Secondary Metabolites of the Sponge-Derived Fungus *Acremonium persicinum*

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ABSTRACT

This study reports the isolation and characterization of six new acremine metabolites, 5-chloroacremine A (**4**), 5-chloroacremine H (**5**), 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 ^{1}H 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 $^{3}J_{\text{H-C}}$ values in acremine P were measured using the pulse sequence EXSIDE, and the observed $^{3}J_{\text{H8-C4}}$ of 5.4 Hz and small $^{3}J_{\text{H-C}}$ values (<1.5 Hz) 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**.

Fungi from the genus *Acremonium* have been reported from both terrestrial[1-3] and marine sources[4-6] and produce unique and biologically active secondary metabolites, the most well known being the antibiotic cephalosporin C.[7] Antioxidant hydroquinone derivatives[8] and a chlorinated polyketide[6] 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]

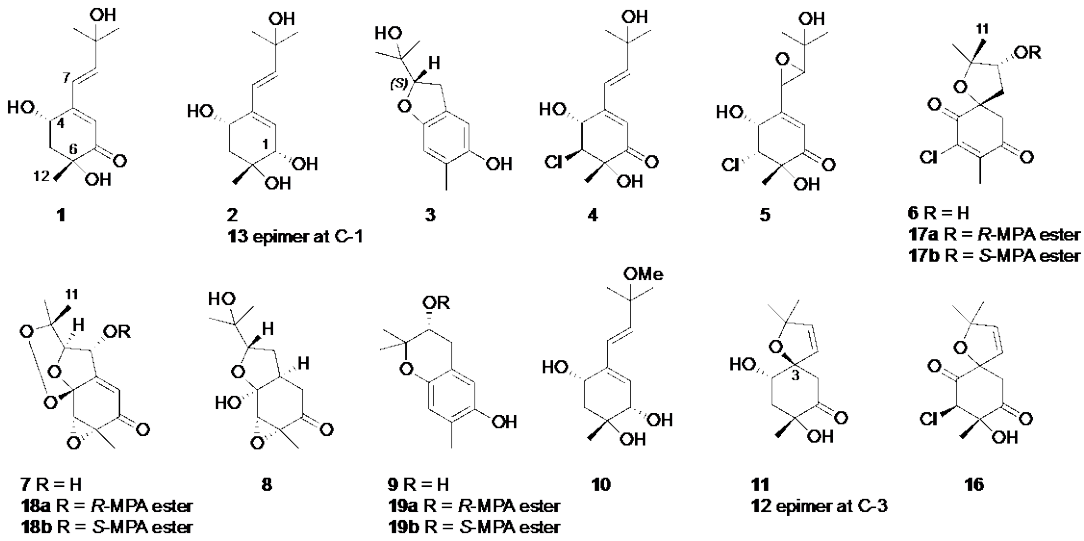
In 2005, Nasini et al. reported a series of 12 meroterpenoids, including acremines A (**1**), F (**2**), and N (**3**), from an endophytic strain of *Acremonium byssoides* isolated from sporangiospores of *Plasmopara viticola* in grapevine leaves.[1,13,14] Malik et al. described the isolation of a "norterpenoid" 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 ^{1}H NMR data of isoacremine D reported by the Russian group were identical to those of acremine D described by the Italian researchers,[1] the ^{13}C 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 carried out in malt extract media (4 L) made up in artificial sea water. 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*-methyl-acremine F (**10**), and spiroacremines A (**11**) and B (**12**) in addition to the known acremines A (**1**), F (**2**), and N (**3**).



The ^{1}H and ^{13}C NMR data (Tables 1 and 2) for acremine A (**1**), the major component in the extract, are reported in $CDCl_3$, while the ^{1}H 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.

