Novel Polyketides Isolated from *Streptomyces* sp.

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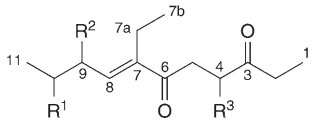
From the endophytic strain *Streptomyces* sp. CS of *Maytenus hookeri*, five novel type III polyketides, compounds **1**–**5**, were isolated. Their structures were elucidated by spectroscopic analyses including 1D- and 2D-NMR experiments, and by HR-ESI-MS.

Introduction. – *Streptomyces* sp. CS is an endophyte isolated from the callus of *Maytenus hookeri*. In our previous work, three new macrolides [1][2], one new polyhydroxyl cyclohexane [3], one new ansamycin named naphthomycin K [4] and several known compounds including naphthomycins A and E were isolated from the fermentation extracts. A PCR-based screening strategy was chosen to detect the biosynthetic gene sequence of 3-amino-5-hydroxybenzoic acid (AHBA) and ketosynthase (KS), which are commitment to ansamycin biosynthesis, indicating the presence of the expected AHBA synthase and KS genes (data not shown). These results suggested that this strain should produce more polyketides.

In our continuous search for ansamycins from this strain, many new metabolites were isolated from the fermentation extracts in *Waksman* media. In this work, we report the isolation and structure elucidation of five novel polyketides, compounds **1**–**5**, from the fermentation products of *Streptomyces* sp. CS.



1 $R^1 = R^3 = H$, $R^2 = OH$
2 $R^1 = OH$, $R^2 = R^3 = H$
3 $R^1 = R^2 = H$, $R^3 = OH$
4 $R^1 = H$, $R^2 = R^3 = OH$
5 $R^1 = R^3 = OH$, $R^2 = H$

Results and Discussion. – The culture broth of *Streptomyces* sp. CS (120 l) was extracted five times with AcOEt, and the crude extract was fractionated by repeated column chromatography (*RP-18*, *Sephadex LH-20*, and SiO_2) to afford five novel polyketides.

The oily compound **1** was determined to have the molecular formula $C_{13}H_{22}O_3$ based on the HR-ESI-MS and NMR data. The ^{13}C-NMR ((D$_6$)acetone) and DEPT spectra showed 13 C-atom signals for three Me, five CH_2, two CH, and three quaternary C-atoms, including two keto CO groups at δ 200.7 and 209.8 (*Table 1*). The ^{1}H,^{1}H-COSY clearly demonstrated the connectivity from H–C(8) to Me(11). The HMBC spectra showed the ^{1}H,^{13}C-long-range correlations of Me(1), CH_2(2), CH_2(4), and CH_2(5) to the CO group at C(3) (δ 209.8), and CH_2(4), CH_2(5), H–C(8), and CH_2(7a) to another CO group at C(6) (δ 200.7). Further analysis of the HMBC and ^{1}H,^{1}H-COSY spectra revealed the connectivities from C(7) to C(7b). The oxymethine H-atom at δ 4.45 (H–C(9)) (*Table 2*) and the olefinic H-atom at δ 6.54 (H–C(8)) showed ^{1}H,^{13}C-long-range correlations with C(7), indicating the linkage between C(7) and C(8). The absolute configuration of C(9) was analyzed by *Mosher*'s method [5]. The reaction of **1** with (*R*)-*Mosher* chloride produced the two diastereoisomers **1a** and **1b**, in a ratio of 5 : 2, as recognized by the integration of ^{1}H-NMR signals. The ^{1}H-NMR signal of H–C(9) of **1b** was shifted downfield +0.043 ppm relative to that of **1a**, and the $\Delta\delta$ value for H–C(8) was ($\Delta\delta = +0.265$ ppm). Thus, the (9*S*) configuration was assigned to **1b**, the (9*R*) configuration was assigned to **1a**. Therefore, compound **1** was determined to be an enantiomeric mixture of (7*E*,9*S*)-7-ethyl-9-hydroxyundec-7-ene-3,6-dione, and (7*E*,9*R*)-7-ethyl-9-hydroxyundec-7-ene-3,6-dione.

Table 1. *^{13}C-NMR Spectroscopic Data of* **1**–**5**. Recorded at 150 MHz in (D$_6$)acetone; δ in ppm, *J* in Hz. Arbitrary atom numbering.

