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# Acremonidins, New Polyketide-derived Antibiotics Produced by *Acremonium* sp., *LL*-Cyan 416

HAIYIN HE,* RAMUNAS BIGELIS, ERIC H. SOLUM, MICHAEL GREENSTEIN and GUY T. CARTER

Natural Products Research, Chemical and Screening Sciences, Wyeth Research,
401 N. Middletown Road, Pearl River, NY 10965

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Acremonidins A~E (**1**~**5**) were produced by fermentation of *Acremonium* sp., *LL*-Cyan 416, in heterogeneous phases. The structures of these compounds, containing a bridging keto group, were determined by spectroscopic analysis. Acremonidins A and B showed moderate activity against Gram-positive bacteria, including the methicillin-resistant staphylococci and vancomycin-resistant enterococci. Selective acylations of acremonidin B afforded ester derivatives **6**~**9** that exhibited improved antibacterial activity.

The chemical diversity and production yields of microbial secondary metabolites are greatly influenced by methods, media, and other conditions used for fermentations. New conditions and approaches are sought that affect the physiological state of microorganisms, and thereby modulate biosynthetic pathways with the capacity to produce useful compounds.[1,2] Previously, we reported the production of two antimicrobial fungal metabolites, pyrrocidines A and B, by fermentation in heterogeneous phases.[3] In the course of our continuing search for novel antibiotics to combat drug-resistance in antibacterial chemotherapy,[4,5] we further investigated this fermentation approach. Among the several fungal cultures studied, an *Acremonium* sp., strain *LL*-Cyan 416, was found to produce much enhanced antibacterial activity when fermented with this method. Five new compounds, designated acremonidins A~E (**1**~**5**), were purified from the methanol extract. Acremonidins A and B showed moderate activity against Gram-positive bacteria, including the methicillin-resistant staphylococci and vancomycin-resistant enterococci. The structures of these compounds, featuring a keto bridge, were determined by spectroscopic analysis. Selective acylations on the acremonidin B afforded some ester derivatives **6**~**9** that exhibited improved antibacterial potency. In this paper, the production, isolation, structural determination, and derivatization of these new antibiotics are reported.

