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Acremoxanthones A and B, novel antibiotic polyketides from the fungus *Acremonium* sp. BCC 31806

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ABSTRACT

Acremoxanthones A and B, novel anthraquinone–xanthone heterodimers with a unique linkage pattern, together with two known compounds, acremonidins A and C, were isolated from the fungus *Acremonium* sp. BCC 31806. The structures of the acremoxanthones were determined by analysis of 2D NMR and mass spectrometric data. Acremoxanthones and acremonidins exhibited antibacterial, antifungal, antiplasmodial, and cytotoxic activities.

As part of our research program on novel bioactive fungal secondary metabolites, we have investigated the constituents of the fungus *Acremonium* sp. BCC 31806 as the ^{1}H NMR spectrum of a mycelial extract from this fungus had shown unique resonance patterns.[1] Chemical studies on a large-scale fermentation broth of BCC 31806 afforded two new heterodimers of aromatic polyketides, acremoxanthones A (**1**) and B (**2**), along with two known compounds acremonidins A (**3**) and C (**4**).[2,3] Details of the isolation, structure elucidation, and biological activities of these compounds are presented here.

Acremonium sp. BCC 31806 was fermented in potato dextrose broth (PDB; 20 × 250 mL) at 25 °C for 28 days under static conditions. The MeOH extract (1.31 g) from mycelia was subjected to fractionation using Sephadex LH-20 and silica gel column chromatography, and the eluted fractions containing products were further purified by preparative HPLC using a reverse phase column to furnish **1** (71 mg), **2** (26 mg), **3** (103 mg), and **4** (75 mg).

The molecular formula of acremoxanthone A (**1**) was determined to be $C_{33}H_{24}O_{11}$ by HRMS (ESI-TOF) and by analysis of ^{1}H, ^{13}C, and DEPT NMR data.[4] The IR spectrum (KBr) shows intense and broad absorption bands at ν_{max} 1731 and 1641 cm^{-1} and a broad hydroxy absorption at 3425 cm^{-1}. The ^{13}C NMR spectrum in $CDCl_3$ shows 33 resolved peaks. DEPT and HMQC experiments enabled the categorization of these carbons into 19 sp^2 quaternary carbons, seven sp^2 methines, one sp^3 quaternary carbon (δ_C 41.6), one oxymethine (δ_C 73.1), one sp^3 methine (δ_C 37.6), one methylene (δ_C 35.2), one methoxy group (δ_C 53.2), and two methyl groups (δ_C 22.1 and 21.2). The ^{1}H NMR spectrum ($CDCl_3$) displayed four phenolic OH signals at δ_H 14.15 (br), 12.81 (s), 11.62 (s), and 7.72 (br). Analysis of the HMBC data revealed the complete structure of **1** as the heterodimer of an anthraquinone moiety (ABC-ring) and a xanthone moiety (DEF-ring). Thus, the A-ring of the anthraquinone was established from the presence of two *meta*-coupled methines, H-5 and H-7, and the HMBC correlations from the chelated OH (δ_H 11.62, 8-O*H*) to C-7, C-8, and C-8a, and from the δ_H 2.40 methyl protons (br s, H-11) to C-5, C-6, and C-7. Weak 4-bond correlations from H-5 and H-7 to the δ_C 186.1 quaternary carbon were observed, hence, this carbon (C-9) should be attached to the quaternary carbon C-8a. The C-ring was composed of a *cis*-olefin (C-3, C-4) connected to a methine (δ_C 37.6, C-2) and a quaternary carbon (δ_C 41.6, C-4a). These carbons, together with two sp^2 carbons at δ_C 185.9 (C-1) and δ_C 105.2 (C-9a), formed a six-membered ring. This connectivity was shown by the COSY correlations (H-2–H-3–H-4) and HMBC correlations from H-2 to C-1 and C-9a, from H-3 to C-4a, and from H-4 to C-2, C-4a, and C-9a. The ABC-ring juncture was confirmed by the HMBC correlations from the oxymethine at δ_H 6.02 (s, H-10) to C-4, C-4a, C-9a, C-10a, C-5, and C-8a. The xanthone structure (DEF-ring) was also deduced from the HMBC correlations. The D-ring portion was assigned from the HMBC correlations from a chelated OH (δ_H 12.81, 1′-O*H*) to C-1′, C-2′, and C-9′a, from H-4′ to C-2′, C-4′a, and C-9′a, and from the non-equivalent methylene protons (δ_H 2.93 and 2.80, H-11′) to C-2′, C-3′, and C-4′. A weak 4-bond HMBC correlation from H-4′ to

