplings to be obtained with a resolution of ± 0.6 Hz.

Biological Material. Sponge specimens were collected at the Gneering reef offshore from Mooloolaba, QLD, in December 2009. The sponges were cut into small pieces, rinsed three times with sterile artificial seawater (ASW, Ocean Nature), and then cut into smaller cubes. Individual cubes were smeared across malt extract and potato dextrose agar (PDA) media or were shaken with sterile glass beads in a Falcon tube containing sterile ASW (5 mL). The cell suspension was then spread onto plates containing malt extract and PDA using a sterile cotton bud. The plates were then incubated at 27 °C for 1-2 weeks. Individual fungal strains were isolated and streak purified onto new media several times. Fungal identification was undertaken using the colony polymerase chain reaction performed with an Eppendorf MasterCycler Epgradient 28 (Eppendorf). Standard reaction mixes contained 0.25 μ L of each primer designed to amplify the conserved rDNA ITS, 5 μ L of template DNA in distilled H₂O, 2.5 μ L of 10× buffer, 2 μ L of dNTPs, and 0.125 μ L of Taq polymerase, with distilled H_2O added to make 25 μL of total volume. PCR products were electrophoresed on 1× TAE 1% agarose gels, stained with ethidium bromide, and visualized on a UV transilluminator. The PCR products were purified using a QIAquick gel extraction kit (QIAGEN GmbH, Hilden DE), then sent to the Australian Genome Research Facility (AGRF) for sequencing (GenBank accession number KF 017582).

Small-Scale Fermentation. Cultures (50 mL) of *A. persicinum* were cultivated in malt extract media made in artificial seawater and were kept in a shaker incubator (180 rpm) at 28 °C for 2 weeks. The culture broths were separated from the mycelia by filtration and then extracted with EtOAc (3×50 mL). The organic layers were collected and dried over anhydrous MgSO₄, then concentrated under reduced pressure to afford brown extracts. The EtOAc extract (30 mg) was then subjected to NP flash column chromatography, eluting with hexanes/EtOAc in order of increasing polarity, to give acremine A (1) (4.3 mg) and spiroacremine B (12) (1.9 mg).

Large-Scale Fermentation. The isolated strain of A. persicinum was cultured in malt extract media (4 L) made in artificial seawater in a shaker incubator (180 rpm) at 28 °C for 4 weeks. Culture medium and mycelia were then separated by filtration, and then the broth was extracted with EtOAc (3 \times 2 L). The aqueous layer was further extracted with *n*-BuOH $(3 \times 1 \text{ L})$. The collected organic layers were then dried over anhydrous MgSO4 and concentrated in vacuo to obtain EtOAc (0.3 g) and BuOH (0.5 g) extracts. The EtOAc and BuOH extracts had a very similar profile by NMR and TLC and were combined for chemical investigation. The combined extract (0.8 g) was subjected to NP flash column chromatography, using a stepwise elution with hexanes/EtOAc, to afford 14 fractions. Fraction 6 was subjected to NP-HPLC using RI detection, eluting with 25% EtOAc/ hexanes, flow rate 2 mL/min over 40 min, to obtain acremine O (6) (0.8 mg). Fraction 7 was purified through NP-HPLC using the same procedure as for fraction 6 and yielded acremine N (3) (2.1 mg). Fractions 8 and 9 (45 mg) were combined and subjected to NP-HPLC, employing 40% EtOAc/hexanes, flow rate 2 mL/min over 40 min, to afford acremine N (3) (2.4 mg), together with a mixture of acremine R (9) and spiroacremine A (11) (7.5 mg), then a fraction containing acremine P (7) (3.0 mg), and finally acremine Q (8) (1.7 mg) in order of elution. The mixture of 9 and 11 was further purified by RP-HPLC using gradient elution from 60% to 80% MeOH/H₂O (20 min), followed by isocratic 80% MeOH/H2O (10 min), flow rate 1.5 mL/min, yielding spiroacremine A (11) (1.1 mg) and acremine R (9) (1.1 mg) in order of elution. The fraction containing acremine P (3.0 mg) was purified with NP-HPLC using isocratic EtOAc/Hex (30:70), flow rate 2 mL/min, yielded 7 (1.1 mg). Fractions 11-13 of the NP flash column chromatography were combined (0.7 g) and subjected to NP flash column chromatography, eluting with CH₂Cl₂/ MeOH in order of increasing polarity, to yield six fractions, coded F13-1 to F13-7. Fraction F13-2 (259 mg) was further chromatographed on an NP flash column employing hexanes/EtOAc in order of increasing polarity to obtain nine fractions. Acremine A (1) (137 mg) was identified in fraction 5, while the third fraction (3.8 mg) was purified on RP-HPLC using gradient elution from 10% to 40% MeOH/H₂O (20 min), followed by isocratic 40% MeOH/H₂O (20 min), flow rate 1.5 mL/min, UV 254 nm, to obtain a mixture of 5chloroacremine H (5), 5-chloroacremine A (4) (0.5 mg), and 9-Omethylacremine F (10) (1.5 mg) in order of elution. 5-Chloroacremine A (4) decomposed to 5-chlorospiroacremine (16) during storage in CDCl₃ for NMR analysis. Fraction F13-3 (154 mg) was also subjected to RP-HPLC employing isocratic 15% MeCN/H2O (20 min), followed by a gradient of 15-30% MeCN/H₂O (10 min), then isocratic 30% MeCN/H2O (10 min), flow rate 1.5 mL/min, to obtain acremine A (1) (22.3 mg), acremine F (2) (13.9 mg), and 9-Omethylacremine F (10) (17.7 mg) in order of elution.