## Results and Discussions

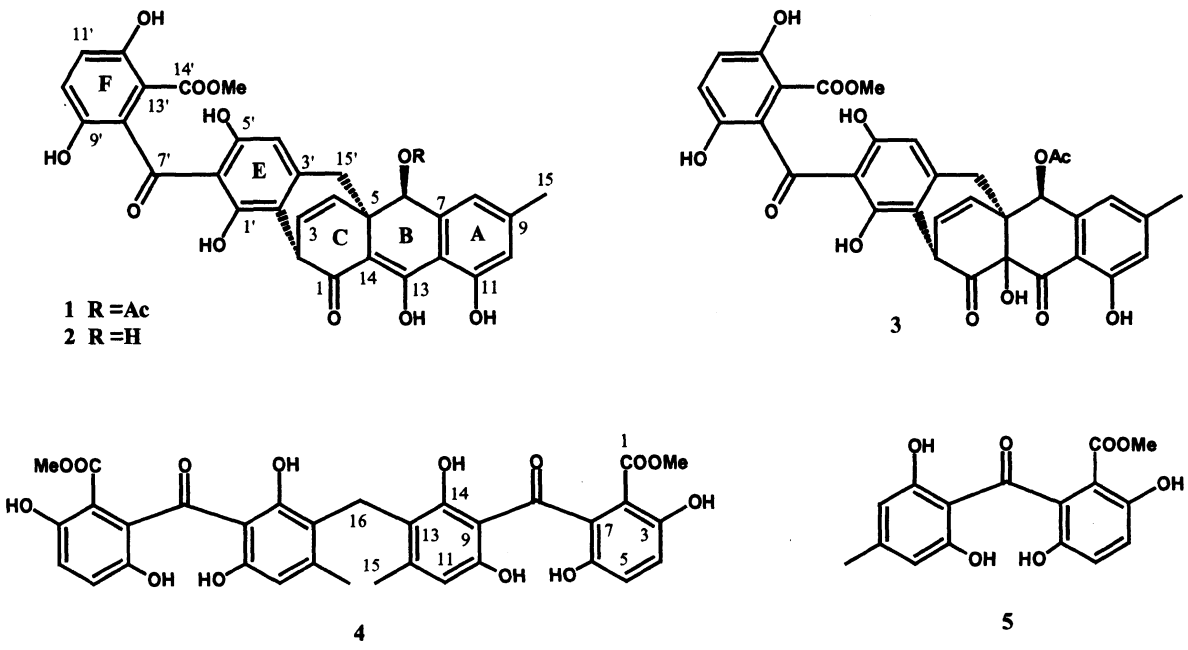
### Production and Structures

Culture *LL*-Cyan 416 was inoculated on moist milk-filter paper placed on the surface of a solid medium containing agar, malt extract, peptone, and yeast extract and incubated under stationary conditions at 22°C. After two weeks of incubation, the milk-filter paper bearing prolific mycelial growth was removed from the surface of the agar, lyophilized, and then extracted with methanol. The extract was chromatographed by reversed phase HPLC on C18 columns using a gradient acidic acetonitrile in water to afford acremonidins A~E (**1**~**5**).

The molecular formula of acremonidin A (**1**) was determined to be $C_{33}H_{26}O_{12}$ by high resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. The $^{13}C$ NMR spectrum displayed 33 signals, which were assigned to 2 $-CH_3$, 1 $OCH_3$, 1 OCH<, 1 $>CH_2$, 1 >CH–, 7 –CH=, and 12 quaternary $sp^2$ carbons using the DEPT experiment. The $^1H$ NMR spectrum indicated the presence of a >CHCH=CH– ($\delta$ 4.72, 6.51, and 6.13), a 1,2,3,4-tetrasubstituted phenyl group (6.94 and 6.82, F ring) and a 1,2,3,5-tetrasubstituted phenyl group (6.92 and 6.86, A ring). In addition, 6 $D_2O$ exchangeable peaks were observed, which were assigned to phenolic OH signals. The $^1H$ and $^{13}C$ NMR spectral data, together with

* Corresponding author: heh@wyeth.com

Fig. 1. Structures of acremonidins A~E (**1**~**5**).




the correlations in an HMBC spectrum of **1** are listed in Table 1.

Detailed analysis of 2-D NMR data, in particular, the multiple bond correlations in the HMBC spectrum, revealed the structure of **1**. The strong HMBC correlations from the methine proton H-2 to C-1′, C-2′, and C-3′, from the methylene protons $CH_2$-15′ to C-2′, C-3′, and C-4′, and from the aromatic proton H-4′ to C-2′ and C-6′, along with those from the phenolic 1′-OH and 5′-OH to the aromatic carbon signals (Table 1) established the structure for the E ring and the connectivities between C-3′ and C-15′, and between C-2 and C-2′. The structure of the F ring, a 1,2,3,4-tetrasubstituted benzene, was easily determined by the 2- and 3-bond HMBC correlations from 9′-OH, 12′-OH, H-10′, and H-11′ to carbon signals in this system. The methoxycarbonyl group, identified by NMR data, was placed at C-13′ by a weak 4-bond coupling between H-11′ and C-14′ observed in the HMBC spectrum. The 4-bond correlations from H-4′ and H-10′ to the carbonyl signal at $\delta$ 201.0 in the HMBC spectrum required a C-7′ keto linkage between C-6′ and C-8′. Therefore, a benzophenone moiety was identified.

The remaining structure, including the A, B, C, and D rings were unambiguously determined by analysis of HMBC data and was found to be reminiscent of the polycyclic moiety contained in the xanthoquinodins,[6,7] a family of anticoccidial antibiotics. In this moiety, compound **1** differed from xanthoquinodins at C-6 where the keto group was replaced by the acetoxy carbinyl. It was noted that the chemical shift data for C-1 at $\delta$ 187.6 and C-13 at 183.8 in **1** clearly indicated the tautomeric nature of the related $\beta$-diketo system.

The relative stereochemistry of C-2, C-5, and C-6 was determined on the basis of an NOE study. The observation of a cross peak between H-6 at $\delta$ 6.01 and H-15′ at 2.89 in the ROESY spectrum required that the H-6 and C-15′ be synfacial, which resulted in a (2*S**,5*R**,6*S**) relative configuration.