Position	**1**	**2**	**3**	**4**	**5**
1	8.2 (*q*)	8.0 (*q*)	7.7 (*q*)	7.7 (*q*)	7.9 (*q*)
2	36.6 (*t*)	36.1 (*t*)	31.5 (*t*)	31.6 (*t*)	31.8 (*t*)
3	209.8 (*s*)	209.8 (*s*)	212.9 (*s*)	213.0 (*s*)	213.1 (*s*)
4	32.3 (*t*)	32.0 (*t*)	74.2 (*d*)	74.1 (*d*)	74.3 (*d*)
5	36.2 (*t*)	36.5 (*t*)	41.8 (*t*)	42.1 (*t*)	42.0 (*t*)
6	200.7 (*s*)	199.8 (*s*)	199.2 (*s*)	199.9 (*s*)	199.4 (*s*)
7	144.6 (*s*)	144.5 (*s*)	144.2 (*s*)	142.8 (*s*)	145.1 (*s*)
7a	20.0 (*t*)	19.6 (*t*)	19.4 (*t*)	19.8 (*t*)	19.7 (*t*)
7b	10.2 (*q*)	14.0 (*q*)	14.1 (*q*)	14.4 (*q*)	14.1 (*q*)
8	142.6 (*d*)	139.7 (*d*)	144.0 (*d*)	145.8 (*d*)	141.4 (*d*)
9	69.9 (*d*)	39.1 (*t*)	31.3 (*t*)	69.8 (*d*)	39.5 (*t*)
10	31.1 (*t*)	67.1 (*d*)	22.8 (*t*)	30.9 (*t*)	67.3 (*d*)
11	14.6 (*q*)	23.9 (*q*)	14.2 (*q*)	10.0 (*q*)	24.0 (*q*)

The oily compound **2** was determined to have the molecular formula $C_{13}H_{22}O_3$ according to the HR-ESI-MS and NMR data. Comparison of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, ^{1}H,^{1}H-COSY) of **2** with those of compound **1** revealed that they were similar, except for the oxymethine H-atom signal, which was at δ 4.45 in **1** and at δ 3.96 in **2**, indicating that the OH group was at a different position. The ^{1}H,^{1}H-COSY correlations indicated that the OH group in **2** was located at C(10); therefore **2** was determined to be (7*E*)-7-ethyl-10-hydroxyundec-7-ene-3,6-dione.

The oily compound **3** was determined to have the molecular formula $C_{13}H_{22}O_3$ according to the HR-ESI-MS and NMR data. Comparison of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, ^{1}H,^{1}H-COSY) of **3** with those of compound **1** revealed that they were

Table 2. *^{1}H-NMR Spectroscopic Data of* **1**–**3**. Recorded at 600 MHz in (D_6)acetone; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**	**2**	**3**
1	0.99 (*t*, *J* = 7.2)	0.99 (*t*, *J* = 7.2)	1.03 (*t*, *J* = 7.2)
2	2.52 (*q*, *J* = 7.2)	2.51 (*q*, *J* = 7.2)	2.31 (*q*, *J* = 7.2)
3	–	–	–
4	2.96 (*dt*, *J* = 6.0, 1.8)	2.96 (*t*, *J* = 6.0)	4.44 (*dd*, *J* = 4.2, 6.0)
5	2.71 (*t*, *J* = 6.0)	2.68 (*t*, *J* = 6.0)	3.21 (*dd*, *J* = 4.2, 16.8), 3.11 (*dd*, *J* = 6.0, 16.8)
6	–	–	–
7	–	–	–
7a	2.30 (*q*, *J* = 7.2)	2.29 (*q*, *J* = 7.2)	2.30 (*q*, *J* = 7.2)
7b	0.96 (*t*, *J* = 7.2)	0.90 (*t*, *J* = 7.2)	0.92 (*t*, *J* = 7.2)
8	6.54 (*d*, *J* = 9.0)	6.86 (*t*, *J* = 7.2)	6.75 (*t*, *J* = 7.2)
9	4.45 (*dt*, *J* = 7.2, 9.0)	2.42 (*dd*, *J* = 6.0, 7.2)	2.72 (*dt*, *J* = 7.2, 7.2)
10	1.66 (*dq*, *J* = 7.2, 7.2), 1.56 (*dq*, *J* = 7.2, 7.2)	3.96 (*tq*, *J* = 6.0, 6.0)	1.55 (*tq*, *J* = 7.2, 7.2)
11	0.91 (*t*, *J* = 7.2)	1.20 (*d*, *J* = 6.0)	0.98 (*t*, *J* = 7.2)

similar, except for an *ABX* coupling system in the spectrum of **3** (δ 3.11 (*dd*, J = 6.0, 16.8), δ 3.21 (*dd*, J = 4.2, 16.8) and δ 4.44 (*dd*, J = 4.2, 6.0)), indicating that the OH group was located at a different position. The ^{1}H,^{1}H-COSY and HMBC correlations revealed that the OH group was attached at C(4). Therefore, **3** was determined as (7*E*)-7-ethyl-4-hydroxyundec-7-ene-3,6-dione.

