Xialenons, New Pentalenons from *Streptomyces*

Yuan-Qing Tang,[a] Isabel Sattler,[a] Ralf Thiericke,*[a] Susanne Grabley,[a] and Xiao-Zhang Feng[b]

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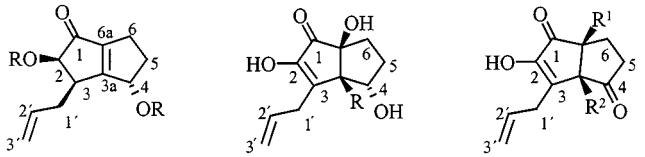
New pentalenons, named xialenon A to E (**1** to **5**), were discovered by a chemical screening of the culture broth of the *Streptomyces sp.* (strain GT 061169). The chemical structures of these secondary metabolites were determined by detailed spectroscopic investigation as well as chemical derivatization reactions. The absolute stereochemistry of **1** was determined by esterification with chiral acids via Helmchen's method. A common structural element of the xialenons is an α,β-unsaturated ketone in one of the two fused 5-membered rings (reduced double bond in **1**), that is substituted with both, a hydroxyl group in α-position, and an additional allyl side chain in β-position.

Introduction

With the application of a chemical screening,[1] new pentalenons that bear striking elements: (i) a bicyclo[3.3.0]octane moiety without methyl substituents and (ii) an α,β-unsaturated ketone in one of the 5-membered rings (reduced double bond in **1**) that is substituted with both a hydroxyl group in α-position and an additional allyl side chain in β-position, have been discovered in the culture broth of a *Streptomyces* species. These secondary metabolites are most likely derived from the polyketide biosynthetic pathway. This paper deals with the fermentation, purification, structure elucidation, physio-chemical properties, the relative and absolute configuration, as well as derivatization attempts of the new pentalenons, named xialenon A, B, C, D, and E (**1** to **5**, Scheme 1).



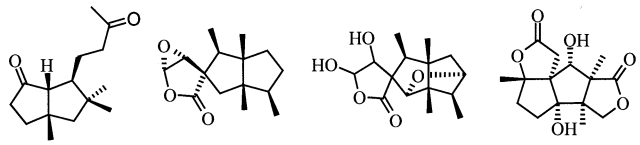
1: R = H
6: R = Ac
7: R = (2*R*)-2-phenylbutyryl
8: R = (2*S*)-2-phenylbutyryl

2: R = H
3: R = OH

4 R^1 = OH, R^2 = H
5 R^1 = H, R^2 = OH

Scheme 1. Structures of xialenon A to E (**1** to **5**) and derivatives of xialenon A (**1**)

The majority of natural products bearing a hexa- or octahydro-pentalene moiety have been isolated from plants. Only a few examples are known to originate from fungi or bacteria. Usually, pentalenes are part of a multi-cyclic chemical structure, and often are biosynthesized via mevalonic acid building blocks using the isoprenoid pathway. Only a few secondary metabolites have a bicyclic pentalenon skeleton. 1,9-Seco-1,9-presilphiperfolanedione[2] (**9**) isolated from the plant *Artemisia chamaemelifolia* bears a simple bicyclo[3.3.0]octane skeleton, while ptychanolide[3] (**10**), and spiropinguisanin[4] (**11**) possess an additional spiro-linked 5-membered ring. In addition, anislactone[5] (**12**) has a larger ring system that integrates in the bicyclo-[3.3.0]octane moiety (Scheme 2).



9 **10** **11** **12**

Scheme 2. Structures of 1,9-seco-1,9-presilphiperfolanedione (**9**), ptychanolide (**10**), spiropinguisanin (**11**), and anislactone (**12**)

Results and Discussion

In our screening routine,[1] extracts of the Streptomyces strain GT 061169 showed striking brown spots [R_f = 0.49 (**1**), 0.39 (**2**), 0.30 (**3**), 0.51 (**4**), and 0.50 (**5**), respectively] on HPTLC silica gel plates ($CHCl_3$/MeOH, 9:1) after staining with anisaldehyde−H_2SO_4. In order to isolate significant amounts of these compounds, cultivation of the producing organism was carried out in three 100-L fermentors containing the yeast-extract medium B (6 d, at 28 °C, 500 rpm, aeration 10 L/min). After harvesting, the culture filtrate (total 200 L) was lyophilized to yield 129 g crude product, which was dissolved in water and extracted with ethyl acetate by an Extra-Flow Membrane Contactor (Liqui-Cel, Hoechst Celanese). Followed by column chromatography on silica gel, gel-permeation chromatography on Sephadex LH-20 and RP-HPLC on a preparative scale, pure xialenons A to E (**1** to **5**) were obtained. The workup procedures yielded 0.40 (**1**), 1.75 (**2**), 0.15 (**3**), 0.10 (**4**), and 0.06 mg/L (**5**) (based on the volume of culture medium),

[a] Hans-Knöll-Institute for Natural Products Research,
Beutenbergstraße 11, D-07745, Jena, Germany
Fax: (internat.) +49 (0)3641/656699
E-mail: thierick@pmail.hki-jena.de
[b] Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College,
Xian Nong Tan 1, 100050, Beijing, P. R. of China

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respectively. The xialenons exhibit good solubility in methanol and chloroform, and appeared to be insoluble in *n*-hexane. Compound **1** was found to be more stable under neutral, weakly basic, and acidic conditions at room temperature, while **2** to **5** were rapidly decomposed by the addition of bases or acids.