The molecular formula of acremonidin B (**2**) was $C_2H_2O$ less than compound **1**. The lack of acetyl signals in the $^1H$ and $^{13}C$ NMR spectral data and the upfield shift of the H-6 signal both indicated that the acetoxy group in **1** was replaced by a hydroxyl in **2**. Acremonidin B (**2**) was obtained quantitatively by hydrolysis of **1** in a solution of 1 : 1 $Et_2O$/MeOH containing 0.5 M HCl at ambient temperature in 16 hours.

The molecular formula of acremonidin C (**3**), $C_{33}H_{26}O_{13}$, contained one more oxygen than compound **1**. The $^{13}C$ chemical shift data for C-1 at $\delta$ 202.8, C-13 at 195.5, and C-14 at 81.5 could all be attributed to the presence of a 14-OH, which prevented the $\beta$-diketone from tautomerization.

The molecular formula of acremonidin E (**4**) was

Table 1. $^{1}H$ and $^{13}C$ NMR spectral data of acremonidin A (**1**) in DMSO-$d_6$.

| No | $^{1}H$ (400 M Hz, mult, $J$ in Hz) | $^{13}C$ ( 75 M Hz) | HMBC correlation ($J$ = 8 Hz) |
|---|---|---|---|
| 1 | | 187.6 | |
| 2 | 4.69 (br d, 6.6) | 37.9 | C-1, C-4, C-13,[b] C-14, C-1′, C-2′, C-3′ |
| 3 | 6.48 (dd, 8.8, 6.6) | 132.0 | C-1, C-2, C-4, C-5, C-14,[b] C-2′,[b] C-15′[b] |
| 4 | 6.10 (d, 8.8) | 132.7 | C-1,[b] C-2, C-3, C-5, C-6, C-14, C-2′ |
| 5 | | 41.4 | |
| 6 | 5.99 (s) | 72.8 | C-1,[b] C-4, C-5, C-7, C-8, C-12, C-14, C-15′, C-1(Ac) |
| 7 | | 137.0 | |
| 8 | 6.89 (br s) | 123.5 | C-6, C-7, C-9, C-10, C-11,[b] C-12, C-13,[b] C-15 |
| 9 | | 147.6 | |
| 10 | 6.83 (br s) | 119.2 | C-8, C-11, C-12, C-13,[b] C-15 |
| 11 | | 160.8 | |
| 11-OH | 11.35 (br s)[a] | | C-10, C-11, C-12 |
| 12 | | 112.4 | |
| 13 | | 183.8 | |
| 13-OH | 13.87 (very broad)[a] | | |
| 14 | | 105.8 | |
| 15 | 2.32 (3 H, br s) | 21.9 | C-8, C-9, C-10 |
| 1′ | | 159.9 | |
| 1′-OH | 13.41 (br s)[a] | | C-1′, C-2′, C-6′ |
| 2′ | | 113.5 | |
| 3′ | | 146.0 | |
| 4′ | 5.78 (br s) | 109.8 | C-2′, C-5′, C-6′, C-7′, C-15′ |
| 5′ | | 158.8 | |
| 5′-OH | 10.13 (br s)[a] | | C-4′, C-5′ |
| 6′ | | 109.0 | |
| 7′ | | 201.0 | |
| 8′ | | 131.4 | |
| 9′ | | 145.9 | |
| 9′-OH | 9.19 (br s)[a] | | C-8′, C-9′, C-10′ |
| 10′ | 6.91 (d, 8.8) | 122.4 | C-7′,[b] C-8′, C-9′, C-12′ |
| 11′ | 6.79 (d, 8.8) | 118.0 | C-9′, C-12′, C-13′, C-14′[b] |
| 12′ | | 151.4 | |
| 12′-OH | 9.60 (br s)[a] | | C-11′, C-12′, C-13′ |
| 13′ | | 112.8 | |
| 14′ | | 168.0 | |
| 15′ | 2.86 (d, 18.0) | 34.2 | C-4, C-5, C-6, C-14, C-2′, C-3′, C-4′ |
| | 2.41 (d, 18.0) | | C-4, C-5, C-6, C-14, C-2′, C-3′, C-4′ |
| OMe | 3.56 (3H, s) | 52.4 | C-14′ |
| OAc | | C-1 170.0 | |
| | 1.93 (3H, s) | C-2 21.1 | C-1 (Ac), C-6 |