Table 1. ^{1}H NMR Assignments for Acremines **1, 2**, **4-9**[a,b]

Position	1	2	4	5	6	7	8	9
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) b) 3.02, d (16.1)	5.20, s	a) 2.90, dd (16.0, 7.5) b) 1.98, dd (16.0, 6.0)	6.49, s
3							2.73, m	
4	4.63, m	4.43, m	4.60, m	4.78, m				
5	a) 2.36, dd (13.0, 5.1) b) 2.10, dd (13.0, 8.8)	a) 2.24, dd (14.5, 2.0) b) 1.80, dd (14.5, 4.0)	4.23, d (8.2)	4.21, d (5.7)		3.57, s	3.51, s	6.61, s
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) b) 1.84, dd (13.7, 4.0)	5.83, br d (8.8)	a) 2.34, ddd (13.1, 7.4, 7.0) b)1.54[c]	a) 2.99, dd (16.8, 5.0) 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)				
6-OH	3.41, s	3.44, br s						
7-OH						3.17, d (8.8)		
8-OH								1.75, d (8.0)

[a] Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.26). [b] At 500 MHz. [c] Obscured by H_2O peak, assigned by DQFCOSY.

Table 2. ^{13}C NMR Assignments for Acremines **1**, **2**, **4-9**[a,b]

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

[a] Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.16). [b] At 500 MHz.

A full NMR assignment of acremine F (**2**) was undertaken using HSQC and HMBC experiments as the ^{13}C NMR data were not available from the literature. The signals for H-7 and H-8 were observed at δ_H 6.19 and 6.08, respectively; on this basis the literature assignments for H-7 (δ_H 6.09) and H-8 (δ_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 $NaBH_4$ in EtOH gave a mixture of two compounds in a 2:1 ratio that were separated by RP HPLC using 15% MeCN/H_2O 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 (δ_H 5.88 (J = 4.8 Hz) vs. δ_H 5.61 (J = 2.5 Hz) in acremine F), and was assigned as the C-1 epimer of acremine F (**13**).

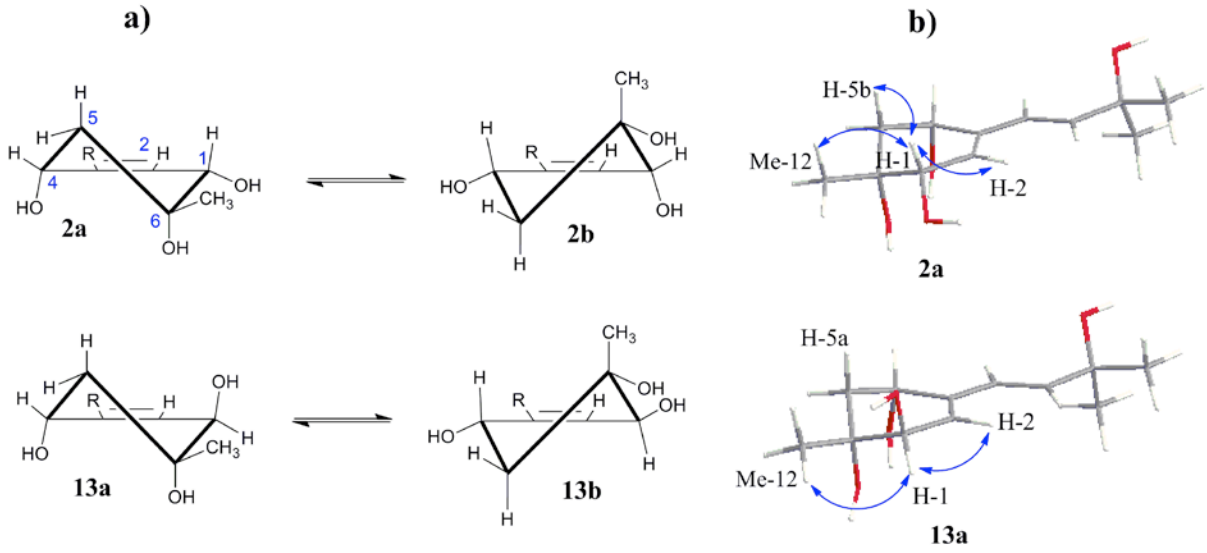


Figure 1. a, Cyclohexene ring conformers for acremine F (**2**) and 1-*epi*-acremine F (**13**) (R = sidechain); **b,** Energy-minimized model structures of **2a** and **13a** showing NOESY correlations observed for **2** and **13**.