Xialenon A (1)

The molecular formula $C_{11}H_{14}O_3$ pointing to 5 double-bond equivalents was deduced from ESI-MS [*m*/*z* = 195 $(M + H)^+$] and HREI-MS [*m*/*z* = 176.0854 $(M - H_2O)^+$] spectra. The IR spectrum shows the presence of both hydroxyl and conjugated carbonyl groups (adsorption bands at: $\tilde{\nu}$ = 3425 and 1696 cm^{-1}). As expected from mass spectrometry the ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **1** exhibits 14 protons signals, which indicated a terminal methylene double bond (δ = 5.81, 5.10/5.05), three methine groups (δ = 5.01, 4.49, and 3.30), as well as three methylene groups (δ = 2.68/2.10, 2.57/2.14, and 2.49/2.34). The protons of two hydroxyl groups are overlapped by the signals at δ = 2.68 and δ = 2.34 (Table 1). Acylation reaction of **1** with acetic acid anhydride in pyridine led to the diacetate **6**, which shows significant downfield shifts in the ^{1}H-NMR spectrum for the two methine protons [Δδ(2-H) = 1.11 ppm and Δδ(4-H) = 0.82 ppm], indicating their linkage to hydroxyl groups.

Table 2. ^{13}C-NMR data of the xialenons A to E (**1** to **5**)

Position	**1** [a]	**2** [b]	**3** [c]	**4** [c]	**5** [a]
1	204.0 (s)	204.5 (s)	202.6 (s)	203.4 (s)	201.2 (s)
2	78.6 (d)	150.0 (s)	152.7 (s)	149.8 (s)	151.0 (s)
3	40.0 (d)	146.4 (s)	145.8 (s)	141.3 (s)	141.0 (s)
3a	183.7 (s)	55.3 (d)	84.2 (s)	59.7 (d)	82.0 (s)
4	73.3 (d)	73.6 (d)	80.6 (d)	214.3 (s)	214.3 (s)
5	37.2 (t)	33.7 (t)	29.2 (t)	37.7 (d)	33.8 (t)
6	23.1 (t)	32.7 (t)	30.9 (t)	31.8 (t)	20.0 (t)
6a	145.5 (s)	82.5 (s)	80.1 (s)	80.0 (s)	50.8 (d)
1′	33.4 (t)	33.8 (t)	32.4 (t)	31.2 (t)	28.5 (t)
2′	136.2 (d)	135.2 (d)	136.0 (d)	133.7 (d)	132.6 (d)
3′	117. 1(t)	117.1 (t)	116.1 (t)	117.9(t)	117.9 (t)

[a] In $CDCl_3$ (125 MHz). – [b] In CD_3OD (75 MHz). – [c] In CD_3OD (125 MHz).

The ^{13}C-NMR spectrum (125.0 MHz, $CDCl_3$) shows the signals of eleven carbon atoms (Table 2). A ^{13}C-DEPT spectrum exhibits four methine groups (δ = 136.2, 78.6, 73.3, and 40.0) as well as four methylene groups (δ = 117.1, 37.2, 33.4, and 23.1) which are in agreement with the ^{1}H-NMR data. Besides the proton-attached carbon atoms, with the signals of three quaternary carbon atoms (δ = 145.5, 183.7, and 204.0) the ^{13}C-NMR spectrum points to an α,β-unsaturated carbonyl group. The assignment of the low-field signal δ = 183.7 to a double bond carbon is justified by comparison to the chemical shift of the double bond carbon atoms in bicyclo[3.3.0]-1(5)-en-2-one[6] (δ = 187.3 and

Table 1. ^{1}H-NMR data of the xialenons A to E (**1** to **5**)

Position	**1** [a]	**2** [b]	**2** [c]	**3** [d]	**3** [c]	**4** [d]	**5** [a]
2	4.49 (d, 6.1)						
3	3.30 (dddd, 7.8, 6.7, 6.1, 2.2)						
3a		2.85 (br, d, 7.3,)	2.69 (d, 7.3)			2.98 (s)	
4	5.01 (ddd, 2.2, 7.3, 5.9)	4.34 (dt, 4.7, 7.3)	4.24 (m)	4.11(dd, 10.2, 6.5)	3.93 (dd, 9.8, 6.0)		
5_α	2.10 (dddd, 13.7, 6.7, 5.9, 2.4)	1.49 (m)	1.29 (m)	1.08 (ddt, 12.4, 10.2, 6.5)	0.90 (dddd, 12.8, 12.3, 9.8, 6.3)	2.40 (ddd, 12.6, 12.0, 6.0)	2.31 (ddd, 12.3, 10.0, 5.5)
5_β	2.68 (dddd, 13.7, 7.3, 5.4, 3.2)	1.93 (m)	1.78 (m)	1.84 (dtd, 12.4, 6.5, 2.4)	1.65 (dddd, 12.3, 6.6, 6.0, 2.1)	2.42 (ddd, 12.6, 6.5, 2.2)	2.52 (ddd, 12.3, 6.8, 1.9)
6_α	2.49 (ddd, 16.4, 5.4, 2.4)	1.90 (m)	1.69 (dt, 13.0, 6.0)	1.96 (ddd, 13.2, 6.5, 2.4)	1.75 (ddd, 12.8, 6.3, 2.1)	2.26 (ddd, 13.2, 6.0, 2.2)	2.19 (m)
6_β	2.34 (ddd, 16.4, 6.7, 3.2)	1.58 (m)	1.43 (ddd, 13.0, 9.4, 6.5)	1.55 (ddd, 13.2, 12.4, 6.5)	1.39 (td, 12.8, 6.6)	2.02 (ddd, 13.2, 12.0, 6.5)	2.17 (m)
6a							2.92 (dd, 6.6, 3.0)
$1'_a$	2.57 (dddt, 14.4, 7.8, 6.4, 1.6)	3.45 (dddd,17.1, 6.3, 1.8, 1.0)	3.11 (ddt, 17.1, 6.8, 1.8)	3.32 (ddt, 13.0, 6.8, 1.5)	3.17 (ddt, 13.0, 6.8, 1.5)	3.35 (ddt, 15.2, 7.7,1.8)	3.14 (ddt, 15.6, 6.8, 1.5)
$1'_b$	2.14 (dddt, 14.4, 7.7, 6.7, 1.6)	3.15 (dddd,17.4, 7.4, 1.8, 1.0)	3.06 (ddt, 17.1, 6.8, 1.8)	3.27 (ddt, 13.0, 6.8, 1.5)	3.13 (ddt, 13.0, 6.8, 1.5)	2.95 (ddt, 15.2, 7.7, 1.8)	3.12 (ddt, 15.6, 6.8, 1.5)
2′	5.81 (dddd, 17.1, 10.0, 7.7, 6.4)	5.95 (dddd, 17.1, 10.0, 7.4, 6.3)	5.87 (ddt, 17.0, 10.1, 6.8)	6.07 (ddt, 17.1, 10.1, 6.8)	5.98 (ddt, 17.1, 10.1, 6.8)	5.85 (ddt, 17.1, 10.1, 7.7)	5.90 (ddt, 17.1, 10.1, 6.8)
$3'_a$	5.10 (dq, 17.1, 1.6)	5.13 (dq, 17.1, 1.8)	5.09 (dq, 17.1, 1.8)	5.13 (ddt, 17.1, 3.5, 1.5)	5.04 (ddt, 17.1, 3.7, 1.5)	5.16 (ddt, 17.1, 3.5, 1.8)	5.21 (ddt, 17.1, 3.0, 1.5)
$3'_b$	5.05 (dq, 10.0, 1.6)	5.07 (dq, 10.0, 1.8)	5.03 (dq, 17.1, 1.8)	4.97 (ddt, 10.1, 3.5, 1.5)	4.91 (ddt, 10.1, 3.7, 1.5)	5.09 (ddt, 10.1, 3.5, 1.8)	5.19 (ddt, 10.1, 3.0, 1.5)
2-OH	2.68 (br, s)		8.95 (br, s)		9.17 (br, s)		
3a-OH	2.34 (br, s)				4.95 (s)		
4-OH			4.75 (br, d, 4.1)		4.47 (s)		
6a-OH			5.37 (s)		5.20 (s)		