[a] --- $D_2O$ exchangeable. [b] --- Weak C-H correlations.

determined to be $C_{33}H_{28}O_{14}$ by high-resolution FTICR mass spectrometry. The analysis of the $^{1}H$ and $^{13}C$ NMR spectral data, and HMBC correlations of **4** (Table 2) revealed the presence of a benzophenone system, similar to the C-1′ to C-15′ moiety in **1**. Interestingly, the integration of the $CH_2$-16 proton signal in **4**, was only about 1/3 of those observed for $CH_3$-15 and for methoxyl signals. Considering the molecular formula, this observation implied that **4** was a symmetric dimer, with C-16 attached to two identical moieties.

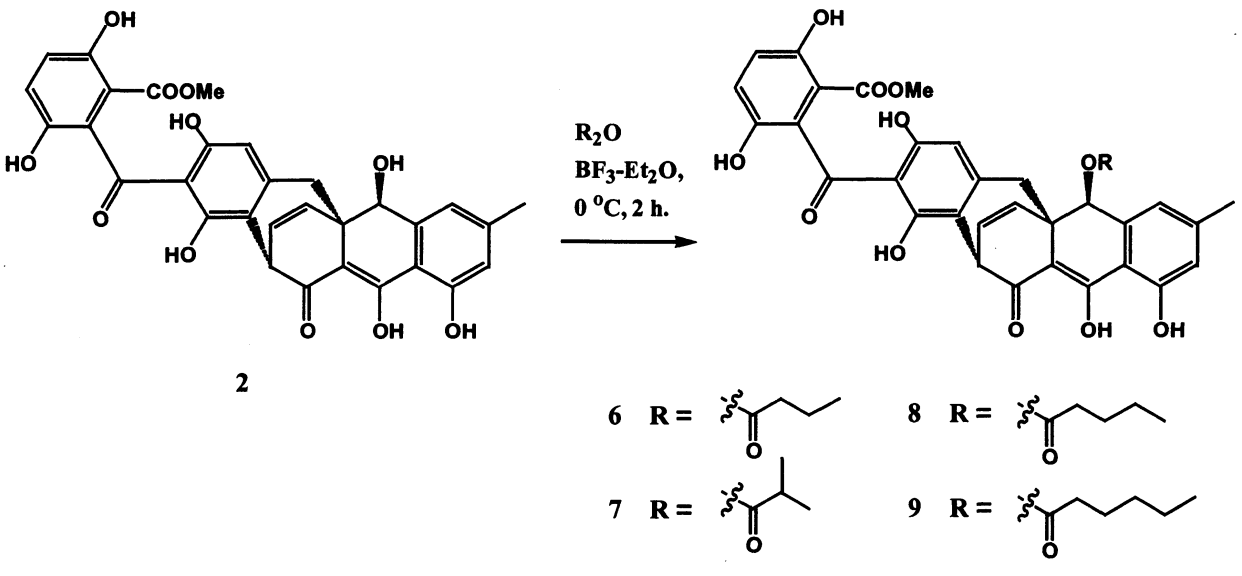
Acremonidin E (**5**) was determined by analysis of high-resolution FTICRMS and NMR spectral data to have a benzophenone structure, which was contained in **4**. The dehydrated form of compound **5**, the 1-carboxylic acid, 2,8-dihydroxy-6-methyl-xanthone methyl ester, or pinselin, was isolated from the roots of *Cassia* sp.[8]

