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Acremines H–N, novel prenylated polyketide metabolites produced by a strain of *Acremonium byssoides*☆

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ABSTRACT

Five novel metabolites, acremines H–N, have been isolated from malt extract–peptone–glucose agar cultures of a strain of *Acremonium byssoides*. Their structures and stereochemistry were elucidated using a combination of ^{13}C and ^{1}H homo and heteronuclear 2D NMR experiments. Acremines H–N inhibited the germination of sporangia of *Plasmopara viticola*.

1. Introduction

Endophytic fungi are a source of intelligent screening, as they grow within the plant hosts in a continuum of interactions with respect to physiological status, colonization pattern and secondary metabolism.[2]

Many endophytic species of the genus *Acremonium* have been proved to be a rich source of biologically active metabolites, i.e., prenylated phenol inhibitors of N-SMase.[3] In a recent investigation, *Acremonium byssoides* was isolated as a residential endophyte in the grapevines of a Sicilian vineyard, never treated with fungicides, and the fungus was found to parasitize *Plasmopara viticola*, growing and sporulating into sporangiophores and sporangia.[4] As a part of a program carried out to study new bioactive metabolites produced by this species, we have recently isolated from *A. byssoides* strain A 20, cultured on corn-step-agar (CSA), a series of structurally related metabolites, acremines A (**1**), B (**2**), C (**6**), D–F, biosynthetically derived from a monoterpene unit and a polyketide moiety.[4] Successively, from the same cultures on CSA medium, acremine G, a new dimeric metabolite generated from acremines A (**1**) and B (**2**) by a Diels–Alder reaction and successive oxidative coupling, was isolated, although in a small amount.[1]

We describe herein the isolation of new acremines (H–N) produced by the fungus on a different culture medium, composed by malt extract–peptone and glucose agar.

2. Results and discussion

A. byssoides A 20 was cultured on malt–peptone–glucose (MPG) agar for 3 weeks and the metabolites were extracted with EtOAc. The crude extracts were submitted to successive chromatographic fractionation and purification, yielding a family of five novel compounds, named acremines H (**3**), I (**4**), L (**5a**), M (**7**) and N (**8**), besides the known acremines B (**2**) and C (**6**) (Scheme 1).

Structure assignments of the isolated compounds were based on spectroscopic data, especially those from NMR and MS analysis, and chemical reactions.

Acremine H (**3**) was isolated as a colourless solid, mp 110–112 °C; $[\alpha]_D$ +22 (*c* 0.06, $CHCl_3$); the CIMS showed an $[MH]^+$ at *m*/*z* 243 corresponding to a molecular formula $C_{12}H_{18}O_5$, confirmed by analysis. Based on ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2), the structural features of **3** were remarkably similar to those of acremine A (**1**), the only difference being the presence in **3** of an epoxy ring in place of the C(1′)H=C(2′)H double bond.

☆ See Ref. 1.

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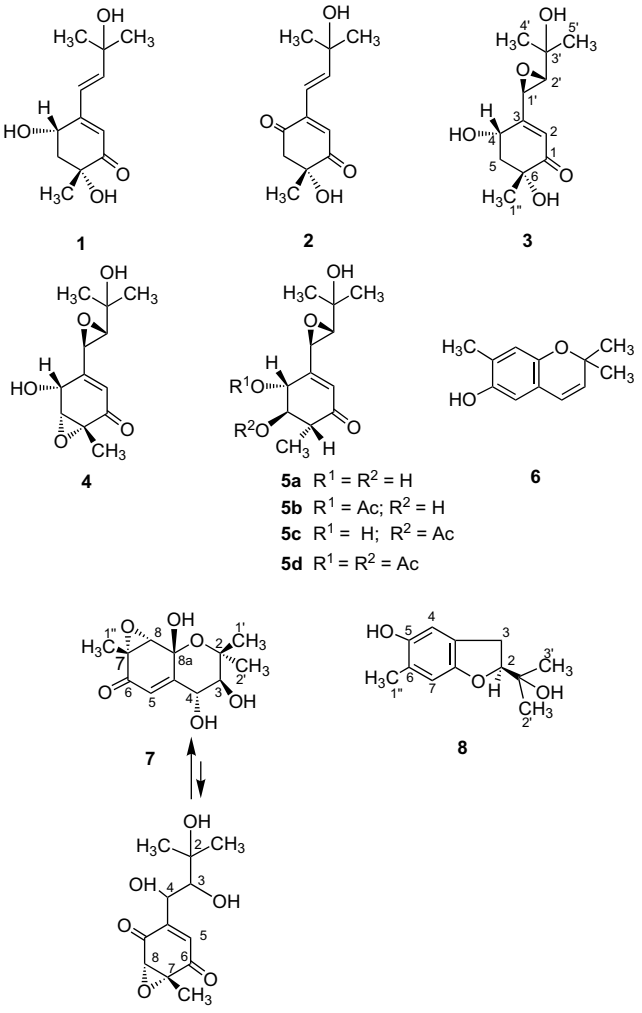


Scheme 1.