The conformational equilibria for acremine F (**2**) and 1-*epi*-acremine 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 Supporting Information), and placed H-1 and H-4 in pseudo-axial and pseudo-equatorial orientations, respectively. Consistent with this interpretation, 1D-NOESY irradiation of the proton signal at δ_H 4.00 (H-1) of **2** led to enhancement of H-2, H-5b, and Me-12 (Figure 1b). For 1-*epi*-acremine 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 matchedtheoretical values anticipated for conformer **13a** in which both H-1 and H-4 were pseudo-equatorial. 1D NOESY irradiation of the signal at δ_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 non-polar 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*-methyl-acremine 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 ^{1}H NMR data were very similar to those of **2**, except for a singlet at δ_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 methanol to form **10**. Treatment of **10** with *p*-bromophenylboronic acid (1.1 equiv.) in CH_2Cl_2 for 24h 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 ^{1}H 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 $C_{13}H_{21}BO_5$. Boric acid is commonly found as a contaminant in commercial samples of boronic acid and related substances.[20] The absolute configuration of **15** was determined by the anomalous dispersion method on a highly redundant data set collected with Cu-Kα radiation and confirmed a (1*S*, 4*S*, 6*R*) configuration (Figure 2).

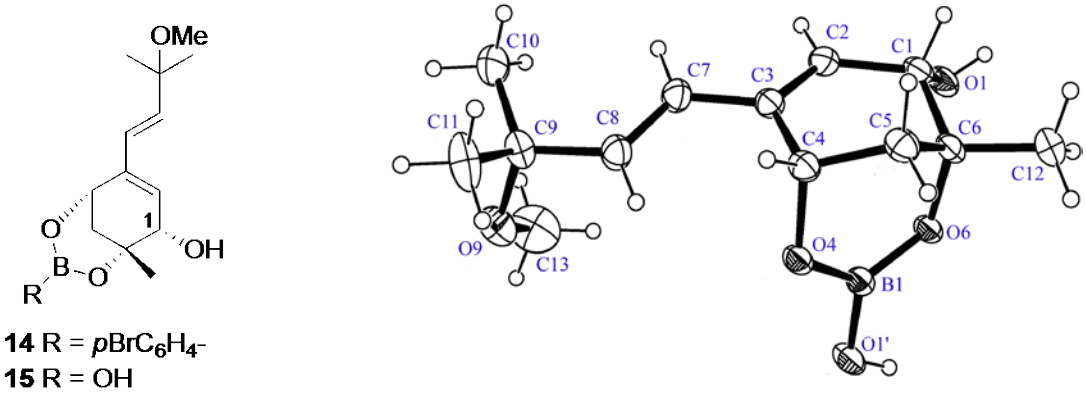


Figure 2. *ORTEP* view of borate ester **15** showing absolute and relative configuration.

5-Chloroacremine A (**4**) and 5-chloroacremine H (**5**) were obtained from RP-HPLC as a mixture in a 1:1 ratio; ESI-MS 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 formulae as $C_{12}H_{17}ClO_4$ and $C_{12}H_{17}ClO_5$, respectively. The ^{1}H and ^{13}C NMR data of 5-chloroacremine A suggested a substituted cyclohexenone ring [δ_H 6.18 (1H), 4.60 (1H), 4.23 (1H); δ_C 198.3 (s), 121.5 (d), 156.2 (s), 72.2 (d), 70.4 (d) and 75.9 (s)] with three methyl groups [δ_H 1.404, 1.400, 1.39; δ_C 29.6, 29.6, 21.0], closely resembling those of **1**[1] except that the methylene group at C-5 was replaced by a methine (δ_H 4.23, δ_C 70.4). The chlorine substituent was placed at C-5 based on HMBC correlations from H-5 to both C-4 (δ_C 72.2) and C-6 (δ_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 δ_H 6.49 and 6.70, each a doublet with *J* of 16 Hz, and linked to signals at δ_C 122.7 and 149.2 by HSQC, respectively, confirmed a *trans* double bond; HMBC correlations from the alkene proton at δ_H 6.70 to C-9, Me-10 and to Me-11 positioned the alkene between C-7 and C-8.