[a] In $CDCl_3$ (500 MHz). – [b] In CD_3OD (300 MHz). – [c] In [D_6]DMSO (500 MHz). – [d] In CD_3OD (500 MHz).

148.8), which bears a similar α,β-unsaturated carbonyl group.

^{1}H-^{1}H as well as ^{1}H-^{13}C correlated 2D NMR spectroscopy demonstrates the connective sequence of the following two segments: allyl group (δ = 5.10/5.05, 5.81 and 2.57/2.14) − methine group (δ = 3.30) − methine group (δ = 4.49) as well as methine group (δ = 5.01) − methylene group (δ = 2.68/2.10) − methylene group (δ = 2.49/2.34). Based on the above mentioned information, and in agreement with the molecular formula $C_{11}H_{14}O_3$, which requires 5 degrees of unsaturation, the molecule is cyclized, forming a bicyclic system with the double bond (δ = 183.7 and 145.5) as a connective moiety (**1**). This was proven by correlation between C-3a (δ = 183.7) and 2-, 3-, 4-, 5-, 6-, and 1′-H as well as correlation between C-6a (δ = 145.5) and 3-, 4-, 5-, and 6-H in the ^{1}H-^{13}C HMBC spectrum (Figure 1).

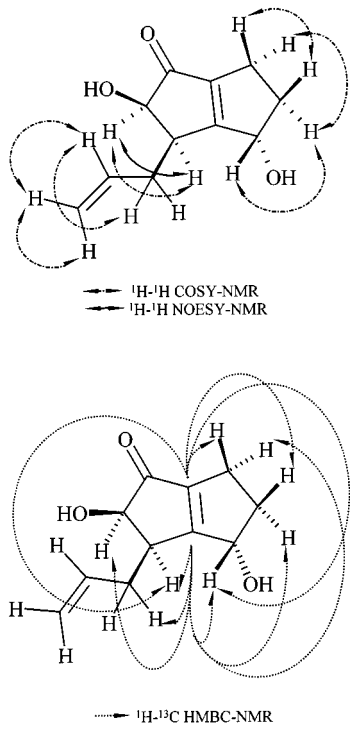

Figure 1. Selected correlation signals for xialenon A (**1**) from 2D NMR spectra

The relative stereochemistry between 2-OH and the 3-allyl moiety was determined as *syn*-facial due to a strong correlation signal between 2-H (δ = 4.49) and 3-H (δ = 3.30) as well as the concurrent lack of a correlation signal between 2-H and 1′-H (δ = 2.57/2.14) in the NOE spectrum. In order to establish the absolute stereochemistry of **1**, esterification of the hydroxyl groups in **1** with each one of the enantiomers of 2-phenylbutyric acid resulted in the bis(2*R*)-2-phenylbutyrate-derivative **7** and bis(2*S*)-2-phenylbutyrate-derivative **8**. The ^{1}H-NMR spectra of **7** and **8** were analyzed according to the rules of Helmchen,[7] which revealed the (*R*)-configuration at C-2 and (*S*)-configuration at C-4 due to significant low-field signal shifts in **7** (Δ 0.41 ppm for 1′-H, Δ 0.39 ppm for 2′-H, Δ 0.25 ppm for 3′-H, and Δ 0.34 ppm for 3-H). The (*R*)-configuration at C-3 was deduced from the *syn*-facial position of 2-OH and the 3-allyl moiety. Thus, the structure of **1** is (2*R*,3*R*,4*S*)-3-allyl-3,4,5,6-tetrahydro-2,4-dihydroxy-2*H*-pentalen-1-one.