In fact, the ^{13}C NMR spectrum of **3** showed the presence of two signals at 52.8 and 69.2 ppm, having one-bond $^{1}J_{CH}$ couplings of 183 and 176 Hz, respectively, characteristic of epoxide carbons. Accordingly, the ^{1}H NMR spectrum revealed the absence of the olefinic protons and the presence of two new epoxy protons at 3.93 and 2.97 ppm having a trans coupling constant of 2.1 Hz.

To confirm the structure of **3**, acremine A (**1**) was treated with 3-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane. Interestingly, the reaction showed a high diastereoselectivity, giving a >95:<0.5 mixture of diastereoisomers **9** and **3**, in which natural compound **3** was the minor isomer (Scheme 2).

There are examples in the literature of *m*-CPBA substrate controlled epoxidation of olefins.[5] A high diastereoselectivity is achieved when a hydroxyl group is present in a suitable position to anchor the peroxyacid, thus giving stereofacial differentiation of the diastereotopic faces of the double bond. We reasoned that the hydroxyl group on C-4 ((*S*) absolute configuration) in acremine A could play an important role in directing the epoxidation reaction, presumably due to hydrogen bond formation with the reagent (Fig. 1). This interaction should preferentially stabilize the transition state related to the electrophilic addition to the *Re*,*Re* face of the double bond.

On the basis of these mechanistic considerations, the configuration of the stereogenic centres in the oxirane ring of the major isomer should be 1′(*S*),2′(*R*). Consequently, the natural compound **3** should have the absolute configuration 1′(*R*),2′(*S*).

This assignment is supported by the analysis of the NOESY NMR spectra.

The NOE enhancements observed for H-1′ and H-2′ upon irradiation of H-4 are 1.5 and 1.5%, respectively, for compound **3**, and 2.5 and 0.5%, respectively, for compound **9**. Molecular mechanics calculations[6] showed that for compound **9** the minimal energy conformation (Fig. 2) may be favoured by an intramolecular hydrogen bond between the oxirane oxygen and the proton of the hydroxy group in position 4. The distances between H-4 and H-1′ and H-2′ (2.98 and 4.40 Å, respectively) are in agreement with the NOE results. Similarly, the distances between the same hydrogens in the minimal energy conformation of compound **3** (3.31 and 3.88 Å, respectively) are in agreement with the NOE measurements.

Acremine I (**4**) was isolated as an oil, $[\alpha]_D$ −126 (*c* 0.1, $CHCl_3$), with an HREIMS consistent with the formula $C_{12}H_{16}O_5$. ^{13}C and ^{1}H NMR spectra revealed its structural similarity with compounds **3** and **9**: two signals at 53.9 and 68.8 ppm, having $^{1}J_{CH}$=181 and 174.5 Hz, respectively, provided evidence of the presence of an epoxy ring in position 1′-2′. The upfield shift of the quaternary C-6 carbon and the presence of a methine carbon at 63.96 ppm, having $^{1}J_{CH}$=181 Hz, in place of the C-5 methylene carbon, suggested the presence of an additional epoxy ring in position 5-6.

The NOE enhancements observed between H_3-1″, assumed as β-disposed, and H-5 and between H-4 and H-5 (see Section 4) suggest that the latter two protons are on the β-side of the molecule.

Acremine L (**5a**) was isolated as an oil; $[\alpha]_D$ +17 (*c* 0.65, MeOH); the ESIMS showed an $[MH+Na]^+$at *m*/*z* 265, consistent with the formula $C_{12}H_{18}O_5$. The ^{1}H and ^{13}C NMR spectra of **5a** were similar to those of **3**, the only relevant difference being the presence in **5a** of a hydroxy group in position 5 rather than in position 6. Typically, the H_3-1″ protons showed a vicinal coupling of 6.8 Hz with H-6, which on its turn exhibited a trans diaxial coupling of 11.1 Hz with H-5. Moreover, the magnitude of the coupling between H-4, assumed as β-disposed, and H-5 suggests that the cyclohexenone ring assumes a half-chair conformation in which the 5-OH and the 6-Me groups are β- and α-disposed, respectively.