Table 2. $^{1}H$ and $^{13}C$ NMR spectral data of acremonidin D (**4**) in DMSO-$d_6$.[a]

| No. | $^{1}H$ (400 M Hz, mult, $J$ in Hz) | $^{13}C$ (100 M Hz) | HMBC ($J$ = 8 Hz) |
|---|---|---|---|
| 1 | | 168.04 | |
| 2 | | 111.98 | |
| 3 | | 151.28 | |
| 3-OH | 9.72 (1 H, br s) | | C-2, C-3, C-4 |
| 4 | 6.79 (1 H, d, 8.8) | 117.24 | C-1, C-2, C-3, C-6 |
| 5 | 6.93 (1 H, d, 8.8) | 122.23 | C-3, C-6, C-7 |
| 6 | | 145.48 | |
| 7 | | 131.44 | |
| 8 | | 199.56 | |
| 9 | | 108.56 | |
| 10 | | 157.65 | |
| 11 | 5.98 (1 H, s) | 108.08 | C-9, C-10, C-13 |
| 12 | | 146.97 | |
| 13 | | 116.67 | |
| 14 | | 160.99 | |
| 15 | 2.09 (3 H, s) | 20.16 | C-11, C-12, C-13 |
| 16 | 3.83 (1 H,[b] s) | 20.52 | C-12, C-13, C-14 |
| OMe | 3.49 (3 H, s) | 51.76 | C-1 |
| OH's | 13.30, 9.82, 9.15 | | |

[a] --- Owing to the symmetry of **4**, only half of the $^{1}H$ and $^{13}C$ signals are listed, with $CH_2$-16 as an exception. [b]--- This integration is accounted for 2 protons.

Fig. 2. Selective acylation of acremonidin B (**2**).




## Biological Activity and SAR

Acremonidins A~E (**1**~**5**) exhibited moderate antibiotic activity against Gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci, but showed poor activity against Gram-negative bacteria. The minimum inhibitory concentrations (MICs) obtained by the broth dilution method,[9] are listed in

Table 3. Antimicrobial activity of acremonidin A~E (**1**~**5**).

| Test organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| *Staphylococcus* aureus GC 4536[a] | 8 | 32 | 32 | 64 | 64 |
| *Staphylococcus* aureus GC 1131[b] | 8 | 32 | 32 | 64 | 64 |
| *Staphylococcus* aureus GC 2216[a] | 8 | 32 | 32 | 64 | 64 |
| *Enterococcus faecalis* GC 842[c] | 16 | 64 | 32 | 64 | 64 |
| *Enterococcus faecalis* GC 2242[d] | 16 | 32 | 32 | 64 | 32 |
| *Enterococcus faecalis* GC 4555[c] | 16 | 64 | 64 | 64 | 64 |
| *Pseudomonas aeruginosa* GC 2214 | >64 | >64 | >64 | 64 | >64 |
| *Escherichia coli* GC 2203 | >64 | >64 | >64 | >64 | >64 |
| *Escherichia coli* GC 4560 (imp) | 32 | 64 | >64 | 64 | 64 |
| *Candida albicans* GC 3066 | >64 | >64 | >64 | 64 | >64 |

[a] methicillin-susceptible. [b] methicillin-resistant. [c] vancomycin-susceptible. [d] vancomycin-resistant.

Table 4. Antimicrobial activity of esters of acremonidin B (**1**, and **6**~**9**).

| Test organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | **1** | **6** | **7** | **8** | **9** |
| *Staphylococcus* aureus GC 4541[b] | 8 | 8 | 2 | 4 | 4 |
| *Staphylococcus* aureus GC 1131[b] | 8 | 8 | 4 | 4 | 4 |
| *Staphylococcus* aureus GC 4543[a] | 8 | 8 | 4 | 4 | 4 |
| *Staphylococcus* aureus GC 2216[a] | 8 | 8 | 4 | 4 | 4 |
| *Staphylococcus* haemolyticus GC 4547[b] | 16 | 16 | 4 | 4 | 4 |
| *Enterococcus faecalis* GC 6189[d] | 16 | 16 | 4 | 4 | 4 |
| *Enterococcus faecalis* GC 4555[c] | 16 | 16 | 4 | 4 | 4 |
| *Enterococcus faecalis* GC 2242[d] | 16 | 8 | 4 | 4 | 4 |
| *Enterococcus faecium* GC 4556[c] | 16 | 8 | 4 | 4 | 4 |
| *Enterococcus faecium* GC 2243[d] | 8 | 16 | 8 | 4 | 4 |
| *Enterococcus faecium* GC 4558[d] | 8 | 8 | 2 | 2 | 2 |
| *Streptococcus pneumoniae* GC 1894 | 8 | 16 | 8 | 8 | 8 |
| *Streptococcus pneumoniae* GC 6242 | 8 | 32 | 16 | 8 | 8 |
| *Escherichia coli* coli GC 2203 | >128 | >128 | >128 | >128 | >128 |
| *Escherichia coli* GC 4560 (imp) | 32 | 16 | 8 | 8 | 8 |
| *Candida albicans* GC 3066 | >128 | >128 | >128 | >128 | >128 |