Acremine A (1) (ref 1): colorless needles (137 mg); $[\alpha]^{24}_{D}$ +13 (*c* 2.52, MeOH), lit.¹ +22.3 (*c* 0.04, MeOH); ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS *m*/*z* 249.1102 [M + Na]⁺ (calcd for C₁₂H₁₈NaO₄, 249.1097).

Acremine F (2) (ref 1): yellow oil (19.2 mg); $[\alpha]^{24}_{D}$ +38 (c 0.47, CHCl₃), lit.¹ +56 (c 0.2, CHCl₃); ¹H and ¹³C NMR see Table 1 and Table 2; LRESIMS m/z 251.2 [M + Na]⁺.

Acremine N (3) (ref 14): brown oil (2.1 mg); $[\alpha]^{24}{}_{\rm D}$ +15 (c 0.08, CHCl₃), lit. +35 (c 0.2, MeOH); ¹H and ¹³C NMR see ref 14; LRESIMS m/z 231.1 [M + Na]⁺.

5-Chloroacremine A (4): colorless oil (0.5 mg) mixture with **5**; ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS m/z 283.0716 [M + Na]⁺ (calcd for C₁₂H₁₇ClNaO₄, 183.0708).

5-Chloroacremine H (5): colorless oil (0.5 mg) mixture with 4; ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS m/z 299.0645 $[M + Na]^+$ (calcd for C₁₂H₁₇ClNaO₅, 299.0657).

Acremine O (6): colorless oil (0.8 mg); $[\alpha]^{24}_D - 8$ (*c* 0.02, CHCl₃); ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS *m*/*z* 281.0565 [M + Na]⁺ (calcd for C₁₂H₁₅ClNaO₄, 281.0551).

Acremine P (7): colorless oil (1.1 mg); $[\alpha]^{24}{}_{\rm D}$ -43 (c 0.05, CHCl₃); ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS m/z 277.0673 [M + Na]⁺ (calcd for C₁₂H₁₄NaO₆, 277.0683).

Acremine Q (8): colorless oil (1.7 mg); $[\alpha]^{24}{}_{\mathrm{D}} -12$ (c 0.08, CHCl₃); ¹H and ¹³C NMR (CDCl₃) see Table 1 and Table 2; HRESIMS m/z 265.1042 $[M + Na]^+$ (calcd for C₁₂H₁₈NaO₅, 265.1046).

Acremine R (9): colorless oil (1.5 mg); $[\alpha]^{24}_{D} - 8$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR see Table 1 and Table 2; HRESIMS *m*/*z* 231.0991 [M + Na]⁺ (calcd for C₁₂H₁₆NaO₃, 231.09992).