The formation of the monoacetates **5b** and **5c** and of the diacetate **5d** with the low-field shift of H-4 and H-5 was in agreement with the proposed structure.

Acremine M (**7**) is an oil. The EIMS showed an $[MH]^+$ at *m*/*z* 257 and the HREIMS confirmed the formula $C_{12}H_{16}O_6$. The ^{13}C NMR spectrum of **7** showed some similarities with that of compound **4**. The presence of a methyl-substituted epoxy ring was inferred from signals at 58.0, 65.6 and 14.7 ppm in ^{13}C NMR spectrum. Further, three signals at 194.8, 156.5 and 118.0 ppm were attributable to an α,β-conjugated ketone moiety. Finally, the ^{13}C spectrum showed the presence of two methyl groups and four oxygen-bearing carbons, one of which, resonating at 93.6 ppm, was assigned to a hemiketalic carbon. This led us to hypothesize that compound **7** derived from compound **4** by an oxidation, followed by a ring closure between C-4 and the oxygen on C-3′, to form a tetrahydrochromen-6-one ring. (In this case, the numbering of the carbon and hydrogen atoms throughout the rings was assigned according to chemical nomenclature and is different from the numbering of the previous acremines.) The ^{1}H NMR spectrum confirmed the above findings. An allylic coupling between H-4 and H-5 indicated a linkage between C-4 and C-4a, whereas the presence of a hemiketalic OH was inferred from an exchangeable singlet at 6.02 ppm. Two exchanging hydrogens coupled with H-3 and H-4 revealed the presence of a diol. The above observations allowed to establish the structure as depicted in Scheme 1.

The NOE enhancements observed between H_3-1″, assumed as β-disposed, and H-8, between H-8 and OH-8a, between H_3-1′ and H-4 and OH-8a place all these protons on the β-side of the molecule.

Table 1
^{1}H NMR spectroscopic data for new compounds (acetone-d_6)

Position	Compound					
	3	**4**	**5a**	**7**	**8**	**9**
	δ (ppm), J (Hz)					
2	5.85 (1H, dd, J=1.7 and 1.0)	5.91 (1H, br d, J=1.0)	5.78 (1H, br dd, J=2.1 and 1.1)		4.49 (1H, dd, J=9.2 and 8.1)	5.80 (1H, dd, J=1.9 and 1.0)
3				3.24 (1H, dd, J=10.2 and 4.0)	3.12 (1H, br dd, J=15.8 and 8.1), 3.02 (1H, br dd, J=15.8, 9.2)	
4	4.59 (1H, dddddd, J=8.9, 5.2, 3.8, 1.7, 0.7 and 0.6)	4.51 (1H, br d, J=1.8)	4.40 (1H, ddd, J=8.5, 6.8 and 2.1)	4.60 (1H, ddd, J=10.2, 4.5 and 2.1)	6.65 (1H, br s)	4.74 (1H, dddd, J=9.8, 7.2, 5.3 and 0.7)
5	2.36 (1H, dd, J=12.9, 5.2), 2.08 (1H, ddd, J=12.9, 8.9 and 0.7)	3.70 (1H, d, J=1.8)	3.51 (1H, ddd, 11.2, 8.5 and 5.5)	5.99 (1H, d, J=2.1)		2.42 (1H, dd, J=12.6 and 5.3), 1.98 (1H, dd, J=12.6 and 9.8)
6			2.32 (1H, dq, J= 11.2 and 6.8)			
7					6.43 (1H, br s)	
8				3.56 (1H, s)		
1′	3.93 (1H, ddd, J=2.1, 1.0 and 0.6)	3.71(1H, dd, J=2.1, 1.0)	3.99 (1H, ddd, J=2.1, 1.1 and 0.7)	1.45 (3H, s)		3.72 (1H, ddd, J=2.1, 1.0 and 0.7)
2′	2.97 (1H, d, J=2.1)	3.12 (1H, d, J=2.1)	2.83 (1H, d, J=2.1)	1.26 (3H, s)	1.20 (3H, s)	2.82 (1H, d, J=2.1)
3′					1.18 (3H, s)	
4′	1.27 (3H, s)	1.25 (3H, s)	1.26 (3H, s)			1.27 (3H, s)
5′	1.25 (3H, s)	1.22 (3H, s)	1.22 (3H, s)			1.27 (3H, s)
1″	1.24 (3H, s)	1.41 (3H, s)	1.15 (3H, d, J=6.8)	1.38 (3H, s)	2.11 (3H, br s)	1.21 (3H, s)
3-OR				4.76 (1H, d, J=4.0)		
4-OR	4.85 (1H, br d, J=3.8)	5.17 (1H, br s)	4.98 (1H, d, J=6.8)	4.79 (1H, d, J=4.5)		4.98 (1H, d, J=7.2)
5-OH			4.71 (1H, d, J=5.5)		7.56 (1H, br s)	
6-OH	4.45 (1H, s)					4.28 (1H, s)
8a-OH				6.02 (1H, br s)		
1′-OH					3.55 (1H, br s)	
3′-OH	3.68 (1H, s)	3.65 (1H, br s)	3.63 (1H, br s)			3.83 (1H, s)