Xialenon B (2)

The molecular formula $C_{11}H_{14}O_4$ resulted from the HREI-mass spectra (*m*/*z* = 210.0896, M^+). The ESI-MS spectra gave characteristic peaks at *m*/*z* = 211.2 $(M + H)^+$ and 233.1 $(M + Na)^+$ and the absorption bands in the IR spectrum indicate the presence of hydroxyl and conjugate carbonyl groups ($\tilde{\nu}$ = 3425 and 1696 cm^{-1}). The ^{1}H-NMR spectrum (500 MHz, [D$_6$]DMSO) of **2** shows 14 proton signals, of which those at δ = 4.75 (d, *J* = 4.1 Hz), δ = 5.37 (s), and δ = 8.95 (s) were exchangeable with D_2O and disappeared in CD_3OD (300 MHz), pointing to three OH groups. One (δ = 4.75) is attached to a methine group, a second (δ = 5.37, s) to a quaternary carbon atom, and a third (δ = 8.95, s) to a double bond due to its significant low-field chemical shift. In addition, an allyl group (δ = 5.87, δ = 5.09/5.03, and δ = 3.06/3.11), two methine groups (δ = 4.24 and δ = 2.69), as well as two methylene groups (δ = 1.78/1.29 and δ = 1.69/1.43) are present in the molecule.

The ^{13}C-NMR (75.0 MHz, CD_3OD, Table 2) and ^{13}C-DEPT spectra indicates three methine groups (δ = 135.2, 73.6 and 55.3), four methylene groups (δ = 117.1, 33.8, 33.7 and 32.7), as well as the signals of three quaternary carbon atoms of an α,β-unsaturated carbonyl moiety at δ = 204.5, δ = 150.0, and δ = 146.4. In comparison to xialenon A (**1**), the signal of C-2 (δ = 150.0) is low-field shifted because of its linkage to a hydroxyl group. Analysis of the ^{1}H-^{1}H COSY spectrum in combination with the data from the ^{1}H-^{13}C-COSY and HMBC spectra (Figure 2) led to the constitution of **2** (Scheme 1).

The relative stereochemistry of **2** was determined from the ^{1}H-^{1}H NOESY spectrum ([D$_6$]DMSO). The correlation signals between δ = 2.69 (3a-H) and δ = 5.37 (6a-OH) and δ = 4.24 (4-H), as well as the concurrent lack of a correlation signal between δ = 2.69 and δ = 4.75 (4-OH) point to a *syn*-facial position of 3a-H, 6a-OH, and 4-H and an *anti*-facial position of 3a-H and 4-OH. Thus, **2** was assigned to be 3-allyl-3a,5,6,6a-tetrahydro-2,4,6a-trihydroxy-4*H*-pentalen-1-one.

Xialenon C (3)

Due to the results from the HREI-MS (*m*/*z* = 226.0850, M^+, $C_{11}H_{14}O_5$), **3** exhibits one more oxygen atom than xialenon B (**2**). The IR-, UV-, ^{13}C-NMR (125 MHz, CD_3OD, Table 2), and ^{1}H-NMR spectra (500 MHz, CD_3OD, Table 1) of **3** otherwise indicate close structural similarities to xialenon B (**2**). One methine group (δ = 2.85, 3a-H; in **2**) is replaced by a quaternary carbon atom (δ = 84.2). The latter is linked to a hydroxyl group (δ = 4.95, s; in [D$_6$]DMSO) while the signal of the tertiary carbon at δ =

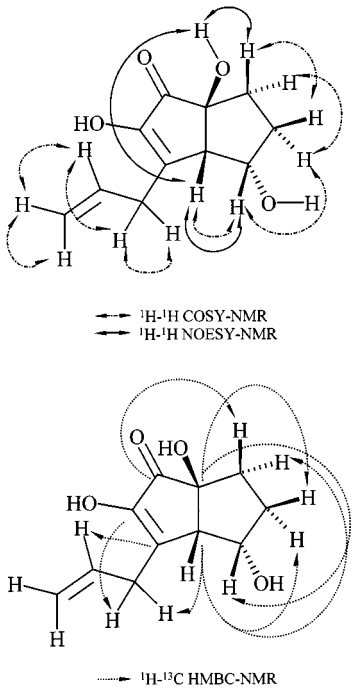

Figure 2. Selected correlation signals for xialenon B (**2**) from 2D NMR spectra

55.3 in **2** is missing in **3**. In comparison to xialenon B (**2**), the analysis of the 2D NMR spectra (see also Figure 3) led to the constitution of xialenon C as depicted in Scheme 1. The *Cotton Effect* in the CD spectrum of **3** is identical with that of **2**, pointing to an analogous stereochemistry at the centers of chirality at C-3a and C-6a, *syn*-facial position of 3a-OH and 6a-OH, which is in agreement with the interpretation of the data of the optical rotation value {**2**: $[\alpha]_D$ = −200.2 (*c* = 2.08 in methanol); **3**: $[\alpha]_D$ = −176.9 (*c* = 1.19 in methanol)}. Therefore, **3** was assigned as 3-allyl-3a,5,6,6a-tetrahydro-2,3a,4,6a-tetrahydroxy-4*H*-pentalen-1-one.