9-O-Methylacremine F (10): yellow oil (17.7 mg); $[\alpha]^{24}_{D}$ +38 (*c* 1.27, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 6.09 (1H, d, *J* = 16.0, H-7), 5.90 (1H, d, *J* = 16.0, H-8), 5.61 (1H, d, *J* = 2.5, H-2), 4.43 (1H, m, H-4), 3.99 (1H, dd, *J* = 9.0, 2.5, H-1), 3.59 (1H, s, 6-OH), 3.48 (1H, d, *J* = 7.5, 4-OH), 3.16 (3H, s, OMe), 2.85 (1H, d, *J* = 9.0, 1-OH), 2.22 (1H, dd, *J* = 15.0, 2.0, H-5a), 1.80 (1H, dd, *J* = 15.0, 4.0, H-5b), 1.31 (3H, s, Me-12), 1.30 (6H, s, Me-11 and Me-10; ¹³C NMR (CDCl₃, 125 MHz) δ 137.7 (C-3), 136.0 (C-8), 130.5 (C-2), 128.7 (C-7), 75.2 (C-9), 73.0 (C-1), 71.0 (C-6), 64.1 (C-4), 50.6 (OMe),

(26.8 (Me-12), 26.0 (Me-10 and Me-11); HRESIMS m/z 265.1422 $[M + Na]^+$ (calcd for $C_{13}H_{22}NaO_4$, 265.1410).

Spiroacremine A (11) (ref 17): white solid (1.4 mg); $[\alpha]^{24}_{D}$ +15 (*c* 0.09, CHCl₃), lit.¹⁹ +18 (*c* 0.3, EtOH); ¹H and ¹³C NMR see ref 17; HRESIMS *m*/*z* 249.1125 [M + Na]⁺ (calcd for C₁₂H₁₈NaO₄, 249.1097).

Spiroacremine B (12) (ref 17): yellow oil (4.7 mg); $[\alpha]^{24}_{D} - 17$ (*c* 0.18, CHCl₃), lit.¹⁹ +4.2 (*c* 0.24, EtOH); ¹H and ¹³C NMR see ref 17; HRESIMS *m*/*z* 249.1100 [M + Na]⁺ (calcd for C₁₂H₁₈NaO₄, 249.1097).

5-Chlorospiroacremine (16): colorless oil (0.5 mg) mixture with 4 and 5; ¹H NMR (CDCl₃, 500 MHz) δ 6.70 (1H, d, *J* = 16.0, H-8), 6.49 (1H, d, *J* = 16.0, H-7), 6.18 (1H, d, *J* = 1.8, H-2), 4.60 (1H, m, H-4), 4.23 (1H, d, *J* = 8.2, H-5), 2.90 (1H, d, *J* = 4.8, 4-OH), 1.404 (3H, s, Me-10), 1.400 (3H, s, Me-11), 1.39 (3H, s, Me-12); ¹³C NMR (CDCl₃, 500 MHz) δ 198.3 (C-1), 156.2 (C-3), 149.2 (C-8), 122.7 (C-7), 121.5 (C-2), 75.9 (C-6), 72.2 (C-4), 71.3 (C-9), 70.4 (C-5), 29.6 (2C, Me-10 and Me-11), 21.0 (C-12).

Reduction of Acremine A with NaBH₄. Acremine A (1) (7.0 mg) dissolved in EtOH (1 mL) was treated with NaBH₄ (1.3 mg, 1.1 equiv) at room temperature (rt). After 1 h, 1 mL of acetone was added, followed by H_2O (2 mL) and extraction with EtOAc (3 × 2 mL). The organic layer was then collected, dried over MgSO₄, and concentrated *in vacuo* to obtain a mixture of acremine F (2) and 1-*epi*-acremine F (13) in a 2:1 ratio. The mixture (5 mg) was then subjected to RP-HPLC, UV 254 nm, using isocratic 15% MeCN/H₂O (25 min), flow rate 1.5 mL/min, to yield acremine F (2.0 mg) and 1-*epi*-acremine F (0.7 mg).

1-epi-Acremine F (13): colorless oil (0.7 mg); $[\alpha]^{24}_{D} - 18$ (*c* 0.05, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 6.24 (1H, d, *J* = 16.1, H-7), 6.16 (1H, d, *J* = 16.1, H-8), 5.88 (1H, d, *J* = 4.8, H-2), 4.49 (1H, ddd, *J* = 7.0, 4.4, 3.0, H-4), 3.92 (1H, br t, *J* = 5.4, H-1), 2.75 (1H, d, *J* = 7.0, 4-OH), 2.59 (1H, s, 6-OH), 2.01 (1H, *J* = dd, 14.4, 4.4, H-5a), 1.96 (1H, ddd, *J* = 14.4, 3.3, 1.1, H-5b), 1.53 (1H, d, *J* = 6.3, 1-OH), 1.38 (3H, s, Me-10), 1.37 (3H, s, Me-11), 1.36 (3H, s, Me-12); LRESIMS m/z 251.2 [M + Na]⁺.