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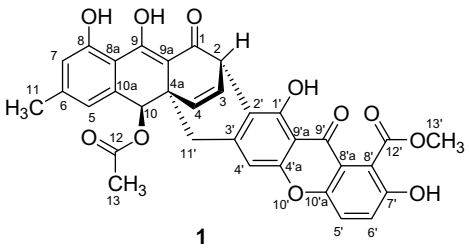

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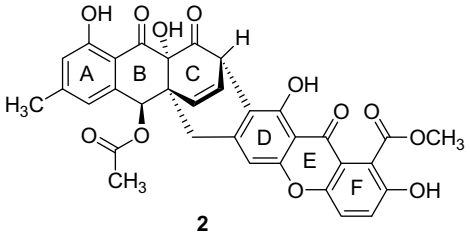

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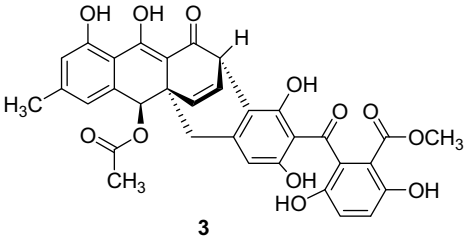

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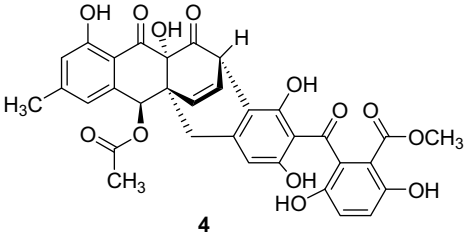

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the δ_C 180.2 quaternary carbon and chelated 1′-OH (δ_H 12.81) indicated the carbonyl linkage at C-9′. The rest of the ^1H and ^{13}C NMR signals were assigned to those for the F-ring portion, which consisted of a tetrasubstituted benzene with vicinally coupled methines at δ_H 7.38 (br d, J = 9.1 Hz, H-5′) and 7.34 (br d, J = 9.1 Hz, H-6′) and substituted with one OH and one methoxycarbonyl group. The molecular formula of **1**, established by HRMS, and the upfield ketone carbonyl (C-9′, δ_C 180.2) resonance required the tricyclic xanthone structure (DEF-ring). The linkage between the two tricyclic units was addressed also from the HMBC correlations: from H-2 to C-1, C-2, and C-3, from H-10 to C-11′, and from H-11′ to C-4, C-4a, C-9a, and C-10. Proton H-4 and one of the diastereotopic methylene protons at δ_H 2.93 (H_β-11′) show a weak NOESY cross-peak, whilst H-10 exhibited NOESY correlations to both H_β-11′ and H_α-11′ (δ_H 2.80) as well as weak cross-peaks with H-4 and H-5. These data indicated that H-10 and C-11′ should be synfacial.

The molecular formula of acremoxanthone B (**2**)[5] was determined by HRMS as $C_{33}H_{24}O_{12}$, containing one more oxygen atom than **1**. The ^1H and ^{13}C NMR data for **2** were similar, in particular the xanthone moiety, to those of **1**. The major differences were the down-field shifted C-1 (δ_C 205.2) and C-9 (δ_C 195.8) when compared to **1** (δ_C 185.9 and 186.1), and the replacement of the sp^2 quaternary carbon at δ_C 105.2 (C-9a) of **1** by a tertiary alcohol (δ_C 82.2). The chelated phenolic proton, 9-OH (δ_H 14.15, br s) for **1**, was absent in **2**. Instead, an OH resonated at δ_H 7.69 (9a-OH), which exhibited HMBC correlations to C-4a and C-9a (δ_C 82.2). Detailed analysis of the 2D NMR data, in particular HMBC correlations (Table 1), revealed that the xanthone part of the molecule was identical to **1**. Therefore, acremoxanthone B (**2**) is the C-9a oxygenated derivative of **1**. The relative configuration of **2** was addressed by analysis of NOESY data (DMSO-d_6). Proton H-4′ showed NOESY correlations to H_α-11′ (δ_H 3.34) and H_β-11′ (δ_H 3.19) with slightly higher cross-peak intensity for the former, while H-10 showed a correlation to H_α-11′ with a higher intensity than that to H_β-11′. Proton H-10 did not show clear NOESY correlations to H-5 or H-4. These data strongly suggested a pseudoaxial orientation of H-10. Unfortunately, there was no NOESY cross-peak from 9a-OH (br s) to any CH or CH_2 group. The NOESY spectrum acquired in acetone-d_6 also provided similar information. However, examination of a molecular model of both the 9a-α-OH and 9a-β-OH isomers demonstrated that only isomer 9a-α-OH is consistent with the above-mentioned NOESY data. In the 9a-α-OH isomer, 9a-OH and H-10 occupy pseudoaxial positions (Fig. 1). In contrast, the 9a-β-OH configuration required pseudoequatorial orientation of H-10.