Based on comparison of its ^{1}H and ^{13}C NMR data with those of **4**, 5-chloroacremine H (**5**) was likewise a chlorinated cyclohexenone, however the prenyl side chain contained an epoxy group (δ_H 3.94, 2.99; δ_C 53.0, 67.9) whose position was determined by HMBC correlations from both Me-10 and Me-11 to the epoxy carbon at δ_C 67.9, as well as from the epoxy proton (δ_H 3.94) to the olefinic carbons (C-2 and C-3). HMBC correlations from the methine proton (δ_H 4.21) to C-1 (δ_C 195.5), C-3 (δ_C 155.7), C-4 (δ_C 70.5) and C-6 (δ_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 centres 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 (δ_H 3.94) to the value of H-7 reported for acremine H (δ_H 3.93)[14] may suggest the same diastereomer.

During NMR analysis of the mixture of **4**/**5** in $CDCl_3$, it was noticed that the signal intensity corresponding to 5-chloroacremine A decreased over time, while signals for a *cis* double bond (δ_H 5.96 and 5.60, d 5.9 Hz), two methines (δ_H 4.18 and 3.84), an AB methylene (δ_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 artefacts,[17] the new product was identified as the spiro compound **16**. The relative configuration at the spiro centre was not determined due to the small amount of material available.

The final chlorinated compound isolated from the *A. persicinum* extract was named as 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 $C_{12}H_{15}ClO_4$ was determined by HRESIMS. The ^1H NMR spectrum showed signals for one oxymethine (δ_H 4.15), two methylene groups (δ_H 3.26, 3.02 and δ_H 3.01, 1.84) and three methyl singlets (δ_H 2.16, 1.27, 1.16). In the ^{13}C NMR spectrum, there were two carbonyl groups (δ_C 189.1, 192.6), while the downfield chemical shift of C-3 (δ_C 83.4) suggested a spiro ring.[17] The carbonyl at δ_C 192.6 was placed at C-1 based on an HMBC correlation to Me-12 (δ_H 2.16), while the carbonyl group at δ_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 (δ_H 1.27, 1.16) to C-8 (δ_C 78.0). As in **4** and **5**, the chlorine substituent was therefore placed at C-5. The relative configuration of the spiro centre followed from NOESY correlations between Me-11/H-8 and Me-11/H-7b which suggested that these protons were all on the same face. Similarly, H-2b and H-7a were on the same face based on the observed NOESY correlations between these two protons, but

opposite to H-8 and Me-11. When **6** was esterified at C-8 to its (*R*)- and (*S*)-*O*-methyl mandelate (MPA) esters **17a** and **17b**, the $\Delta\delta^{RS}$ values (where $\Delta\delta^{RS} = \delta R - \delta S$) were positive for H_2-2 and H_2-7, while negative for Me-10 and Me-11, which was consistent with an 8*R* configuration. The absolute configuration of acremine O is thus (3*R*, 8*R*). The natural product status of acremine O is unclear.

A molecular formula of $C_{12}H_{14}O_6$ was determined for acremine P (**7**) from the adduct ion at *m*/*z* 277.0673 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra supported the presence of a substituted cyclohexenone ring [δ_H 5.20 (1H); δ_C 192.3 (s), 162.5 (s), 102.4] containing epoxy [δ_H 3.57 (1H); 59.1 (d), 57.4 (s)] and acetal (δ_C 99.0) groups. Three methyl groups observed at δ_H 1.51, 1.47, and 1.43 were linked to signals at δ_C 14.5, 25.8 and 23.4 by HSQC, respectively, and there were two oxymethines (δ_H 5.83, 4.15; δ_C 95.0, 86.2) and one other oxygenated carbon (δ_C 78.2). HMBC correlations from the epoxy proton to C-3 (δ_C 162.5), C-4 (δ_C 99.0), C-6 (δ_C 57.4) and Me-12 (δ_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 δ_C 99.0 which was therefore assigned to C-4. A hydroxy group (δ_H 3.17) was located at C-7 from its coupling to H-7 and by HMBC correlations to C-7 (δ_C 95.0) and C-8 (δ_C 86.2), therefore an ether linkage between C-4 and C-8 accounted for the HMBC correlation between H-8 and C-4. Based on 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 (H_2, 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*-methyl mandelate (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