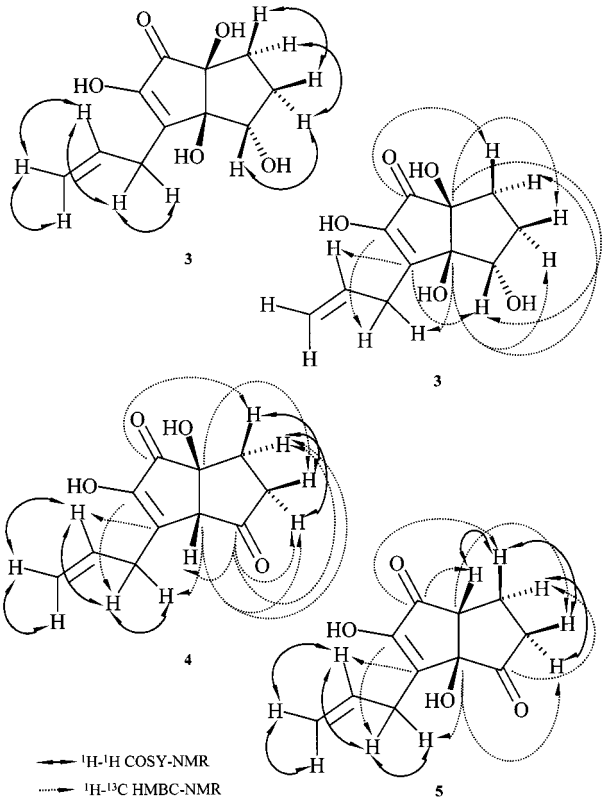

Figure 3. Selected correlation signals for xialenon C to E (**3** to **5**) from 2D NMR spectra

Xialenon D (4)

In comparison to xialenon B (**2**), two hydrogen atoms are less in the molecular formula $C_{11}H_{12}O_4$ [HREI-MS (*m*/*z* = 208.0725, M^+); ESI-MS *m*/*z* = 207 $(M - H)^-$] of **4**. In the ^{1}H- and ^{13}C-NMR spectra differences are downfield shifts of the proton signals of two methylene groups, the lack of the signal of a methine group (δ_H = 4.34 and δ_C = 73.6 in **2**), and the appearance of an additional carbonyl group (δ = 214.3). Thus, in **4** the hydroxyl group at C-4 is oxidized to a carbonyl group. Close similarities in the CD spectra of **4** and **2** suggested identity of the stereochemistry at C-3a and C-6a and thus, 3a-H and 6a-OH are in *syn*-facial position. Therefore, the structure of **4** was assigned to be 3-allyl-3a,5,6,6a-tetrahydro-2,6a-dihydroxy-pentalene-1,4-dione.

Xialenon E (5)

The last isolated compound of strain GT 061169 (**5**) show an identical molecular formula as xialenon D (**4**) [HREI-MS (*m*/*z* = 207.0646, $C_{11}H_{11}O_4$, $M - H]^+$); ESI-MS (*m*/*z* = 207 $[M - H]^-$): $C_{11}H_{12}O_4$]. A comparison of the NMR data, especially the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC data identified the difference of both molecules in the position of a hydroxyl group which is moved from C-6a in **4** to C-3a in **5**. In the ^{1}H-^{1}H COSY spectrum of **4** no correlation signals can be observed between 3a-H (δ = 2.98) and other protons, whereas correlations between 6a-H (δ = 2.92) and both, 5_α-H (δ = 2.31), and 5_β-H (δ = 2.52) are found in **5** (Figure 3). Identical CD spectra of **5** and **3** suggested the stereochemistry at C-3a and C-6a of the two compounds to be the same, which led to the *syn*-facial relationship between 3a-H and 6a-OH. The structure of xialenon E is therefore 3-allyl-3a,5,6,6a-tetrahydro-2,3a-dihydroxy-pentalen-1,4-dione (**5**).

Biological Activity

The xialenons A (**1**) and B (**2**) were tested in a number of different biological assays including basic antibacterial, antifungal, antiviral, and cytotoxicity assays each performed with a number of different test organisms or cell-lines. Both metabolites appeared to be inactive except xialenon B (**2**) exhibiting a weak cytotoxicity on the L-929 mouse fibroblastoma cell line and Hela human cervix carcinoma cell line (IC_{50} = 88.2 μg/mL and 95.9 μg/mL, respectively).

Experimental Section

General Methods: ^{1}H- and ^{13}C NMR spectra: Bruker Avance DPX 300 (300 MHz) and Avance DRX 500 (500 MHz) instruments. The multiplicities of the ^{13}C NMR values were assigned by distortionless enhancement by polarization transfer (DEPT) techniques. − MS: High-resolution EI mass spectra: AMD-402 instrument of BE geometry equipped with direct inlet system (AMD Intectra Harpstedt, Germany). Electrospray MS spectra: triple quadrupole mass spectrometer Quattro (VG Biotech, Altrincham, England). − IR spectra: Shimadzu, Model IR 470 (KBr, discs). − UV/Vis spectra: Varian CARY 1/3 Bio UV/Vis spectrophotometer. − Optical rotation values: Perkin−Elmer 241. CD spectra: Jasco J 500 A, and Jasco 750. − Analytical HPLC: Hewlett−Packard 1050 equipped with DAD-detector. Preparative scale HPLC: ABiMED Gilson Instruments [306 pump; 811C Dynamic Mixer; 806 Manometric Module; column: LiChrosorb RP-18 (7 μm), 250/25, Merck]. − Fermentation: 100-L fermentor (Braun Diessel). TLC: silica gel plates (HPTLC ready-to-use plates, silica gel 60F_{254} on aluminum foil or glass, Merck). LC: silica gel 60 (0.040−0.063 mm, Merck). Sephadex-LH 20 (Pharmacia).

Culture Media: Medium A: Soluble starch (10 g/L), $(NH_4)_2SO_4$ (2 g/L), K_2HPO_4 (1 g/L), NaCl (1 g/L), $Mg_2SO_4 \cdot 7\,H_2O$ (1 g/L), $CaCO_3$ (2 g/L), trace element solution (5 mL/L) of 3 g/L of $CaCl_2 \cdot H_2O$, 1 g/L of Fe^{III} citrate, 0.2 g/L of $MnSO_4$, 0.1 g/L of $ZnCl_2$, 0.025 g/L of $CuSO_4 \cdot 5\,H_2O$, 0.02 g/L of $Na_2B_4O_7 \cdot 10\,H_2O$, 0.004 g/L of $CoCl_2$, 0.01 g/L of $Na_2MoO_4 \cdot 2\,H_2O$, pH = 7.0 prior to sterilization. Medium B: Yeast extract 20 g/L, trace element solution (5 mL/L) identical to medium A, pH = 7.8 prior to sterilization.