The structures of compounds **3** and **4** were elucidated on the basis of HRMS and 2D NMR data.[6,7] The ^1H and ^{13}C NMR spectral data for these isolated compounds in DMSO-d_6 were identical to those of acremonidins A and C, respectively.[2] Acremonidins A–E were previously isolated from *Acremonium* sp. *LL*-Cyan 416. In the original report,[2] the relative configuration of C-2, C-4a, and C-10 for acremonidins A and C was proposed as shown in the structures **3** and **4**, respectively, whereas the configuration of the additional chiral carbon center, C-9a, in **4** was not presented. Since the ^1H and ^{13}C NMR data for the ABC-ring moiety are very close between acremoxanthone B (**2**) and acremonidin C (**4**) (in DMSO-d_6), and considering also their co-occurrence in BCC 31806, we propose that these compounds possess the same stereochemistry at C-9a.

A unique structural feature of acremoxanthones is the linkage pattern of two tricyclic units. Ring junctions similar to those of acremoxanthones/acremonidins are shown only in xanthoquinodins (anticoccidial antibiotics from *Humicola* sp. FO-888),[8] beticolins/cebetins (yellow toxins produced by *Cercospora beticola*),[9,10] and chaetomanone (from *Chaetomium globosum* KMITL-N0802).[11] Similar to the biosynthesis of xanthoquinodins,[8b] acremoxanthones are probably formed via coupling between the xanthone (elminthosporin) and the xanthone (pinselin) moieties.[12] Xanthone and benzophenone monomers, such as those corresponding, respectively, to acremoxanthones and acremonidins, are known to be produced by oxidative cleavage of common anthraquinones.[8b,12,13]

Compounds **1**–**4** were subjected to in vitro biological assays: antibacterial (*Staphylococcus aureus*, *Bacilus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli*),[14] antifungal (*Candida albicans*, *Magnaporthe grisea*),[14] antimycobacterial (*Mycobacterium tuberculosis* H37Ra), and antiplasmodial (*Plasmodium falciparum* K1)[15] activities, and cytotoxicity to three cancer cell-lines (KB, BC, and NCI-H187)[14] and non-cancerous Vero cells. Table 2 shows selected data. Compounds **1**–**4** exhibited moderate antibacterial activity against S. aureus and B. cereus, but they were inactive against *P. aeruginosa* and *E. coli*. Only acremoxanthone A (**1**) displayed significant activity against C. albicans (IC_{50} 1.7 μg/mL), while all the compounds were inactive against the plant pathogenic fungus *M. grisea*. All these compounds lacked antituberculosis activity; however, the antimalarial activity of **2** and **4** should be noted. Whereas compounds **1**–**4** exhibited significant antibiotic activities, they also showed cytotoxicity to all tested cell-lines. Acremonidins A (**3**) and B (deacetyl analogue of **3**) are known to show moderate activity against Gram-positive bacteria, including the methicillin-resistant staphylococci and vancomycin-resistant enterococci.[2]

Table 1
NMR data for acremoxanthones A (**1**) and B (**2**) (500 MHz for ^{1}H, and 125 MHz for ^{13}C)