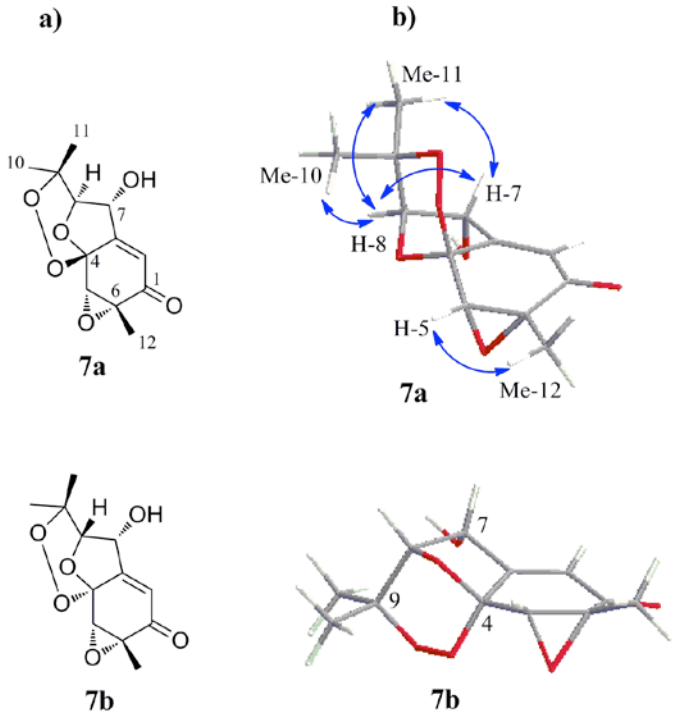


Figure 3. a, Structures of candidate diastereomers **7a** and **7b** for acremine P; **b,** Energy-minimized model structures of diastereomers of acremine P showing NOESY correlations for preferred conformer **7a**.

2,3,8-trioxabicyclo[3.2.1]octane ring system, two candidate diastereomers **7a** and **7b** (Figure 3a) were next considered. In the ^{1}H NMR spectrum of **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 approx. 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; (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). Based on the measured dihedral angles, diagnostic $^{3}J_{\text{H-C}}$ values were

predicted by application of the Karplus equation, and revealed that stereoisomer **7a** should give a medium $^{3}J_{H8\text{-}C4}$ value (4-5 Hz) and small couplings (0-3 Hz) from H-8 to Me-10 and to Me-11.[21] The $^{3}J_{H\text{-}C}$ values in acremine P were measured using the pulse sequence EXSIDE;[22] the observed $^{3}J_{H8\text{-}C4}$ of 5.4 Hz and small $^{3}J_{H\text{-}C}$ values (<1.5 Hz) 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 ^{1}H and ^{13}C NMR spectra revealed a saturated ketone (δ_C 205.3 (s)), an acetal (δ_C 102.7 (s)), an epoxy group [δ_H 3.51 (1H); 66.0 (d), 60.7 (s)], an oxymethine (δ_H 3.98; δ_C 86.2) and one other oxygenated carbon (δ_C 71.3). Three methyl groups at δ_H 1.44, 1.14, and 1.32 were linked to signals at δ_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 from the epoxy proton (H-5) to C-3, C-4, C-6, and to 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-7a, which was obscured by the H_2O peak in the ^{1}H NMR spectra recorded in $CDCl_3$, 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 δ_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 (5*S*, 6*R*) configuration were considered on the reasonable expectation that **8** would share the same configuration at C-6 as other acremines. In $CDCl_3$, 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, that 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 inter-proton distances of 2.4 Å and 2.7 Å between H-2b and H-8, respectively; for each of the two diastereomers **8b**–**8c**, the inter-proton 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 modelled using Macromodel version 9.9 (New York)[23] and the structures further optimized with Gaussian version 09 (Wallingford),[24] resulting in 12 and 8 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 not be conclusively assigned to acremine Q. However the Boltzmann weighted proton NMR coupling constants calculated in vacuum using the method of Jain *et al.*[25] gave values that supported **8a** as the preferred diasteromer. (see Table S3 in Supporting Information). The noticeable difference in chemical shift values for the Me-10 and Me-11 signals (δ_H 1.32 and 1.14 in $CDCl_3$) may be a consequence of the hydrogen bonding between the C-4 and C-9 hydroxy groups. The