[a] methicillin-susceptible. [b] methicillin-resistant. [c] vancomycin-susceptible. [d] vancomycin-resistant.

Table 3. A comparison of the MICs of **1** and **2** revealed the importance of the C-6 acetyl group for retaining antimicrobial potency. In order to investigate the potential of these of antibiotics, a series of ester derivatives **6**~**9** with an acyl group at 6-OH were synthesized by selective acylations on **2**.

Acremonidin B (**2**) was treated with an anhydride in the presence of boron trifluoride diethyl etherate ($BF_3 \cdot Et_2O$)[10] to afford an ester as shown in Fig. 2. The products **6**~**9**, generated by several analogous reactions, were tested against a panel of bacteria. The SAR data (Table 4) implied that the attachment of a longer hydrophobic acyl group at C-6 enhanced antibacterial potency against streptococci and enterococci, but the enhancement quickly reached the maximum with the isobutyrate **7** and pentanoate derivative **8**.

In summary, acremonidins A~E (**1**~**5**), featuring a bridging keto group in their structures, were produced by a fungus *Acremonium* sp. when fermented in heterogeneous phases. Acremonidins A and B showed moderate activity against Gram-positive bacteria, including the methicillin-resistant staphylococci and vancomycin-resistant enterococci. Selective acylations of acremonidin B afforded some ester derivatives **6**~**9** that exhibited improved antibacterial activity.

The biosynthesis of the benzophenone moieties in acremonidins **1**~**5** quite likely involves the octaketide-based anthraquinone as a precursor by an oxidative cleavage, as reported for xanthones and other related compounds.[11] Similar to the xanthoquinodins,[7] compounds **1**~**3** are probably formed by a coupling between the benzophenone and anthraquinone moieties and compound **4**, formed by a coupling between two units of benzophenone.

## Experimental Section

### Inoculum Preparation

Fungal culture *Acremonium* sp., strain *LL*-Cyan 416, was plated on Bennett's agar medium (10 g/liter Sigma D-glucose, 1 g/liter Difco beef extract, 1 g/liter Difco yeast extract, 2 g/liter N-Z amine A, 20 g/liter Difco agar) from a frozen 25% glycerol stock culture and then incubated at 22°C. A small agar slice bearing mycelial growth was used to inoculate 50 ml of Difco potato - dextrose broth in a 250-ml Erlenmeyer flask. This liquid seed culture was shaken at 200 rpm at 22°C for one week, and then used to inoculate production medium.

### Fermentation

Production medium (1 liter) consisted of malt extract agar (25 g Difco malt extract, 5 g Difco peptone, 0.5 g Difco yeast extract, 20 g Difco agar) that had been sterilized and poured into a 30×20×13 cm polypropylene tray covered with aluminum foil. The solidified agar was then overlaid with a sterile 28×46 cm sheet of nongauze milk-filter paper cut from 18×22 in strips (KenAG Animal Care Group, Ashland, Ohio) that had been sterilized separately. The production medium was inoculated by transferring 50 ml of seed culture fluid onto the sheet of milk-filter paper. The inoculated tray culture was incubated stationary at 22°C. After two weeks of incubation, the milk-filter paper bearing prolific mycelial growth was removed from the surface of the agar, lyophilized for 5 days, and then extracted with methanol (1.2 liters).