Position	**1** (in $CDCl_3$)			**2** (in DMSO-d_6)		
	δ_C mult.	δ_H mult. (*J* in Hz)	HMBC	δ_C mult.	δ_H mult. (*J* in Hz)	HMBC
1	185.9 qC			205.2 qC		
2	37.6 CH	4.94 dd (6.6, 0.6)	1,3,4,9a,1′,2′,3′	44.0 CH	4.69 d (6.1)	1,3,4,9a,1′,2′,3′
3	131.9 CH	6.49 dd (8.4, 6.7)	2,4,4a,5	134.9 CH	6.54 dd (9.1, 6.6)	1,2,4a
4	132.5 CH	6.13 dd (8.4, 0.6)	2,3,4a,9a,10	129.6 CH	5.84 d (9.1)	2,4a,9a,10,
4a	41.6 qC			47.2 qC		
5	123.2 CH	6.93 br s	6,7,8a,9,10,10a,11	118.8 CH	6.65 br s	7,8a,10,11
6	147.8 qC			150.0 qC		
7	119.4 CH	6.82 br s	5,8,8a,9,11	117.9 CH	6.84 brs	5,8a,11
8	161.8 qC			163.2 qC		
8-O*H*		11.62 s	7,8,8a		11.40 s	7,8,8a
8a	112.7 qC			110.4 qC		
9	186.1 qC			195.8 qC		
9-O*H*		14.15 br s				
9a	105.2 qC			82.2 qC		
9a-O*H*					7.69 s	4a,9a
10	73.1 CH	6.02 s	4,4a,5,8a,10a,12,11′	70.5 CH	6.33 br s	4,4a,10a,12,11′
10a	136.5 qC			140.0 qC		
11	22.1 CH_3	2.40 s	5,6,7	22.4 CH_3	2.37 br s	5, 6, 7
12	170.6 qC			171.5 qC		
13	21.2 CH_3	2.03 s	12	21.4 CH_3	2.38 s	12
1′	157.8 qC			156.7 qC		
1′-O*H*		12.81 s	1′,2′,9′a		12.83 s	1′,2′,9′a
2′	117.4 qC			118.4 qC		
3′	146.4 qC			147.1 qC		
4′	109.8 CH	6.47 br s	2′,4′a,9′,9′a,11′	109.9 CH	6.99 s	2′,4′a,9′a,11′
4′a	154.0 qC			154.3 qC		
5′	122.0 CH	7.38 br d (9.1)	7′,8′a,10′a	120.6 CH	7.61 d (9.2)	7′,8′a
6′	125.7 CH	7.34 br d (9.1)	8′,10′a	126.1 CH	7.48 d (9.2)	8′,10′a
7′	152.3[a] qC			151.3[c] qC		
7′-O*H*		7.72 br s			10.53 br s	
8′	114.2 qC			117.50[b] qC		
8′a	118.4 qC			117.53[b] qC		
9′	180.2 qC			180.9 qC		
9′a	107.0 qC			106.2 qC		
10′a	150.6[a] qC			149.4[c] qC		
11′	35.2 CH_2	2.93 d (17.7)	4,4a,9a,10,2′,4′	32.3 CH_2	3.34 d (19.2)	4,4a,3′,4′
		2.80 d (17.7)	4,4a,9a,10,2′,3′,4′		3.19 d (19.2)	4a,9a,2′,3′
12′	169.0 qC			167.1 qC		
13′	53.2 CH_3	4.01 s	12′	52.7 CH_3	3.85 s	12′

[a–c] Assignment of carbons can be interchanged.

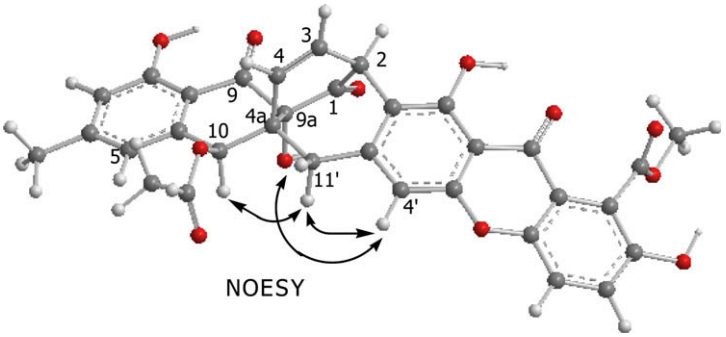

Figure 1. Probable stereo structure of acremoxanthone B (**2**).