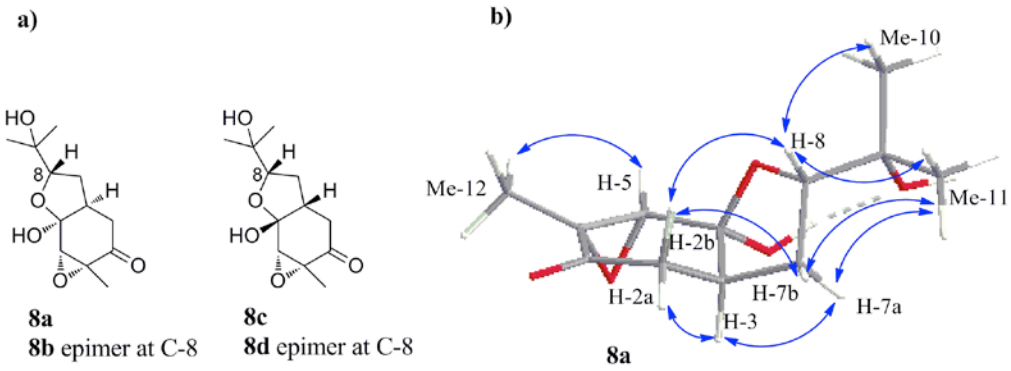


Figure 4. a, Candidate diastereomers of acremine Q; **b,** Energy-minimized model structures of preferred diastereomer of acremine Q (**8a**) showing observed NOESY correlations.

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 ^{13}C NMR spectra of this compound also revealed a close relationship to acremine N, notably the signals for a tetrasubstituted benzene ring [δ_H 6.61 (1H), 6.49 (1H); δ_C 147.8 (s), 146.3 (s), 123.7 (s), 119.2 (d), 116.8 (s), and 115.6 (d)], a quaternary carbon (δ_C 76.3), one oxymethine (δ_H 3.73, δ_C 69.8), one methylene group (δ_H 2.99, 2.69; δ_C 31.3) and three methyl singlets (δ_H 2.17, 1.34, 1.27; δ_C 15.7, 22.6, 24.5). Two methyl groups (δ_H 1.34, 1.27) and the oxymethine showed HMBC correlations to the quaternary carbon at δ_C 76.3, assigned as C-9. The downfield chemical shift of C-9 suggested a chromane ring.[26] The signal at δ_C 69.8 was assigned as C-8 based on HMBC correlations to Me-10 (δ_H 1.34), Me-11 (δ_H 1.27) and to the methylene group (δ_H 2.99, 2.67). A DQFCOSY experiment located the hydroxy group (δ_H 1.75 d, 8.0) at C-8. When **9** was converted to its (*R*)- and (*S*)-*O*-methyl mandelate (MPA) esters **19a** and **19b**, the $\Delta\delta^{RS}$ values (where $\Delta\delta^{RS} = \delta R - \delta S$) were positive for H-2 and H_2-7 and negative for H-5, Me-10, Me-11 and Me-12. These data supported an 8*R* configuration. Also isolated from the fungal extract was acremine N (**3**) with ^{1}H and ^{13}C 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). Based on a comparison of its ECD data with those of other 2,3-dihydrobenzofurans, Nasini et al proposed that (+)-acremine N has an 8*S* configuration.[14]