The oily compound **4** was determined to have the molecular formula $C_{13}H_{22}O_4$ based on the HR-ESI-MS and NMR data. Inspection of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, ^{1}H,^{1}H-COSY) indicated that the spectrum of **4** showed an *ABX* coupling system similar to the one of **3** (*Table 3*). Additionally, **4** had one more OH substituent than **3**. Comparison of the NMR data of **4** and **1** indicated that they contained the

Table 3. *^{1}H-NMR Spectroscopic Data of* **4**–**5**. Recorded at 600 MHz in (D_6)acetone; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**4**	**5**
1	1.02 (*t*, *J* = 7.2)	1.01 (*t*, *J* = 7.2)
2	2.65 (*q*, *J* = 7.2)	2.68 (*q*, *J* = 7.2)
3	–	–
4	4.44 (*dd*, *J* = 4.2, 6.0)	4.41 (*dd*, *J* = 4.2, 6.0)
5	3.21 (*dd*, *J* = 4.2, 16.8)	3.19 (*dd*, *J* = 17.2, 4.2)
	3.15 (*dd*, *J* = 6.0, 16.8)	3.09 (*dd*, *J* = 17.2, 6.0)
6	–	–
7	–	–
7a	2.31 (*q*, *J* = 7.2)	2.29 (*q*, *J* = 7.2)
7b	0.93 (*t*, *J* = 7.2)	0.90 (*t*, *J* = 7.2)
8	6.54 (*d*, *J* = 7.2)	6.84 (*t*, *J* = 7.2)
9	4.45 (*dt*, *J* = 7.2, 7.2)	2.42 (*t*, *J* = 6.6)
10	1.72 (*dq*, *J* = 7.2, 7.2), 1.60 (*dq*, *J* = 7.2, 7.2)	3.94 (*tq*, *J* = 6.6, 6.0)
11	0.96 (*t*, *J* = 7.2)	1.85 (*d*, *J* = 6.0)

identical moiety including the eight C-atoms C(6), C(7), C(7a), C(7b), C(8), C(9), C(10), and C(11), assigning one OH substituent at C(9). The relative configuration of **4** has not been established. Therefore, compound **4** was determined to be (7*E*)-7-ethyl-4,9-dihydroxyundec-7-ene-3,6-dione.

The oily compound **5** was determined to have the molecular formula $C_{13}H_{22}O_4$ according to the HR-ESI-MS and NMR data. Inspection of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, ^{1}H,^{1}H-COSY) indicated that the spectrum of **5** showed an *ABX* coupling system similar to those of **3** and **4**. Additionally, **5** had one more OH substituent than **3**. Comparison of the NMR data of **5** and **2** indicated that they contained the identical moiety of eight C-atoms, including C(6), C(7), C(7a), C(8), C(9), C(10), and C(11), with a OH substituent at C(10). The relative configuration of **5** has not been established. Therefore compound **5** was determined to be (7*E*)-7-ethyl-4,10-dihydroxyundec-7-ene-3,6-dione.

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Experimental Part

General. Fermentor (Model *GUJS 50-200*, *Zhenjiang East Biotech Equipment and Technology*). TLC: Precoated silica gel GF_{254} plates (0.20–0.25 mm, *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Column chromatography (CC): SiO_2 (200–300, and 80–100 mesh; *Qingdao Marine Chemical Factory*), SiO_2 GF_{254} (*Merck*), *RP-18* (40–63 μm *Merck*), and *Sephadex LH-20* (*Amersham Biosciences*). Optical rotations: *Perkin-Elmer 341* polarimeter with $CHCl_3$ as solvent. UV Spectra: *UNICO single-beam 210A* spectral photometer; 190–1100 nm, in MeOH. IR: in KBr on a *Nicolet FT-IR 360*; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AV-600* spectrometer, at 600/150 MHz, resp., in (D_6)acetone; in ppm rel. to Me_4Si, *J* in Hz. ESI-Q-TOF-MS: *Bruker ESI-Bio-Q-TOF* mass spectrometer; in *m*/*z*.

Isolation and Fermentation of the Strain Streptomyces *sp. CS.* The strain *Streptomyces* sp. CS was isolated from the callus of *Maytenus hookeri* [1]. A stock of *Streptomyces* sp. CS was cultured on ISP2 agar plates (yeast extract 4 g/l, malt extract 10 g/l, glucose 4 g/l, and agar 20 g/l, pH 7.2–7.4), at 28° for 7 d and a single colony was inoculated to 500 ml *Erlenmeyer* flasks containing 50 ml ISP2 broth as a seed medium. After 48 h on a rotary shaker (180 rpm, 28°), 10 l of the precultures were fermented in a 150 l fermentor (*Zhenjiang*) containing 120 l of sterilized *Waksman Synthetic* medium (made from glycerol 30 g/l, $K_2HPO_4 \cdot 3\ H_2O$ 1 g/l, $MgSO_4 \cdot 7\ H_2O$ 0.5 g/l, KCl 0.5 g/l, $FeSO_4 \cdot 7\ H_2O$ 0.01 g/l, $NaNO_3$ 2 g/l, pH 7.2–7.4). Fermentation was carried out at 28° for 1 week with aeration (10 l/min) under constant agitation (240 rpm).