Preparation of *p*-Bromophenylboronate and Borate Esters of 9-O-Methylacremine F. 9-O-Methylacremine F (10) (2.6 mg) was treated with *p*-bromophenylboronic acid (2.4 mg, 1.1 equiv) in CH_2Cl_2 (1 mL) at rt. After 24 h, the sample was evaporated under N_2 to obtain a mixed ester product (4.4 mg) containing the *p*bromophenylboronate ester of 9-O-methylacremine F (14). The mixture was then subjected to NP pipet column chromatography, employing gradient elution with hexanes/EtOAc to obtain nine fractions. Fractions 7 to 9 were combined (2.1 mg) to give a mixture of the boronate ester 14 and the borate ester 15 in a 3:2 ratio. This sample was subjected to recrystallization without further purification in EtOAc using the vapor diffusion method, yielding single crystals of the borate ester 15.

p-Bromophenylboronate ester of 9-O-Methylacremine F (14): white, amorphous solid; ¹H NMR (CDCl₃, 500 MHz) δ 7.62 (2H, m, Ph), 7.47 (2H, m, Ph), 6.06 (2H, s, H-7 and H-8), 5.61 (1H, d, J = 2.5, H-2), 4.81 (1H, t, J = 2.7, H-4), 4.14 (1H, dd, J = 11.7, 2.5, H-1), 3.18 (3H, s, OMe), 2.18 (1H, dd, J = 14.0, 3.3, H-5a), 2.16 (1H, d, J = 11.7, 1-OH), 2.04 (dd, J = 14.0, 2.4, H-5b), 1.54 (3H, s, Me-12), 1.33 (3H, s, Me-10), 1.33 (3H, s, Me-11); ¹³C NMR (CDCl₃, 500 MHz) δ 138.5 (C-3), 136.8 (C-8), 135.5 (Ph), 131.0 (Ph), 130.8 (Ph), 130.1 (C-2), 127.9 (C-7), 125.9 (Ph), 75.0 (C-9), 74.7 (C-1), 72.6 (C-6), 63.4 (C-4), 50.5 (OMe), 38.5 (C-5), 26.4 (C-11), 25.2 (C-10), 25.1 (C-12); LRESIMS *m*/*z* 429.0458/431.0848 [M + Na]⁺ (calcd for C₁₉H₂₄BBrNaO₄, 429.0849/431.0828).

Borate ester of 9-O-methylacremine F (15): colorless crystals; ¹H NMR (CDCl₃, 500 MHz) δ 6.10 (1H, d, J = 16.4, H-7), 5.92 (1H, d, J = 16.4, H-8), 5.63 (1H, d, J = 2.5, H-2), 4.47 (1H, m, H-4), 4.01 (1H, dd, J = 8.8, 2.5, H-1), 3.17 (3H, s, OMe), 2.56 (1H, dd, J = 14.9, 2.2, H-5a), 2.41 (1H, d, J = 8.8, 1-OH), 1.82 (dd, J = 14.9, 4.3, H-5b), 1.33 (3H, s, Me-12), 1.314 (3H, s, Me-10), 1.309 (3H, s, Me-11); ¹³C NMR (CDCl₃, 500 MHz) δ 137.6 (C-3), 136.1 (C-8), 130.4 (C-2), 128.5 (C-7), 75.0 (C-9), 72.9 (C-1), 70.7 (C-6), 64.0 (C-4), 50.5 (OMe), 40.4 (C-5), 26.7 (C-12), 26.0 (C-10), 26.0 (C-11); LRESIMS m/z 291.1 [M + Na]⁺; HRESIMS m/z 291.1350 [M + Na]⁺ (calcd for C₁₃H₂₁BNaO₅, 291.1374).