CONCLUSIONS

This study reported six new acremine metabolites, 5-chloroacremine A (**4**), 5-chloroacremine H (**5**), 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 confirmed as (1*S*, 4*S*, 6*R*) by X-ray crystallographic analysis of borate ester **15**. Acremines O, P and R were all shown to be of 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. EXSIDE[22] 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 ºC 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 μL of each primer designed to amplify the conserved rDNA ITS, 5 μL template DNA in distilled H_2O, 2.5 μL of 10× Buffer, 2 μL dNTPs and 0.125 μL Taq polymerase and added distilled H_2O 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 x 50 mL). The organic layers were collected and dried over anhydrous $MgSO_4$, 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**)[17] (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 x 2 L). The aqueous layer was further extracted with *n*-BuOH (3 x 1L). The collected organic layers were then dried over anhydrous $MgSO_4$ and concentrated under *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 60-80% MeOH/H_2O (20 min), followed by isocratic 80% MeOH/H_2O (10 min), flow rate 1.5 mL/min, and 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, yielding **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 CH_2Cl_2/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 NP-flash column employing hexanes/EtOAc in order of increasing polarity to obtain nine fractions. Acremine A (**1**) (137 mg) was identified in fraction five, while the third fraction (3.8 mg) was purified on RP-HPLC using gradient elution from 10-40% MeOH/H_2O (20 min), followed by isocratic 40% MeOH/H_2O (20 min), flow rate 1.5 mL/min, UV 254 nm to obtain a mixture of 5-chloroacremine H (**5**), 5-chloroacremine A (**4**) (0.5 mg) and 9-*O*-methyl acremine F (**10**) (1.5 mg) in order of elution. 5-Chloroacremine A (**4**) decomposed to 5-chlorospiroacremine (**16**) during storage in $CDCl_3$ for NMR analysis. Fraction F13-3 (154 mg) was also subjected to RP-HPLC employing isocratic 15% MeCN/H_2O (20 min),

followed by a gradient of 15-30% MeCN/H_2O (10 min), then isocratic 30% MeCN/H_2O (10 min), flow rate 1.5 mL/min to obtain acremine A (**1**) (22.3 mg), acremine F (**2**) (13.9 mg) and 9-*O*-methyl acremine F (**10**) (17.7 mg) in order of elution.

Acremine A (1):[1] colorless needles (137 mg); $[\alpha]^{24}_D$ +13 (*c* 2.52, MeOH), Lit[1] +22.3 (*c* 0.04, MeOH); ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 249.1102 $[M+Na]^+$ (calcd for $C_{12}H_{18}NaO_4$, 249.1097).

Acremine F (2):[1] yellow oil (19.2 mg); $[\alpha]^{24}_D$ +38 (*c* 0.47, $CHCl_3$), Lit[1] +56 (*c* 0.2, $CHCl_3$); ^{1}H and ^{13}C NMR see Table 1 and Table 2; LRESIMS *m/z* 251.2 $[M+Na]^+$.

Acremine N (3):[14] brown oil (2.1 mg); $[\alpha]^{24}_D$ +15 (*c* 0.08, $CHCl_3$), Lit +35 (*c* 0.2, MeOH); ^{1}H and ^{13}C NMR see ref 14; LRESIMS *m/z* 231.1 $[M+Na]^+$.

5-Chloroacremine A (4): colorless oil (0.5 mg) mixture with **5**; ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 283.0716 $[M+Na]^+$ (calcd for $C_{12}H_{17}ClO_4Na$, 183.0708).

5-Chloroacremine H (5): colorless oil (0.5 mg) mixture with **4**; ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 299.0645 $[M+Na]^+$ (calcd for $C_{12}H_{17}ClO_5Na$, 299.0657).

Acremine O (6): colorless oil (0.8 mg); $[\alpha]^{24}_D$ -8 (*c* 0.02, $CHCl_3$); ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 281.0565 $[M+Na]^+$ (calcd for $C_{12}H_{15}O_4ClNa$, 281.0551).

Acremine P (7): colorless oil (1.1 mg); $\alpha]^{24}_D$ -43 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 277.0673 $[M+Na]^+$ (calcd for $C_{12}H_{14}O_6Na$, 277.0683).

Acremine Q (8): colorless oil (1.7 mg); $[\alpha]^{24}_D$ -12 (*c* 0.08, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1 and Table 2; HRESIMS *m/z* 265.1042 $[M+Na]^+$ (calcd for $C_{12}H_{18}O_5Na$, 265.1046).

Acremine R (9): colorless oil (1.5 mg); $[\alpha]^{24}_D$ -8 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR see Table 1 and Table 2; HRESIMS *m/z* 231.0991 $[M+Na]^+$ (calcd for $C_{12}H_{16}O_3Na$, 231.09992).