### Purification of Acremonidins A, B, and C (**1**~**3**)

The methanol extract was chromatographed by reverse phase HPLC on a C18 column (YMC ODS-A, 10 $\mu$m particle size, 70×500 mm), using a linear gradient of 30~100% acetonitrile in water containing 0.01% trifluoroacetic acid (TFA) over 35 minutes. Four fractions at 27.5, 30.5, 35.0, and 38.37 minutes were collected. The materials from the later three fractions were respectively purified by a different HPLC system (YMC ODS-A, 5 $\mu$m, 30×250 mm column, 40~75% acetonitrile in water containing 0.01% TFA over 30 minutes) to afford acremonidins B (**2**, 4.5 mg), C (**3**, 4.2 mg), and A (**1**, 130.8 mg), all as yellow amorphous powders.

### Purification of Acremonidins D (**4**) and E (**5**)

The material from the first fraction at 27.5 minutes was further separated by HPLC (YMC ODS-A, 5 $\mu$m, 30×250 mm column, 30~100% acetonitrile in water containing 0.01% TFA over 30 minutes) to afford pure acremonidins E (**5**, 21.0 mg) and D (**4**, 3.1 mg), both as pale yellow amorphous powders.

### Acremonidin A (**1**)

High-resolution FTICRMS (positive): *m/z* 615.14913 ($MH^+$, $C_{33}H_{27}O_{12}$ requires 615.14970); UV $\lambda_{max}$ (1 : 1 MeCN/$H_2O$, HP Diode Array Detector) 280, 291, 361 nm; $^1H$ and $^{13}C$ NMR data see Table 1.

### Acremonidin B (**2**)

High-resolution FTICRMS (positive): *m/z* 573.13900 ($MH^+$, $C_{33}H_{25}O_{11}$ requires 573.13968); UV $\lambda_{max}$ (1 : 1 MeCN/$H_2O$, HP Diode Array Detector) 282, 289, 359 nm; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 14.00 (br s, $D_2O$ exchangeable, 13-OH), 13.38 (s, $D_2O$ exchangeable, 1′-OH), 11.40 (s, $D_2O$ exchangeable, 11-OH), 10.06 (s, $D_2O$ exchangeable, 5′-OH), 9.61 (s, $D_2O$ exchangeable, 13-OH), 9.17 (s, $D_2O$ exchangeable, 13-OH), 6.91 (d, $J$=8.8 Hz, H-10′), 6.80 (br s, H-8), 6.79 (d, $J$=8.8 Hz, H-11′), 6.74 (br s, H-10), 6.42 (2H, m, H-3, H-4), 5.57 (br s, $D_2O$ exchangeable, 6-OH), 4.65 (dd, $J$=5.4, 2.5 Hz, H-2), 4.47 (br s, H-6), 3.56 (3H, s, $OCH_3$), 2.67, 2.30 (d, $J$=18.0 Hz, $H_2$-15′), 2.33 (3H, s, $CH_3$-15); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 199.86 (C-7′), 187.03 (C-1), 184.84 (C-13), 167.76 (C-14′), 160.51 (C-11), 159.50 (C-1′), 158.39 (C-5′), 151.04 (C-12′), 147.07 (C-9), 146.31 (C-3′), 145.53 (C-9′), 142.73 (C-7), 134.69 (C-4), 131.16 (C-8′), 130.18 (C-3), 122.22 (C-10′), 122.08 (C-8), 117.64 (C-11′), 117.36 (C-10), 113.77 (C-2′), 112.45 (C-13′), 111.63 (C-12), 109.45 (C-4′), 108.64 (C-6′), 106.16 (C-14), 71.39 (C-6), 52.02 ($OCH_3$), 42.37 (C-5), 37.41 (C-2), 34.44 (C-15′),

21.56 (C-15).

Acremonidin C (**3**)