Fermentation: A 1 cm^2 slant of agar from 7 d old cultures grown on medium A was used to inoculate a 300-mL Erlenmeyer flask containing 100 mL of medium B. The flask was cultivated for 6 d at 28 °C on a rotary shaker (180 rpm). The shaking culture was used for both, TLC analysis in the screening routine and for inoculation of eight inoculation flasks (400 mL) which were used for inoculation of three 100-L fermentors containing medium B (duration of fermentation: 6 d, at 28 °C, 500 rpm, aeration 10 L/min).

Isolation and Purification: After harvesting the filtered and lyophilized culture broth (about 200 L) yielded 129 g of a crude product, which was dissolved in 4 L of water and extracted with ethyl acetate (4 L × 8, ca. 2 L/min, 15 h) by an Extra-Flow Membrane Contactor (Liqui-Cel, Hoechst Celanese). The ethyl acetate extract was evaporated to dryness to yield 17.4 g of dark oily enriched material, that was purified by chromatography on a silica gel column (7.5 × 40 cm, chloroform/methanol, gradient 0 to 5%). 30 mL fractions were collected and analyzed by TLC ($CHCl_3$/MeOH, 9:1; anisaldehyde−H_2SO_4). Fractions 166 to 183 (0.3 g) contained compound **1**, which after combined re-purification using gel permeation chromatography on Sephadex LH-20 (2.5 × 100 cm, MeOH) and HPLC (RP-C_{18}, 2.1 × 25 cm, 7 um, MeOH/H_2O, 1:4) gave 80 mg of colorless oily **1** (0.40 mg/L). Pooled fractions 321 to 380 (1.5 g) were purified by column chromatography on Sephadex LH-20 column (2.5 × 100 cm, MeOH, twice) and HPLC (RP-C_{18}, 2.1 × 25 cm, 7 μm, MeOH/H_2O, 3:17) to yield 350 mg of yellowish oily **2** (1.75 mg/L). From fractions 381 to 440 (500 mg) compound **3** was isolated by column chromatography on Sephadex LH-20 (2.5 × 100 cm, MeOH, twice) and HPLC (RP-C_{18}, 2.1 × 25 cm, 7 μm, MeOH/H_2O, 1:9) in 30 mg yield (yellow oil, 0.15 mg/L). The combined fractions 81 to 104 (1.2 g) were further purified by chromatography on a silica gel column (4.0 × 50 cm, petroleum ether/ethyl acetate/MeOH, gradient from 4:1:0 to 2:1:0.1), on Sephadex LH-20 (MeOH), and HPLC (H_2O/MeOH, 4:1) to yield 20 mg (0.10 mg/L) of **4** and 12 mg (0.06 mg/L) of **5**.

Xialenon A [(2*R*,3*R*,4*S*)-3-Allyl-3,4,5,6-tetrahydro-2,4-dihydroxy-2*H*-pentalen-1-one] (1): Colorless oil. $[\alpha]_D$ = −108.0 (*c* = 0.66, methanol). − IR (KBr): $\tilde{\nu}$ = 3425, 2965, 2935, 2860, 1696, 1625, 1435, 1050, 995, 917, 625 cm^{-1}. − UV (ethanol): λ_{max} (lg ε) = 199 nm (4.82), 240 (4.90). − UV (ethanol, H^+): λ_{max} (lg ε) = 207 nm (4.76), 240 (4.93), − UV (ethanol, OH^-): λ_{max} (lg ε) = 302 nm (4.67). − CD (ethanol): λ_{max} (θ) = 244 nm (−3,062). − HREI MS: calcd. for $C_{11}H_{12}O_2$ 176.0837, found 176.0854 [M^+ − H_2O]; calcd. for $C_8H_9O_3$ 153.0552, found 153.0554 [M^+ − C_3H_5]; calcd. for $C_8H_7O_2$ 135.0446, found 135.0450 [M^+ − H_2O − C_3H_5]. − ESI MS (positive ion); *m*/*z*: 195 [M^+ + H], 217 [M^+ + Na], 411 [2M + Na]$^+$. − ^{13}C NMR and ^{1}H NMR: see Table 1 and 2.

Xialenon B [3-Allyl-3a,5,6,6a-tetrahydro-2,4,6a-trihydroxy-4H-pentalen-1-one] (2): Yellowish oil. $[\alpha]_D$ = −200.2 (*c* = 2.08, methanol). − IR (KBr): $\tilde{\nu}$ = 3425, 2955, 2865, 1696, 1633, 1391, 1297, 1230, 1093, 1044, 963, 918 cm−1. − UV (ethanol): λmax (lg ε) = 199 nm (4.23), 273 (4.48). − UV (ethanol, H^+): λmax (lg ε) = 198 nm (4.15), 273 (4.59). − UV (ethanol, OH^-): λmax (lg ε) = 314 nm (4.51). − CD (ethanol): λmax (θ) = 224 nm (−971), 280 (+241), 332 (−367). − ESI MS (positive ion); *m*/*z*: 211.2 [M^+ + H], 233.1 [M^+ + Na]. − HREI MS: calcd. for $C_{11}H_{14}O_4$ 210.0892, found 210.0896 [M^+]; calcd. for $C_{11}H_{12}O_3$ 192.0786, found 192.0789 [M^+ − H_2O]. − ^{13}C NMR and ^{1}H NMR: see Table 1 and 2.