Extraction and Isolation. After filtration of the harvested culture of the strain *Streptomyces* sp. CS, the culture filtrate was extracted with AcOEt (100 l). The org. soln. was collected and evaporated to dryness *in vacuo* to afford 75 g (dry weight) of extract. The AcOEt extract (75 g) was subjected to MPLC (130 g, *RP-18*), and eluted with H_2O, and 30, 50, 70, and 100% acetone, resp., which yielded 5 fractions of each 2 l: *Fr. 1–5*. *Fr. 2* (28 g) was subjected to MPLC (130 g, *RP-18*), eluting with H_2O, and 30, 50, 70, and 100% MeOH, resp. (2 l each) to yield 4 fractions; *Fr. 2a–2d*. *Fr. 2b* (1.9 g) was subjected to CC (100 g *Sephadex LH-20*; MeOH). All fractions were analyzed by TLC ($CHCl_3$/MeOH 10:1) and pooled accordingly into seven portions (*Fr. 2b1–2b7*). *Fr. 2b4* (57 mg) was further purified by CC (silica gel, petroleum ether/AcOEt 5:1) to yield **1** (18 mg) and **2** (23 mg). *Fr. 2b2* (33 mg) was further purified by CC (silica gel, petroleum ether/AcOEt 5:1) to yield **3** (25 mg). *Fr. 2b3* (100 mg) was further purified by CC (silica gel, petroleum ether/AcOEt 5:1) to yield **4** (56 mg) and **5** (43 mg).

*(7*E*)-7-Ethyl-9-hydroxyundec-7-ene-3,6-dione* (**1**). Colorless oil. $[\alpha]_D^{20} = -5.5$ ($c = 4.0$, $CHCl_3$). UV (MeOH): 229.5 ($\varepsilon = 1.64$). IR (KBr): 3439, 2968, 1713, 1671, 1407, 1099. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-ESI-MS: 249.1858 ($[M + Na]^+$, $C_{13}H_{22}NaO_3^+$; calc. 249.1856).

(7E)-7-Ethyl-10-hydroxyundec-7-ene-3,6-dione (**2**). Colorless oil. $[\alpha]_D^{20} = +4.6$ ($c = 5.0$, $CHCl_3$). UV (MeOH): 231.5 ($\varepsilon = 0.87$). IR (KBr): 3436, 2970, 1712, 1666, 1408, 1117. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-ESI-MS: 249.1717 ($[M + Na]^+$, $C_{13}H_{22}NaO_3^+$; calc. 249.1714).

(7E)-7-Ethyl-4-hydroxyundec-7-ene-3,6-dione (**3**). Colorless oil. $[\alpha]_D^{20} = +5.6$ ($c = 4.0$, $CHCl_3$). UV (MeOH): 231.6 ($\varepsilon = 0.87$). IR (KBr): 3467, 2964, 1713, 1665, 1380, 1099. ^{1}H-and ^{13}C-NMR: see *Tables 1* and *2*. HR-ESI-MS: 249.1539 ($[M + Na]^+$, $C_{13}H_{22}NaO_3^+$; calc. 249.1541).

(7E)-7-Ethyl-4,9-dihydroxyundec-7-ene-3,6-dione (**4**). Colorless oil. $[\alpha]_D^{20} = -7.5$ ($c = 2.0$, $CHCl_3$). UV (MeOH): 230.5 ($\varepsilon = 1.31$). IR (KBr): 3433, 2970, 1712, 1666, 1382, 1095. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-MS: 249.1812 ($[M + Na]^+$, $C_{13}H_{22}NaO_4^+$; calc. 249.1808).

(7E)-7-Ethyl-4,10-dihydroxyundec-7-ene-3,6-dione (**5**). Colorless oil. $[\alpha]_D^{20} = +9.0$ ($c = 2.0$, $CHCl_3$). UV (MeOH): 232.0 ($\varepsilon = 0.26$). IR (KBr): 3435, 2970, 1712, 1664, 1382, 1099. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-MS: 249.1616 ($[M + Na]^+$, $C_{13}H_{22}NaO_4^+$; calc. 249.1615).

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