Finally, the vicinal coupling of 10.2 Hz observed between H-3 and H-4 indicates that these protons are trans diaxially disposed (Fig. 3).

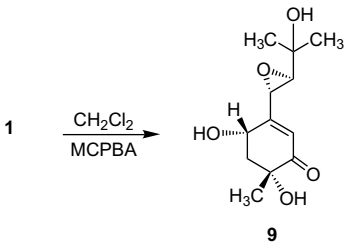
Acremine N (**8**) was obtained as a solid, mp 150–153 °C, $[\alpha]_D$ +35 (c 0.2, MeOH) and analyzed for $C_{12}H_{16}O_3$; no carbonyl stretching band was present in the IR spectrum.

The ^{13}C NMR spectrum of **8** exhibited signals due to six sp^2 and six sp^3 carbon atoms. The sp^2 resonances indicated the presence of a tetrasubstituted benzene ring while the sp^3 resonances were assigned to three methyl, one methylene, one methine and one quaternary carbon atom, the last two bearing an oxygen atom.

Table 2
^{13}C NMR spectroscopic data for new compounds (acetone-d_6)

Position	Compound					
	3	**4**	**5a**	**7**	**8**	**9**
	δ (ppm), J (Hz)					
1	200.4 (s)	194.5 (s)	198.5 (s)			201.3 (s)
2	120.3 (d, J=163)	122.2 (d, J=163.5)	122.2 (d, J=163)	78.07 (s)	89.8 (d, J=147.5)	119.0 (d, J=164)
3	163.0 (s)	155.7 (s)	160.4 (s)	79.9[a] (d, 141.5)	31.4 (t, J=133)	164.9 (s)
3a					125.9[a] (s)	
4	66.1 (d, J=142)	63.5 (d, J=144)	78.3[a] (d, J=143)	68.9[a] (d, J=142)	112.2 (d, J=156.5)	67.0 (d, J=143)
4a				156.5 (s)		
5	46.1 (t, J=131)	64.0 (d, J=181)	73.8[a] (d, J=142)	118.0 (d, J=168)	149.7[b] (s)	46.6 (t, J=131)
6	73.4 (s)	58.2 (s)	48.5 (d, J=125)	194.8 (s)	123.6[a] (s)	73.6 (s)
7				58.0 (s)	111.1 (d, J=157.5)	
7a					154.1[b] (s)	
8				65.6 (d, J=181.5)		
8a				93.6 (s)		
1′	52.8 (d, J=183)	53.9 (d, J=181)	52.3 (d, J=184)	29.6 (q, J=127.5)	71.5 (s)	53.8 (d, J=181)
2′	69.2 (d, J=176)	68.8 (d, J=174.5)	69.6 (d, J=175)	22.7 (q, J=127.5)	26.0 (q, J=125.5)	69.7 (d, J=175)
3′	67.9 (s)	68.3 (s)	67.9 (s)		25.5 (q, J=125.5	68.2 (s)
4′	26.6 (q, J=126)	26.8 (q, J=126.5)	26.6 (q, J=126)			27.0 (q, J=127)
5′	25.5 (q, J=126)	26.0 (q, J=126.5)	25.4 (q, J=126)			25.1 (q, J=127)
1″	24.6 (q, J=127)	15.0 (q, J=127.5)	10.9 (q, J=127)	14.7 (q, J=129.5)	16.6(q, J=126.5)	25.0 (q, J=127)

[a] May be interchanged.
[b] May be interchanged.



Scheme 2.