High-resolution FTICRMS (positive): *m/z* 631.14490 ($MH^+$, $C_{33}H_{27}O_{13}$ requires 631.14462); UV $\lambda_{max}$ (1:1 MeCN/$H_2O$, HP Diode Array Detector) 281, 346; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.38 (s, $D_2O$ exchangeable, 1′-OH), 11.41 (s, $D_2O$ exchangeable, 11-OH), 10.20 (s, $D_2O$ exchangeable, 5′-OH), 9.64 (br s, $D_2O$ exchangeable, 13-OH), 9.22 (s, $D_2O$ exchangeable, 13-OH), 7.56 (br s, $D_2O$ exchangeable, 14-OH), 6.93 (d, *J*=8.8 Hz, H-10′), 6.82 (br s, H-8), 6.81 (d, *J*=8.8 Hz, H-11′), 6.63 (br s, H-10), 6.52 (dd, *J*=9.2, 6.7 Hz, H-3), 6.29 (br s, H-6), 5.99 (br s, H-4′), 5.79 (d, *J*=9.2 Hz, H-4), 4.62 (br d, *J*=6.7 Hz, H-2), 3.56 (3H, s, $OCH_3$), 3.08, 2.86 (d, *J*=18.7 Hz, $H_2$-15′), 2.35 (3H, s, $COCH_3$), 2.34 (3H, s, $CH_3$-15); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 202.71 (C-1), 199.83 (C-7′), 195.54 (C-13), 171.03 ($COCH_3$), 167.72 (C-14′), 162.68m (C-11), 158.96 (C-1′), 158.55 (C-5′), 151.09 (C-12′), 149.44 (C-9), 145.55 (C-9′), 145.00 (C-9), 139.53 (C-7), 134.60 (C-4), 131.00 (C-8′), 128.72 (C-3), 122.14 (C-10′), 118.24 (C-11′), 117.69 (C-8), 117.38 (C-10), 114.67 (C-3′), 112.33 (C-13′), 109.90 (C-12), 108.85 (C-6′), 108.60 (C-4′), 81.51 (C-14), 70.12 (C-6), 51.95 ($OCH_3$), 46.74 (C-5), 43.40 (C-2), 31.51 (15′), 21.84 (C-15), 20.86 ($COCH_3$).

Acremonidin D (**4**)

High-resolution FTICRMS (negative): *m/z* 647.14154 ($M-H^-$, $C_{33}H_{27}O_{14}$ requires 647.14016); UV $\lambda_{max}$ (1:1 MeCN/$H_2O$, HP Diode Array Detector) 288, 350 nm; $^1H$ and $^{13}C$ NMR data see Table 2.

Acremonidin E (**5**)

High-resolution FTICRMS (positive): *m/z* 319.08104 ($MH^+$, $C_{16}H_{15}O_7$ requires 319.08177); UV $\lambda_{max}$ (1:1 MeCN/$H_2O$, HP Diode Array Detector) 288, 348 nm; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.34 (2H, br s, $D_2O$ exchangeable, 10-OH, 14-OH), 9.71 (s, $D_2O$ exchangeable, 3-OH), 9.25 (br s, $D_2O$ exchangeable, 6-OH), 6.94 (d, *J*=8.8 Hz, H-5), 6.80 (d, *J*=8.8 Hz, H-4), 6.10 (2H, s, H-11, H-13), 3.65 (3H, s, $OCH_3$), 2.15 (3H, s, $CH_3$-15); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 199.35 (C-8), 168.05 (C-1), 161.57 (C-10, C-14), 151.22 (C-3), 147.56 (C-12), 145.65 (C-6), 131.24 (C-7), 122.21 (C-5), 117.52 (C-4), 112.36 (C-2), 108.96 (C-9), 107.53 (C-11, C-13), 51.94 ($OCH_3$), 21.65 (C-15).

Acremonidin B Butyrate (**6**)

To a solution of acremonidin B (**2**, 20.0 mg) in dry tetrahydrofuran (0.5 ml), was added dropwise a solution of 7% (v/v) of $BF_3 \cdot Et_2O$ in butyric anhydride (0.2 ml) at 0°C. The reaction mixture was stirred at this temperature for 2 hours before methanol (2.0 ml) was added. The resulting solution was stirred at ambient temperature for 0.5 hour and then chromatographed by reversed phase HPLC on a C18 column to afford acremonidin B butyrate (**6**, 15.3 mg). ESIMS (negative) *m/z* 641, $(M-H)^-$.

Acremonidin B Isobutyrate (**7**)

[ESIMS (negative) *m/z* 641, $(M-H)^-$], pentanoate (**8**) [ESIMS (negative) *m/z* 655, $(M-H)^-$], and hexanoate (**9**) [ESIMS (negative) *m/z* 669, $(M-H)^-$] were synthesized using an appropriate anhydride to replace the butyric anhydride in production of **6**.

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