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Acremonol and Acremodiol, New Fungal Bislactones

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In the course of our continuing search for new fungal metabolites with antibacterial properties we isolated new bislactones, acremonol (**1**) and acremodiol (**2**) (Fig. 1). In this paper we describe the isolation and structural elucidation of **1** and **2** by ESI-MS, FAB-MS and NMR spectroscopy.

The new bislactones **1** and **2** possess structures related to grahamimycin[1] and the colletodiol family[2,3]. For the latter compounds chemical synthesis was reported[4]. Biosynthetic studies[5] revealed that these compounds are polyketides. The producer strain of **1** and **2** was isolated from a soil sample of the Bermuda Islands as a taxonomically unidentified *Acremonium*-like anamorphic fungus. It forms mycelial colonies colored pale pink to pale brown. The hyphae are mostly narrow, seldom up to 6 μm in diameter. The characteristic conidia are ellipsoid to short cylindrical, mostly 3.5~6.5/2~3 μm.

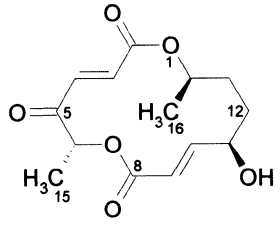
The producer strain was cultivated as surface culture at 24°C in 500 ml Erlenmeyer bottles containing 100 ml medium composed as follows (g/liter): malt extract 20, glucose 10, yeast extract 1, pH 6.0. Each bottle was inoculated with a 1 cm^2 area of a 15 days agar culture. The cultivation time as stationary culture was three weeks at 24°C. Acremonol (**1**) and acremodiol (**2**) were extracted from 40 liters culture broth with 20 liters of ethyl acetate, which was subsequently dried (Na_2SO_4) and evaporated to yield 2.2 g of an oily residue. This was chromatographed on silica gel 60 (Merck, 0.063~0.1 mm, column 600×30 mm). Sequential elution occurred with 40 ml portions of $CHCl_3$, $CHCl_3$-MeOH (95 : 5, 80 : 20, 60 : 40, 0 : 100, v/v). Fractions of similar composition, as determined by TLC, were pooled to yield 63 mg of a mixture of **1** and **2**. Further purification was carried out by chromatography on Sephadex LH-20 (column 400×20 mm, eluent MeOH - $CHCl_3$, 8 : 2, v/v) and preparative TLC (Merck, silica gel 60 F_{254}, 20×20 cm, eluent $CHCl_3$-MeOH, 9 : 1, v/v). Yield: 11 mg of acremonol (**1**) and 23 mg of acremodiol (**2**). The physicochemical properties of **1** and **2** are shown in Table 1. The molecular weight and the chemical formula of the new metabolites were determined by HREI-MS (double-focussing mass spectrometer MAT 95XL, Finnigan, Bremen, Germany) (Table 1) from *m/z* 282.1200 (**1**; M^+; calcd. 282.1103) and *m/z* 286.1402 (**2**; M^+; calcd. 286.1416). The positive ion ESI-MS (triple quadrupole mass spectrometer Quattro 400, VG Biotech, Altrincham, U.K.) showed *m/z* 283.2 $[M+H]^+$ for **1** and *m/z* 287.4 $[M+H]^+$ for **2**.

The structures of compounds **1** and **2** were determined by one- and two-dimensional NMR spectroscopy (^1H, ^{13}C, DEPT, COSY, HMQC, HMBC, Table 2, Bruker Avance DRX 500, Germany).

The positions of substituents and double bonds in the 14-membered ring system were assigned due to observable cross peaks in the ^1H, ^1H-COSY spectrum and C, H long-range correlations (HMBC).

The values of coupling constants of neighbouring

Fig 1. Structures of acremonol (**1**) and acremodiol (**2**).