Accordingly, the ^{1}H NMR spectrum showed the presence of two *para* aromatic protons at 6.65 and 6.43 ppm, assigned to H-4 and H-7, two methylene protons at 3.02 and 3.06 ppm (H_2-3) and three methyl protons at 2.11 ppm (H_3-1″), presenting benzylic couplings with H-4 and H-7, respectively. Moreover, the spectrum showed

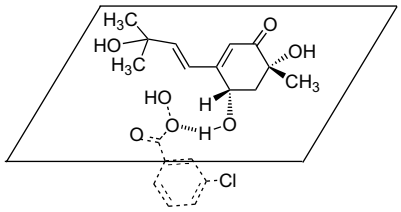


Figure 1. Model of the possible diastereoselective epoxidation of acremine A.

one methine proton, having vicinal couplings with H_2-3, two tertiary methyl groups, a phenolic and aliphatic hydroxy protons at 7.56 and 3.55 ppm, respectively. The NOE enhancements observed between the phenolic OH and H-4 and H_3-1″, together with those observed between H-4 and H_2-3 and H-7 and H_3-1″, permitted us to locate the H_2-3, the OH and the H_3-1″groups at C-3, -5 and -6, respectively.

The above observations suggested for compound **8** a bicyclic structure correlated to the previously described acremine D,[4] due

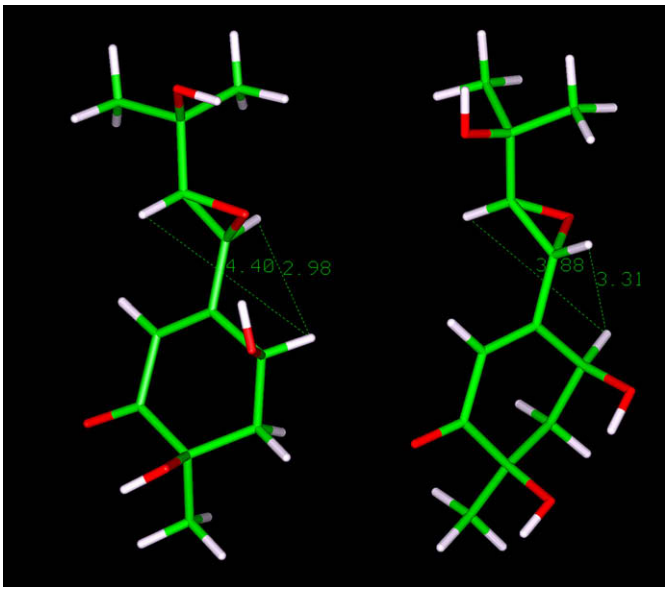


Figure 2. Model of compounds **3** (right) and **9** (left).

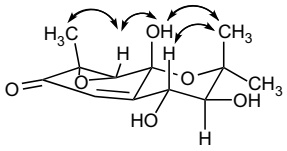
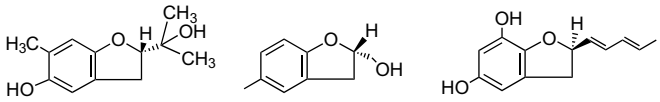


Figure 3. Selected NOE correlations for compound **7**.

to an intramolecular cyclization of the isoprenyl side chain. The presence of two signals at 3.02 and 3.06 ppm, showing a geminal coupling constant (J=15.8 ppm) and a further coupling with a hydrogen resonating at 4.49 ppm, along with the absence of the olefinic proton, led us to assume a reduction of the double bond in the benzofuran ring with respect to acremine D.

The absolute configuration determination was based on analogy to other natural compounds. It is well known that for structurally similar compounds, similarity in sign of the specific rotation and CD curves indicates a similarity in configuration at the stereogenic centre. Ramadas and co-workers[7] reported the specific rotation and the CD spectra of a series of substituted 2-hydroxymethyl-2,3-dihydrobenzofurans with known (*S*) absolute configuration; the compounds showed a (+) sign of specific rotation. The reported CD of compound **10** (Scheme 3) showed a positive Cotton effect with maxima at 238 nm (θ=+18.52×10^{-3} deg cm^2 dmol^{-1}) and 292 nm (θ=+6.74×10^{-3} deg cm^2 dmol^{-1}).

Pfefferle and co-workers[8] reported the CD of (−) (*R*)-arthrographol (**11**), which showed a (−) sign of specific rotation and the mirror image CD curve with respect to the previous 2-hydroxymethyl-2,3-dihydrobenzofurans.



Scheme 3.

Since, compound **8** showed a (+) sign of specific rotation (+35) as well as a similar CD curve (see Section 4) with respect to compound **10**, we may safely deduce that the absolute configuration of compound **8** is *S*.