Crystallographic data of the borate ester 15: $C_{13}H_{21}BO_5$, *M* 268.11, *T* 293(2) K, monoclinic, space group *C2*, *a* 22.3089(7) Å, *b* 7.0719(2) Å, *c* 9.8434(3) Å, *V* 1486.28(8) Å³, D_c (*Z* = 4) 1.198 g cm⁻³, *F*(000) 576, μ (Cu K α) 0.738 mm⁻¹, 10 093 data ($2\theta_{max} = 62^{\circ}$), R_{int} 0.0204, 2282 with *I* > $2\sigma(I)$; *R* 0.0248 (obsd data), wR_2 0.0659 (all data), goodness of fit 1.068. CCDC number 928199. Data were collected on an Oxford Diffraction Gemini CCD diffractometer with Cu K α radiation (1.5418 Å). The structure was solved by direct methods and refined with SHELX.²⁸ A complete sphere of data was collected, and the absolute structure was determined by analysis of 1001 Bijvoet pairs using the method of Hooft et al.²⁹ implemented within PLATON.³⁰ The probability of the correct enantiomer (P2) was 1.000 using Student's t-statistics with a ν value of 10 and a Hooft parameter of -0.01(6). All calculations were carried out within the WinGX³¹ program, and the thermal ellipsoid plot (Figure 2) was produced with ORTEP3.³²

Preparation of MPA Esters of Acremine O (17a/17b), Acremine P (18a/18b), and Acremine R (19a/19b). Acremine O (6) (0.6 mg) was divided into two portions, and each sample (approximately 0.3 mg) was treated with either (R)- or (S)-MPA (0.4 mg, 2 equiv), followed by DCC (0.5 mg, 2 equiv) and DMAP (0.3 mg, 2 equiv) in dry CH₂Cl₂ (0.5 mL). The reaction was stirred overnight at rt, which was then filtered through a small plug of silica eluting with CHCl₃. The solvent was then dried in vacuo, and each product was then purified by RP-HPLC, eluting with 60-100% MeCN/H₂O for 35 min, flow rate 1.5 mL/min, to yield the (R)-MPA ester (17a) (0.2 mg)and (S)-MPA ester (17b) (0.2 mg). A portion of acremine P (7) was likewise divided into two, and each sample (approximately 0.4 mg) was treated with either (R)- or (S)-MPA, using the same procedures for 6 to obtain the (R)-MPA ester (18a) (0.4 mg) and (S)-MPA ester (18b) (0.4 mg). A portion of acremine R (9) (0.5 mg each) was reacted with either (R)- or (S)-MPA using the same procedure as for 6 and 7 to obtain the (R)-MPA ester (19a) (0.3 mg) and (S)-MPA ester (19b) (0.3 mg).

(*R*)-MPA ester (17a): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.46–7.35 (5H, m, MPA phenyl protons), 5.16 (1H, dd, J = 5.8, 2.0, H-8), 4.79 (1H, CH of MPA), 3.43 (3H, s, OMe of MPA), 3.22 (1H, dd, J = 14.6, 5.8, H-7a), 3.12 (1H, d. J = 16.1, H-2a), 2.89 (1H, d, J =16.1, H-2b), 2.15 (3H, s, Me-12), 1.80 (1H, dd, J = 14.6, 2.0, H-7b), 1.08 (3H, s, Me-10), 0.80 (3H, s, Me-11); LRESIMS m/z 429.2 [M + Na]⁺.

(S)-MPA ester (17b): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.46–7.35 (5H, m, MPA phenyl protons), 5.15 (1H, dd, J = 5.8, 1.5, H-8), 4.80 (1H, CH of MPA), 3.42 (3H, s, OMe of MPA), 3.19 (1H, dd, J = 15.0, 5.8, H-7a), 2.73 (1H, d. J = 16.3, H-2a), 2.51 (1H, d, J =16.3, H-2b), 2.13 (3H, s, Me-12), 1.48 (1H, dd, J = 15.0, 1.5, H-7b), 1.18 (3H, s, Me-10), 1.13 (3H, s, Me-11); LRESIMS m/z 429.2 [M + Na]⁺.

(*R*)-MPA ester (18a): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.48–7.37 (5H, phenyl protons), 6.603 (1H, s, H-7), 5.22 (1H, s, H-2), 4.83 (1H, s, CH of MPA), 4.17 (1H, s, H-8), 3.44 (3H, s, OMe of MPA), 1.51 (3H, s, Me-12), 1.45 (3H, s, Me-10), 1.42 (3H, s, Me-11); LRESIMS *m*/*z* 425.0 [M + Na]⁺.