9-*O*-Methyl Acremine F (10): yellow oil (17.7 mg); $[\alpha]^{24}_D$ +38 (*c* 1.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 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; ^{13}C NMR ($CDCl_3$, 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 $C_{13}H_{22}O_4Na$, 265.1410).

Spiroacremine A (11):[17] white solid (1.4 mg); $[\alpha]^{24}_D$ +15 (*c* 0.09, $CHCl_3$), Lit[19] +18 (*c* 0.3, EtOH); ^{1}H and ^{13}C NMR see ref 17; HRESIMS *m/z* 249.1125 $[M+Na]^+$ (calcd for $C_{12}H_{18}O_4Na$, 249.1097).

Spiroacremine B (12):[17] yellow oil (4.7 mg); $[\alpha]^{24}_D$ -17 (*c* 0.18, $CHCl_3$), Lit[19] +4.2 (*c* 0.24, EtOH); ^{1}H and ^{13}C NMR see ref 17; HRESIMS *m/z* 249.1100 $[M+Na]^+$ (calcd for $C_{12}H_{18}O_4Na$, 249.1097).

5-Chlorospiroacremine (16): colorless oil (0.5 mg) mixture with **4** and **5**; ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 & Me-11), 21.0 (C-12).

Reduction of Acremine A with $NaBH_4$. Acremine A (**1**) (7.0 mg) dissolved in EtOH (1 mL) was treated with $NaBH_4$ (1.3 mg, 1.1 equiv) at room temperature (rt). After 1 h, 1 mL acetone was added, followed by H_2O (2 mL), and extraction with EtOAc (3 x 2mL). The organic layer was then collected, dried over $MgSO_4$ and concentrated under *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/H_2O (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, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 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*-Methyl Acremine F. 9-*O*-Methyl-acremine F (**10**) (2.6 mg) was treated with *p*-bromophenyl boronic 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*-methyl-acremine F (**14**). The mixture was then subjected to NP-pipette 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*-Methyl Acremine F (14)**: white amorphous solid; ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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* 429.2/431.1

$[M+Na]^+$, 445.1/457.1 $[M+K]^+$. HRESIMS *m/z* 429.0858/431.0848 $[M+Na]^+$ (calcd for $C_{19}H_{24}BBrO_4Na$, 429.0849/431.0828).

Borate Ester of 9-*O*-Methyl Acremine F (15): colorless crystals; ^1H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{13}H_{21}BO_5Na$, 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 *C* 2, *a* 22.3089 (7) Å, *b* 7.0719 (2) Å, *c* 9.8434 (3) Å, *V* 1486.28 (8) $Å^3$, D*c* (Z = 4) 1.198 g cm^{-3}, F(*000*) 576, μ(Cu-Kα) 0.738 mm^{-1}, 10093 data ($2\theta_{max}$ = 62°), R_{int} 0.0204, 2282 with $I>2\sigma(I)$; *R* 0.0248 (obs. 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-Ka radiation (1.5418 Å). The structure was solved by direct methods and refined with SHELX.[28] A complete sphere of data were 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 WinGX[31] 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, 2equiv) in dry CH_2Cl_2 (0.5 mL). The reaction was stirred overnight at rt, which was then filtered through a small plug of silica eluting with $CHCl_3$. The solvent was then dried in *vacuo*, and each product was then purified by RP-HPLC, eluting with 60-100% MeCN/H_2O 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; ^{1}H NMR ($CDCl_3$, 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; ^{1}H NMR ($CDCl_3$, 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; ^{1}H NMR ($CDCl_3$, 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; ^{1}H NMR ($CDCl_3$, 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; ^{1}H NMR ($CDCl_3$, 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; ^{1}H NMR ($CDCl_3$, 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 N_2 for about 2 min. Dry EtOAc (2 mL) was then added, and reaction flushed with H_2 gas for 2 min. Catalyst Pd/C (1.3 mg) was then added, and the reaction flushed with H_2 gas for about 5 min to remove air. After 22h, the product was filtered through a short plug of celite eluting with EtOAc (15 mL). The collected product was then dried under N_2. When ^{1}H 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/H_2O 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

Supporting Information. Figures **S1**-**S25**. ^{1}H 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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GRAPHICAL ABSTRACT