Acremines H, I, L and N were tested for their capability to inhibit germination of *P. viticola* sporangia.

The results of the biological assays are summarized in Table 3. All the new acremines affected germinability of sporangia more or less efficiently.

Table 3
Mean values (%) of the inhibition of *P. viticola* sporangia germination by acremines H, I, L and N

Germinability % inhibition		Control
Water		7.7
DMSO		8.9
	Metabolite concentration	
	0.5 mM	1 mM
Acremine H	15.6	30.4
Acremine I	23.2	32.8
Acremine L	49.6	55.2
Acremine N	54.6	69.4

Selected compounds were tested for their cytotoxicity against non-small cell lung tumour cell line H460, and showed modest activity. [IC_{50} (μM): for **2**: 35.7; for **3** >100 and **4**: 56].

3. Conclusion

We have established the structure and stereochemistry of a further group of acremines (H–N), produced by a strain of *A. byssoides* on a culture medium composed by malt extract–peptone and glucose agar. The oxygenation pattern of these compounds may suggest that they are formed by bioconversion of the main metabolite acremine A (**1**) first to the epoxide **3** and subsequently to compounds **4**, **5a** and **7** by the monooxygenase enzymes of the fungus, grown in particular conditions (MPGA cultures). It is well known that these enzymes are able to activate molecular oxygen in order to transfer it to an organic compound.[9]

4. Experimental

4.1. General

Flash column chromatography was performed with Merck silica gel (0.040–0.63 mm); thin and preparative layer chromatography (TLC and PLC) were performed on precoated Merck silica gel 60 F_{254} plates. The IR spectra were measured on a Perkin–Elmer 177 spectrophotometer. MS spectra were recorded with a Bruker Esquire 3000 Plus instrument, HRMS with a Bruker APEX-QZT ICR. The NMR spectra were recorded with a Bruker AMX-600 spectrometer, at 600.13 MHz for ^1H and 150.92 MHz for ^{13}C.

4.2. Culture of *A. byssoides*, extraction and isolation of acremines H–N

The fungal strain A20 was isolated in pure culture from grapevine leaves infected by *P. viticola* and identified as *A. byssoides*, by conventional taxonomy.[1] For chemical investigations, the fungus was grown in batches of 40 Roux flasks containing 100 mL MPGA (malt extract–peptone–glucose agar, 20, 2, 20 and 15 g L^{-1}). After two-week-growth period at 24 °C, the cultures were extracted twice with EtOAc–MeOH (100:1). The extracts (1.8 g) were dried on Na_2SO_4, evaporated to dryness and chromatographed on a silica gel flash column eluted with hexane–EtOAc at increasing polarity. Collected fractions were further purified by means of PLC with CH_2Cl_2–MeOH 9:1 to give the pure metabolites in order of decreasing R_f value: acremine N (**8**) (75 mg, R_f 0.5), acremine B (**2**) (60 mg), acremine I (**4**) (140 mg, R_f 0.4), acremine C (**6**) (65 mg), acremine H (**3**) (186 mg, R_f 0.3), acremine L (**5a**) (220 mg, R_f 0.3) and acremine M (**7**) (15 mg, R_f 0.2).

4.2.1. Acremine H (*3*)

UV: λ_{max} 220 and 280 nm (ε 1875 and 17.620); IR: ν_{max} (KBr) 1685 cm^{-1}, conj. CO group; CIMS, *m*/*z* 243 $(MH)^+$ (22%), 227 (100) and 209 (30). (Found: C, 59.6; H, 7.6; $C_{12}H_{18}O_5$ requires C, 59.49; H, 7.48.) The ^1H and ^{13}C NMR data are listed in Tables 1 and 2. NOEs (acetone-d_6+D_2O): {H-2} enhanced H-1′ (2%) and H-2′ (2.5%), {H-4} enhanced H-5a (4.5%), H-5b (0.5%), H-1′ (1.5%), H-2′ (1.5%) and H_3-1″ (1%), {5a} enhanced H-4 (3%), H-5b (7%) and H_3-1″ (1%), {H-5b} enhanced H-4 (0.5%), H-5a (9%) and H_3-1″ (0.5%), {H-1′} enhanced H-2 (2.5%), H-4 (1%), H-2′ (1%), H_3-4′ (0.5%) and H_3-5′ (0.5%), {H-2′} enhanced H-2 (3%), H-4 (1%), H-1′ (1%), H_3-4′ (0.5%) and H_3-5′ (0.5%), {H_3-4′ and H_3-5′} enhanced H-1′ (8%) and H-2′ (11), {H_3-1″} enhanced H-4 (8%), H-5a (3%) and H-5b (0.5%).