(S)-MPA ester (18b): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.48–7.38 (5H, phenyl protons), 6.597 (1H, s, H-7), 5.19 (1H, s, H-2), 4.82 (1H, s, CH of MPA), 4.34 (1H, s, H-8), 3.43 (3H, s, OMe of MPA), 1.45 (3H, s, Me-12), 1.48 (3H, s, Me-10), 1.47 (3H, s, Me-11); LRESIMS m/z 425.0 [M + Na]⁺.

(*R*)-MPA ester (19a): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.43–7.30 (5H, m, phenyl protons), 6.58 (2H, br s, H-2 and H-5), 4.92 (1H, t, *J* = 5.5, H-8), 4.75 (1H, CH of MPA), 3.50 (3H, s, OMe of MPA), 3.03 (1H, dd, *J* = 17.3, 5.5, H-7a), 2.71 (1H, dd, *J* = 17.3, 5.5, H-7b), 1.75 (3H, s, Me-12), 1.02 (3H, s, Me-10), 0.92 (3H, s, Me-11); LRESIMS *m*/*z* 379.1 [M + Na]⁺.

(S)-MPA ester (19b): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.43–7.30 (5H, m, phenyl protons), 6.59 (1H, s, H-5), 6.37 (1H, s, H-2), 4.98 (1H, t, J = 5.1, H-8), 4.75 (1H, CH of MPA), 3.50 (3H, s, OMe of MPA), 3.87 (1H, dd, J = 17.2, 5.1, H-7a), 2.32 (1H, dd, J = 17.2, 5.1, H-7b), 1.78 (3H, s, Me-12), 1.28 (3H, s, Me-10), 1.23 (3H, s, Me-11); LRESIMS m/z 379.1 [M + Na]⁺.

Catalytic Hydrogenation of Acremine P (7) and Acremine Q (8). Acremine P (7) (0.7 mg) was first dried under N_2 followed by high-vacuum treatment overnight. The round-bottom flask (5 mL) containing the sample was flushed with N2 for about 2 min. Dry EtOAc (2 mL) was then added, and reaction flushed with H₂ gas for 2 min. Catalyst Pd/C (1.3 mg) was then added, and the reaction flushed with H₂ gas for about 5 min to remove air. After 22 h, the product was filtered through a short plug of Celite eluting with EtOAc (15 mL). The collected product was then dried under N2. When ¹H NMR showed that no reaction had occurred, hydrogenation was carried out for a further 24 h under the same conditions. The reaction was filtered through Celite eluting with EtOAc to give acremine A (1), which was purified by RP-HPLC, using a gradient elution of MeCN/H2O, to obtain acremine A (1) (0.5 mg). Acremine Q (0.5 mg) was treated similarly, but the reaction product (0.5 mg) obtained contained a mixture of products.

ASSOCIATED CONTENT

S Supporting Information

Figures S1–S25. ¹H and selected 2D NMR data for compounds 1-19, molecular modeling details for 2 and 8, and crystallographic data for the borate ester 15. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Assante, G.; Dallavalle, S.; Malpezzi, L.; Nasini, G.; Burruano, S.; Torta, L. *Tetrahedron* **2005**, *61*, 7686–7692.

(2) Evans, L.; Gedger, J. N.; Brayford, D.; Stavri, M.; Smith, E.; O'Donnell, G.; Gray, A. I.; Griffith, G. W.; Gibbons, S. *Phytochemistry* **2006**, *67*, 2110–2114.

(3) Isaka, M.; Palasarn, S.; Auncharoen, P.; Kmowijit, S.; Jones, E. B. G. *Tetrahedron Lett.* **2009**, *50*, 284–287.

(4) Julianti, E.; Oh, H.; Lee, H.-S.; Oh, D.-C.; Oh, K.-B.; Shin, J. *Tetrahedron Lett.* **2012**, *53*, 2885–2886.

(5) Boot, C. M.; Tenney, K.; Valeriote, F. A.; Crews, P. J. Nat. Prod. 2006, 69, 83–92.

(6) Pontius, A.; Mohamed, I.; Krick, A.; Kehraus, S.; König, G. M. J. Nat. Prod. 2008, 71, 272–274.