Xialenon C [3-Allyl-3a,5,6,6a-tetrahydro-2,4,3a,6a-tetrahydroxy-4*H*-pentalen-1-one] (3): Yellow oil. $[\alpha]_D$ = −176.9 (*c* = 1.19, methanol). − IR (KBr): $\tilde{\nu}$ = 3430, 2920, 1701, 1634, 1442, 1391, 1299, 1104, 1067, 933, 918 cm^{-1}. − UV (ethanol): λ_{max} (lg ε) = 199.2 nm (4.32), 266 (4.71). − UV (ethanol, H^+): λ_{max} (lg ε) = 199 nm (4.21), 269 (4.71). − UV (ethanol, OH^-): λ_{max} (lg ε) = 308 nm (4.64). − CD (ethanol): λ_{max} (θ) = 230 nm (−824.5), 274 (+2,556.9), 337 (−1,443.4). − ESI MS (positive ion); *m*/*z*: 227 [M^+ + H], 244 [M^+ + NH_4], 249 [M^+ + Na]. − HREI MS: calcd. for $C_{11}H_{14}O_5$ 226.0841, found 226.0850 [M^+]; calcd. for $C_{11}H_{12}O_4$ 208.0736, found 208.0734 [M^+ − H_2O]. − ^{13}C NMR and ^{1}H NMR: see Table 1 and 2.

Xialenon D [3-Allyl-3a,5,6,6a-tetrahydro-2,6a-dihydroxypentalene-1,4-dione] (4): Pale yellow oil. $[\alpha]_D$ = −436.9 (*c* = 2.23, methanol). − IR (KBr): $\tilde{\nu}$ = 3410, 2930, 1730, 1707, 1632, 1439, 1389, 1229, 1168, 1054, 918 cm^{-1}. − UV (ethanol): λ_{max} (lg ε) = 200 nm (4.51), 273 (4.71). − UV (ethanol, H^+): λ_{max} (lg ε) = 200 nm (4.47), 273 (4.71). − UV (ethanol, OH^-): λ_{max} (lg ε) = 313.5 nm (4.63). − CD (ethanol): λ_{max} (θ) = 222 nm (−1,239), 278 (+2,434), 320 (−1,991). − ESI MS (negative ion); *m*/*z*: 207 [M^- − H]. − ESI MS (positive ion); *m*/*z*: 209 [M^+ + H]. − HREI MS: calcd. for $C_{11}H_{12}O_4$ 208.0736, found 208.0725 [M^+]; calcd. for $C_{11}H_{10}O_3$ 190.0630, found 190.0650 [M^+ − H_2O]. − ^{13}C NMR and ^{1}H NMR: see Table 1 and 2.

Xialenon E [3-Allyl-3a,5,6,6a-tetrahydro-2,3a-dihydroxypentalene-1,4-dione] (5): Pale yellow oil. $[\alpha]_D$ = −285.7 (*c* = 0.14, methanol). − IR (KBr): $\tilde{\nu}$ = 3410, 2945, 1729, 1706, 1651, 1566, 1384, 1177, 1074, 918 cm^{-1}. − UV (ethanol): λ_{max} (lg ε) = 199 nm (4.27), 269 (4.35). − UV (ethanol, H^+): λ_{max} (lg ε) = 199 nm (4.11), 269 (4.32). − UV (ethanol, OH^-): λ_{max} (lg ε) = 296 nm (4.22), 326 (4.16). − CD (ethanol): λ_{max} (θ) = 222 nm (−260), 275 (+2,704), 330 (−1,716). − ESI MS (negative ion); *m*/*z*: 207 [M^- − H]. − HRFABMS (negative ion): cacld. for $C_{11}H_{11}O_4$ 207.0658, found 207.0646 [M^- − H]. − ^{13}C NMR and ^{1}H NMR: see Table 1 and 2.

(2*R*,3*R*,4*S*)-2,4-Diacetoxy-3-allyl-3,4,5,6-tetrahydro-2*H*-pentalen-1-one (6): 11 mg of **1** was dissolved in 0.25 mL of pyridine and 50 µL of acetic anhydride was added. The mixture was stirred at room temperature for 24 h and dried under a flow of nitrogen. The crude product was purified by chromatography on silica gel (column: 50 × 1 cm, chloroform/methanol, 99:1) to yield 14.5 mg of pure **6** (91%). HREI MS: calcd. for $C_{15}H_{18}O_5$ 278.1118, found 278.1136 [M^+]; calcd. for $C_{13}H_{16}O_4$ 236.1005, found 236.1027 [$M^+ - C_2H_2O$]; calcd. for $C_{13}H_{14}O_3$ 218.0903, found 218.0923 [$M^+ - C_2H_4O_2$]. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.83, (1 H, t, *J* = 6.1 Hz, 4-H), 5.64 (1 H, m, 2′-H), 5.60 (1 H, d, *J* = 6.3 Hz, 2-H), 5.06 (1 H, ddd, *J* = 17.0, 3.2, and 1.6 Hz, 3′-H_a), 5.02 (1 H, ddd, *J* = 10.2, 3.2, and 1.6 Hz, 3′-H_b), 3.32 (1 H, m, 3-H), 2.73 (1 H, m, 5-H_β), 2.58 (1 H, m, 1′-H_a), 2.42 (1 H, m, 6-H_α), 2.35 (1 H, m, 6-H_β), 2.23 (1 H, m, 1′-H_b), 2.19 (1 H, m, 5-H_α), 2.18 (3 H, s, OAc), 2.09 (3 H, s, OAc). − ^{13}C NMR (125 MHz, $CDCl_3$): δ 197.5 (C-1), 178.6 (C-3a), 170.4 (OAc), 170.2 (OAc), 148.9 (C-6a), 134.7 (C-2′), 117.6 (C-3′), 77.6 (C-2), 74.7 (C-4), 39.2 (C-3), 34.0 (C-5), 33.2 (C-1′), 23.5 (C-6), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3).