Table 2
Biological activities of compounds **1**–**4**

Compound	*S. aureus*[a]	*B. cereus*[a]	*C. albicans*[b]	*P. falciparum*[c]	Cytotoxicity (IC_{50}, μg/mL)[d]			
	(MIC, μg/mL)	(MIC, μg/mL)	(IC_{50}, μg/mL)	(IC_{50}, μg/mL)	KB	BC	NCI-H187	Vero
1	12.5	>100	1.7	>10	3.3	1.1	0.87	1.2
2	6.25	6.25	>50	3.0	18	9.4	14	12
3	3.13	1.56	>50	5.4	13	5.0	4.5	6.6
4	3.13	3.13	>50	>10	>20	9.3	16	23

[a] Antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* was evaluated using the resazurin microplate assay (REMA). MIC values of a standard antibacterial vancomycin for *S. aureus* and *B. cereus* were 1.0 and 4.0 μg/mL, respectively.
[b] Antifungal activity against *Candida albicans*. Standard compound, amphotericin B, showed an IC_{50} value of 0.047 μg/mL.
[c] Antimalarial activity against *Plasmodium falciparum* K1. Standard antimalarial drug, dihydroartemisinin, showed an IC_{50} value of 0.0011 μg/mL.
[d] The IC_{50} values of a standard compound, ellipticine, against KB, BC, NCI-H187 and Vero cells were 0.27, 0.21, 0.15 and 0.60 μg/mL, respectively.

References and notes

1. This fungus was isolated from indoor air at BIOTEC, Thailand, and is deposited at the BIOTEC Culture Collection as BCC 31806.
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3. Bigelis, R.; He, H.; Yang, H. Y.; Chang, L.-P.; Greenstein, M. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 815–826.
4. Acremoxanthone A (**1**): Yellow powder; $[\alpha]_D^{26}$ +649 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (4.26), 245 (4.46), 273 (4.47), 330 (4.18), 366 (4.37) nm; IR (KBr) ν_{max} 3425, 1731, 1641, 1612, 1577, 1220, 1015 cm^{-1}; HRMS (ESI-TOF) *m*/*z* 595.1277 $[M-H]^-$ (calcd for $C_{33}H_{23}O_{11}$, 595.1246); ^1H and ^{13}C NMR data in $CDCl_3$, Table 1.
5. Acremoxanthone B (**2**): Yellow powder; $[\alpha]_D^{27}$ +52 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (4.12), 241 (4.21), 275 (4.45), 302 (sh) (4.00), 397 (sh) (3.59) nm; IR (KBr) ν_{max} 3443, 1737, 1639, 1433, 1233, 1211 cm^{-1}; HRMS (ESI-TOF) *m*/*z* 635.1175 $[M+Na]^+$ (calcd for $C_{33}H_{24}O_{12}Na$, 635.1160); ^1H and ^{13}C NMR data in DMSO-d_6), Table 1.
6. Acremonidin A (**3**): Yellow powder; $[\alpha]_D^{26}$ +422 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.04), 241 (4.23), 276 (4.15), 290 (sh) (4.11), 362 (4.29) nm; IR (KBr) ν_{max} 3426, 3204, 1731, 1681, 1614, 1462, 1217 cm^{-1}; HRMS (ESI-TOF) *m*/*z* 637.1311 $[M+Na]^+$ (calcd for $C_{33}H_{26}O_{12}Na$, 637.1316); ^1H and ^{13}C NMR spectral data in DMSO-d_6 were identical to those reported in the literature.
7. Acremonidin C (**4**): Yellow powder; $[\alpha]_D^{26}$ −20 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (4.17), 241 (4.33), 278 (4.43), 349 (4.18) nm; IR (KBr) ν_{max} 3426, 3208, 1736, 1680, 1624, 1207 cm^{-1}; HRMS (ESI-TOF) *m*/*z* 629.1318 $[M-H]^-$ (calcd for $C_{33}H_{25}O_{13}$, 629.1301); ^1H and ^{13}C NMR spectral data in DMSO-d_6 were identical to those reported in the literature.
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