4.2.2. Synthesis of compound (*9*) from acremine A (*1*)

Acremine A (50 mg) was dissolved in dry CH_2Cl_2 (5 mL) and treated with *m*-CPBA (60 mg) for 3 h at rt; the solution was washed with a solution of $NaHCO_3$, dried and evaporated. The residue was purified on silica gel (plates 1 mm) with CH_2Cl_2–MeOH (9:1) as eluant to obtain 35 mg of the epoxide **9** as an oil and 1.5 mg of acremine H; ESIMS *m*/*z* 265 $(M+Na)^+$. The ^1H and ^{13}C NMR data are listed on Tables 1 and 2. NOEs (acetone-d_6+D_2O): {H-2} enhanced H-1′ (0.5%) and H-2′ (1.5%), {H-4} enhanced H-5a (3%), H-1′ (2.5%), H-2′ (0.5%) and H_3-1″(1%), {H-1′} enhanced H-4 (2%), H-2′ (0.5%), H_3-4′ (0.5%) and H_3-5′ (1%), {H-2′} enhanced H-2 (2%), H-1′ (0.5%), H_3-4′ (1%) and H_3-5′ (1%), {H_3-4′} enhanced H-1′ (1.5%) and H-2′ (4.5%), {H_3-5′} enhanced H-1′ (3%) and H-2′ (4.5%), {H_3-1″} enhanced H-4 (5%) and H-5a (2.5%).

4.2.3. Acremine I (*4*)

CIMS, *m*/*z* 241 $(MH)^+$ (100%), 223 $(MH-18)^+$ (78) and 183 (65); HREIMS, *m*/*z* 240.0972 (calcd for $C_{12}H_{16}O_5$ 240.0997). The ^1H and ^{13}C NMR data are listed in Tables 1 and 2. NOEs ($CDCl_3$+D_2O): {H-2} enhanced H-1′ (4.5%) and H-2′ (2%), {H-4} enhanced H-5 (7.5%), H-1′ (4%) and H-2′ (2.5%), {H-5} enhanced H-4 (5.5%) and H_3-1″ (1%), {H-1′} enhanced H-2 (5.5%), H-4 (3.5%) and H-2′ (1%), {H-2′} enhanced H-2 (3.5%), H-1′ (2.5%), H_3-4′ (1%) and H_3-5′ (1%), {H_3-4′} enhanced H-1′ (3%) and H-2′ (6%), {H_3-5′} enhanced H-4 (1%), H-1′ (3.5%) and H-2′ (5%), {H_3-1″} enhanced H-5 (7%).

4.2.4. Acremine L (*5a*)

HREIMS, *m*/*z* 242.1136 (calcd for $C_{12}H_{18}O_5$ 242.1154). The ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

4.2.5. Acetylation of compound *5a*

Compound **5a** (30 mg) was dissolved in dry pyridine (0.2 mL) and treated with Ac_2O (0.5 mL) overnight at 0 °C. Standard work-up followed by PLC on silica gel in hexane–EtOAc (2:1) gave mainly the diacetate **5d** (18 mg) as a solid, mp 120–125 °C; ESIMS, *m*/*z* 349 $(M+Na)^+$ and 675 $(2M+Na)^+$ and the monoacetates **5b** and **5c** in the ratio 3:1. Compound **5b**. R_f (hexane–ethyl acetate 2:1) 0.4; ^1H NMR (acetone-d_6) δ: 1.17 (3H, s, *Me*), 1.24 (3H, s, *Me*), 1.19 (3H, d, *J* 6.8 Hz, MeC*H*), 2.16 (3H, s, OCO*Me*), 2.51 (1H, dq, *J* 6.8, 11.1 Hz, CH*Me*), 2.84 (1H, d, *J* 2.1 Hz, C*H*O), 3.43 (1H, s, OH), 3.57 (1H, ddd, *J* 0.8, 1.0, 2.1 Hz, C*H*O), 3.77 (1H, ddd, *J* 5.5, 8.2, 11.1 Hz, C*H*OH), 4.83 (1H, d, *J* 5.5 Hz, OH), 5.80 (1H, ddd, *J* 0.8, 2.3, 8.2 Hz, C*H*OCO), 5.92 (1H, dd, *J* 1.0, 2.3 Hz, C*H*=C).