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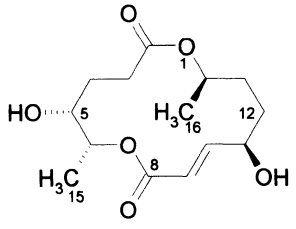

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Table 1. Physico-chemical properties of acremonol (**1**) and acremodiol (**2**).

	1	**2**
Appearance	colorless solid	colorless solid
Melting point[a]	83 - 84 °C	110 - 112 °C
Chemical formula	$C_{14}H_{18}O_6$	$C_{14}H_{22}O_6$
HREI-MS *m/z*	282.1200 $[M]^+$	286.1402 $[M]^+$
	calcd. 282.1103	calcd. 286.1416
$[\alpha]_D^{25}$ (c 0.3, MeOH)	+ 40 °	+ 98 °
IR (KBr) cm^{-1}	1715, 1651, 1648	1690, 1655, 1647
R_f values[b]	0.51	0.38

[a] Büchi Melting Point B 540

[b] $CHCl_3$-MeOH (9:1, v/v)

Table 2. ^1H and ^{13}C assignments of acremonol (**1**) and acremodiol (**2**) (in DMSO-d_6, chemical shifts in ppm, multiplicity in parentheses (s: singlet, d: doublet, t: triplet, m: multiplet), coupling constants in Hz).

Position	**1**			**2**		
	δ_C	δ_H	**multiplicity coupling constants**	δ_C	δ_H	**multiplicity coupling constants**
2	163.9	-	-	174.7	-	-
3	136.7	7.35	d, 16.2	32.0	2.56, 2.86	m, m
4	129.8	6.37	d, 16.2	26.3	1.90, 2.12	m, m
5	200.1	-	-	72.7	3.70	m
6	75.3	5.15	q, 7.2	73.6	5.27	d, q, 6.5, 2.1
8	165.7	-	-	164.9	-	-
9	118.1	5.92	dd, 16.3, 1.7	120.6	6.02	dd, 15.9, 2.2
10	154.3	7.18	dd, 16.3, 3.1	149.2	6.67	dd, 15.9, 2.6
11	68.8	4.35	m	69.2	4.60	m
12	30.4	1.48, 1.87	m, m	28.2	1.78	m
13	27.6	1.66, 1.55	m, m	26.9	1.52, 1.82	m, m
14	71.9	4.89	m	70.6	4.95	m
15	15.8	1.48	d, 7.2	16.7	1.24	d, 6.4
16	18.5	1.20	t, 7.0	19.2	1.16	d, 6.6

olefinic protons (Table 2) confirmed *E*-configuration for the double bonds. The absence of NOE effects between H-11/H-16 and H-15/H-16, respectively, in the NOESY spectra was compatible with the view that relative stereochemistry of **1** and **2** is the same as was reported for colletodiol[2,3]. The $^3J_{H\text{-}5,H\text{-}6}$=2.1 Hz suggested synclinal proton conformation and α-positions of substituents at C-5 and C-6.

Table 3. Antimicrobial activity of acremonol (**1**) and acremodiol (**2**).

Microorganisms	Diameter of inhibition zone (mm)	
	1	**2**
Bacillus subtilis ATCC 6633	24	22
Sporobolomyces salmonicolor	0	0
Penicillium notatum JP 36	18 p[a]	16 p
Escherichia coli SG 458	14 p	13 p
Enterococcus faecalis	21	16
Staphylococcus aureus	26	20
Pseudomonas aeruginosa	13 p	12 p

Each well contained 50 μg of 1 or 2 dissolved in 50 μl methanol

[a] p = partial inhibition

The new metabolites **1** and **2** display antimicrobial activity against a series of Gram-positive bacteria and fungi in the agar well diffusion assay[6,7] (Table 3). In contrast, colletodiol described as biologically inactive.

Moreover, **1** and **2** were active in cellular phagocytosis assay using dog PMNL cells[8] decreasing the oxidative burst at concentrations ≥4 μg/ml.

Acknowledgements

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