(7) Patterson, E. L.; Van Meter, J. C.; Bohonos, N. J. Med. Chem. 1964, 7, 689.

(8) Abdel-Lateff, A.; König, G. M.; Fisch, K. M.; Höller, U.; Jones, P. G.; Wright, A. D. J. Nat. Prod. 2002, 65, 1605–1611.

(9) Belofsky, G. N.; Anguera, M.; Jensen, P. R.; Fenical, W.; Köck, M. *Chem. Eur. J.* **2000**, *6*, 1335–1360.

(10) Ratnayake, R.; Fremlin, L. J.; Lacey, E.; Gill, J. H.; Capon, R. J. J. Nat. Prod. 2008, 71, 403–408.

(11) Chen, Z.; Song, Y.; Chen, Y.; Huang, H.; Zhang, W.; Ju, J. J. Nat. Prod. 2012, 75, 1215–1219.

(12) Julianti, E.; Oh, H.; Jang, K. W.; Lee, K. J.; Lee, S. K.; Oh, D.-C.; Oh, K.-B.; Shin, J. J. Nat. Prod. **2011**, 74, 2592–2594.

(13) Arnone, A.; Nasini, G.; Panzeri, W.; de Pava, O. V.; Malpezzi, L.J. Nat. Prod. 2008, 71, 146–149.

(14) Arnone, A.; Assante, G.; Bava, A.; Dallavalle, S.; Nasini, G. *Tetrahedron* **2009**, *65*, 786–791.

(15) Rehman, A.; Malik, A.; Riaz, N.; Nawaz, H. R.; Ahmad, H.; Nawaz, S. A.; Choudhary, M. I. *J. Nat. Prod.* **2004**, *67*, 1450–1454.

(16) Hoarau, C.; Pettus, T. R. R. Org. Lett. 2006, 8, 2843–2846.
 (17) Smetanina, O. F.; Yurchenko, A. N.; Kalinovskii, A. I.;

Berdyshev, D. V.; Gerasimenko, A. V.; Pivkin, M. V.; Slinkina, N. N.; Dmitrenok, P. S.; Menzorova, N. I.; Kuznetsova, T. A.; Afiyatullov, S. S. *Chem. Nat. Compd.* **2011**, *47*, 385–390.

(18) Mehta, G.; Kumar, Y. C. S.; Khan, T. B. *Tetrahedron Lett.* **2010**, *51*, 5112–5115.

(19) Abraham, R. J.; Chambers, E. J.; Thomas, W. A. J. Chem. Soc., Perkin Trans. 1993, 2, 1061–1066.

(20) Bishop, M.; Shahid, N.; Yang, J.; Barron, A. R. Dalton Trans. 2004, 33, 2621–2634.

(21) Bifulco, G.; Dambruoso, P.; Gomez-Paloma, L.; Riccio, R. Chem. Rev. 2007, 107, 3744–3779.

(22) Krishnamurthy, V. V. J. Magn. Reson. 1996, 121A, 33-41.

(23) MacroModel, version 9.9; Schrödinger LLC: New York, NY, 2012.

(24) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennuci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, P.; Ixmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jamarillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision A.02; Gaussian, Inc.: Wallingford, CT, 2009.

(25) Jain, R.; Bally, T.; Rablen, P. R. J. Org. Chem. 2011, 74, 4017–4023.

(26) Zainuddin, N.; Alias, S. A.; Lee, C. W.; Ebel, R.; Othman, N. A.; Mukhtar, M. R.; Awang, K. *Bot. Mar.* **2010**, *53*, 507–513.

(27) Yong, K. W. L.; De Voss, J. J.; Hooper, J. N. A.; Garson, M. J. J. Nat. Prod. **2011**, 74, 194–207.

(28) Sheldrick, G. M. Acta Crystallogr., Sect. A 2008, 64, 112.

(29) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. J. Appl. Crystallogr. 2008, 41, 96-103.

(30) Spek, A. L. PLATON, A Multipurpose Crystallographic Tool; Utrecht University: Utrecht, The Netherlands, 1998.

(31) Farrugia, L. J. J. Appl. Crystallogr. 1999, 32, 837-838.

(32) Farrugia, L. J. J. Appl. Crystallogr. 1997, 30, 565.