4.2.6. Compound *5c*

R_f (hexane–ethyl acetate 2:1) 0.3; ^1H NMR (acetone-d_6) δ: 1.06 (3H, d, *J* 6.8 Hz, *Me*CH), 1.22 (3H, s, *Me*), 1.26 (3H, s, *Me*), 2.09 (3H, s, OCO*Me*), 2.56 (1H, dq, *J* 6.8, 11.2 Hz, C*H*Me), 2.88 (1H, d, *J* 2.1 Hz, C*H*O), 3.51 (1H, s, OH), 3.98 (1H, ddd, *J* 0.8, 1.1, 2.1 Hz, C*H*O), 4.65 (1H, dddd, *J* 0.8, 2.2, 6.8, 8.3 Hz, C*H*OH), 5.05 (1H, dd, *J* 8.3 11.2 Hz, C*H*OCO), 5.13 (1H, d, *J* 6.8 Hz, OH), 5.88 (1H, dd, *J* 1.1, 2.2 Hz, C*H*=C).

4.2.7. Compound *5d*

R_f (hexane–ethyl acetate 2:1) 0.6; ^1H NMR (acetone-d_6) δ: 1.10 (3H, d, *J* 6.8 Hz, MeC*H*), 1.17 (3H, s, *Me*), 1.24 (3H, s, *Me*), 2.05 (3H, s, OCO*Me*), 2.12 (3H, s, OCO*Me*), 2.76 (1H, dq, *J* 6.8, 11.1 Hz, C*H*Me), 2.87 (1H, d, *J* 2.2 Hz, C*H*O), 3.49 (1H, s, OH), 3.61 (1H, ddd, *J* 0.7, 1.2, 2.2 Hz, C*H*O), 5.22 (1H, dd, *J* 8.2, 11.1 Hz, C*H*OCO), 5.98 (1H, ddd, *J* 0.7, 2.2, 8.2 Hz, CHOCO), 6.01 (1H, dd, *J* 1.2, 2.2 Hz, H-2).

4.2.8. Acremine M (*7*)

Oil; UV: λ_{max} 204, 245 and 290 sh (ε 9720, 10,000 and 6350); IR: ν_{max} 1682 cm^{-1}, conj. CO group; EIMS, *m*/*z* 257 $(MH)^+$ (10%), 239 (40), 221 (18) and 59 (100); HREIMS: 242.1146 (calcd for $C_{12}H_{18}O_5$

242.1154). The ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2; NOEs (acetone-d_6): {H-3} enhanced H_3-2′ (1.5%), {H-4} enhanced H-5 (2.5%) and H_3-1′ (1.5%), {H-5} enhanced H-4 (1.5%) and H-8 (1%), {H-8} enhanced H_3-1″ (1.5%), {H_3-1′} enhanced H-4 (10%) and OH-8a (1%), {H_3-2′} enhanced H-3 (8%), {H_3-1″} enhanced H-8 (7%), {OH-4 and OH-8a} enhanced H-3 (9%), H-5 (3%), H-8 (5.5%), H_3-1′ (1%), H_3-2′ (0.5%).

*4.2.9. Acremine N (**8**)*

UV: λ_{max} 206 and 304 nm (ε 13.830 and 3500); CD (EtOH) $\Delta\varepsilon$: 216.4, 238.0, 301.4 and 322 nm (−3.02, +2.82, −1.49 and +0.1). (Foud: C, 69.3; H, 7.6; $C_{12}H_{16}O_3$ requires C, 69.20; H, 7.74%.) CISM, *m*/*z* 209 $(MH)^+$, 208, 191, 156 and 100. The ^{1}H and ^{13}C NMR data are in Tables 1 and 2. NOEs (acetone-d_6): {H-2} enhanced H-3a (0.5%), H-3b (3.5%), H-7 (0.5%), H_3-2′ (1%) and H_3-3′ (1%), {H-4} enhanced H_2-3 (1%), and OH-5 (1.5%), {H-6} enhanced H_3-1″ (2%), {H_3-2′ and H_3-3′} enhanced H-2 (6.5%), H_2-3 (3%), H-7 (0.5%) and OH-5 (2.5%), {H_3-1″} enhanced H-7 (7%).

4.3. Bioassay on germination of *P. viticola* sporangia

The test of *P. viticola* sporangia germination inhibition was performed as described previously.[4] The analysis of the germination percentages was performed on data collected from two independent experiments, comprising readings on 1200 sporangia for each treatment and the results are presented in Table 3.

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