(2*R*,3*R*,4*S*)-3-Allyl-3,4,5,6-tetrahydro-2,4-bis[(2*R*)-2-phenylbutyryl]-2*H*-pentalen-1-one (7): 12 mg of *N,N*-dicyclohexylcarbodiimide (DCC), 8 mg of 4-(dimethylamino)pyridine (DMAP), and 10 mg of (2*R*)-2-phenylbutyric acid were added to a 10-mL flask which contained 3 mL of dichloromethane. After cooling to −5°C, 10 mg of **1** in 2 mL of dichloromethane was added. After warming to room temperature the mixture was stirred for 14 h. 4 mL of water was added and the solution was extracted 4 times with chloroform (4 mL each). The combined organic layer was dried with Na_2SO_4, filtered, the filtrate was dried in vacuum, and purified by chromatography on silica gel [column 10 × 0.5 cm, chloroform/methanol, 99:1] to yield 9 mg (34%) of colorless oily **7**. HREI MS: calcd. for $C_{31}H_{34}O_5$ 486.2406, found 486.2406 [M^+]; calcd. for $C_{21}H_{22}O_3$ 322.1543, found 322.1556 [$M^+ - C_{10}H_{12}O_2$]. − ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25−7.35 (10, m, aromatic H), 5.78 (1 H, t, *J* = 6.1 Hz, 4-H), 5.56 (1 H, d, *J* = 6.4 Hz, 2-H), 5.44 (1 H, m, 2′-H), 4.94 (1 H, dq, *J* = 17.0 and 1.5 Hz, 3′-H_a), 4.89 (1 H, dq, *J* = 10.2 and 1.5 Hz, 3′-H_b), 3.59 (1 H, t, *J* = 7.7 Hz, 2′′-H), 3.45 (1 H, t, *J* = 7.7 Hz, 2′′′-H), 3.16 (1 H, m, 3-H), 2.64 (1 H, m, 5-H_β), 2.49 (1 H, m, 6-H_α), 2.30 (1 H, m, 6-H_β), 2.25 (1 H, m, 1′-H_a), 2.18 (1 H, m, 3′′′-H_a), 2.09 (1 H, m, 3′′-H_a), 2.01 (1 H, m, 1′-H_b), 2.00 (1 H, m, 5-H_α), 1.88 (1 H, m, 3′′′-H_b), 1.80 (1 H, m, 3′′-H_b), 0.95 (3 H, t, *J* = 7.3 Hz, 4′′-H), 0.89 (3 H, t, *J* = 7.5 Hz, 4′′′-H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.9 (C-1), 178.4 (C-3a), 173.4 (C-1′′), 173.1 (C-1′′′), 149.0 (C-6a), 138.5 (C-1′′′′), 138.2 (C-1′′′′′), 134.5 (C-2′), 128.6−127.1 (10 C, aromatic C), 117.5 (C-3′), 77.6 (C-2), 75.0 (C-4), 53.5 (C-2′′), 53.4 (C-2′′′), 39.2 (C-3), 33.9 (C-5), 32.8 (C-1′), 26.5 (C-3′′), 26.2 (C-3′′′), 23.4 (C-6), 12.2 (C-4′′), 12.1 (C-4′′′).

(2*R*,3*R*,4*S*)-3-Allyl-3,4,5,6-tetrahydro-2,4-bis[(2*S*)-2-phenylbutyryl]-2*H*-pentalen-1-one (8): Using the same conditions and workup procedure as described for the preparation of **7**, 8 mg of colorless oily **8** (30%) was obtained from 10 mg of **1** and 10 mg of (2*S*)-2-phenylbutyric acid. HREI MS: calcd. for $C_{31}H_{34}O_5$ 486.2406, found 486.2406 [M^+]; calcd. for $C_{21}H_{24}O_4$ 340.1675, found 340.1666 [$M^+ - C_{10}H_{10}O$]. − ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25−7.35 (10 H, m, aromatic H), 5.74 (1 H, dd, *J* = 5.3 and 7.4 Hz, 4-H), 5.46 (1 H, d, *J* = 6.4 Hz, 2-H), 5.05 (1 H, m, 2′-H), 4.68 (1 H, ddt, *J* = 7.0, 3.2 and 1.5 Hz, 3′-H_a), 4.64 (1 H, ddt, *J* = 10.2, 3.2 and 1.5 Hz, 3′-H_b), 3.56 (1 H, t, *J* = 7.4 Hz, 2′′-H), 3.42 (1 H, t, *J* = 7.7 Hz, 2′′′-H), 2.82 (1 H, m, 3-H), 2.71 (1 H, m, 5-H_β), 2.48 (1 H, m, 6-H_α), 2.35 (1 H, m, 6-H_β), 2.20 (1 H, m, 3′′-H_a), 2.14 (1 H, m, 3′′′-H_a), 2.16 (1 H, m, 5-H_α), 1.86 (1 H, m, 3′′-H_b), 1.84 (1 H, m, 1′-H_a), 1.80 (1 H, m, 3′′′-H_b), 1.60 (1 H, m, 1′-H_b), 0.91 (3 H, t, *J* = 7.3 Hz, 4′′-H), 0.88 (3 H, t, *J* = 7.3 Hz, 4′′′-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.3 (C-1), 179.0 (C-3a), 173.4 (C-1′′), 173.2 (C-1′′′), 148.5 (C-6a), 138.7 (C-1′′′′), 138.6 (C-1′′′′′), 134.3 (C-2′), 128.6−127.4 (10 C, aromatic C), 117.2 (C-3′), 77.4 (C-2), 75.0 (C-4), 53.4 (C-2′′′), 53.3 (C-2′′), 39.1 (C-3), 34.2 (C-5), 32.7 (C-1′), 26.3 (C-3′′), 26.1 (C-3′′′), 23.5 (C-6), 12.3 (C-4′′), 12.1 (C-4′′′).

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