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内生链霉菌 *Streptomyces* sp. Ly221的次生代谢成分

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摘 要:从云南美登木茎皮组织分离得到内生菌 Ly221,经 16S rDNA 部分核酸序列鉴定该菌属于链霉菌属(*Streptomyces*)。从其液体发酵提取物中分离得到 5个化合物,经波谱分析鉴定其结构分别为: 4, 10-dihydroxy-10-methyl-dodec-2-en-1, 4-olide(**1**)、两个非对映异构体 4, 11-dihydroxy-10-methyldodec-2-en-1, 4-olides(**2**/**3**)、4-hydroxy-10-methyl-11-oxo-dodec-2-en-1, 4-olide(**4**)和 N-acetyltyramine(**5**)。

关键词:内生菌;云南美登木;烯丁酸内酯

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Metabolites from Endophytic Streptomyces sp. Ly221

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Abstract: The strain Ly221 was isolated from the branch tissue of *Maytenus hookeri* and determined to be a member of *Streptomyces* according to the 16S rDNA sequence. The extracts from the fermentation broth of *Streptomyces* sp. Ly221 were purified, and five compounds were obtained. They were identified to be 4, 10-dihydroxy-10-methyl-dodec-2-en-1, 4-olide (**1**), two diastereomeric 4, 11-dihydroxy-10-methyldodec-2-en-1, 4-olides (**2**/**3**), 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1, 4-olide (**4**) and N-acetyltyramine (**5**) based on spectral data, respectively.

Key words: endophytes; *Maytenus hookeri*; butenolides

Introduction

Endophytes, ubiquitously present in almost all plants, are important sources of natural products [1]. Metabolites of some endophytic microorganisms have proven useful for new drug discovery [2,3]. *Maytenus hookeri* (Celastraceae) is well-recognized for producing the anticancer compound maytansine [4,5]. During our search for new bioactive natural products from the endophytic microorganisms of *M. hookeri*, a series of novel compounds were obtained [6-9]. In the further study about chemical constituents of endophytic microorganisms from the plant *M. hookeri*, we investigated the secondary metabolites produced by the strain Ly221. Herein, we described the identification of the strain, and the isolation and structural elucidation of five compounds from Ly221.

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Materials and Methods

General procedures

Optical rotations were measured on a Jasco DIP-370 polarimeter. NMR spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal standard. ESI-MS (positive ion mode) were performed on Finnigan LCQ-Advantage mass spectrometers. Column chromatography (CC) was performed on silica gel (200-300 mesh, 10-40 μm; Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Amersham Pharmacia, Sweden), and reverse-phase C_{18} (RP-18) silica gel (40-70 μm, Merck, Germany). TLC was performed on silica gel GF_{254} (10-40 μm, Qingdao). All solvents were distilled before use.

Microbial material

The branches of *Maytenus hookeri* were collected at Xishuangbanna, Yunnan, People's Republic of China, in April 2003. The plant materials were washed by running tap water and were sterilized successively with 75% ethanol for 1 min and 1.2% sodium hypochlorite for 8 min, then rinsed in sterile water for five times and cut into small pieces. These small pieces were incubated at 25 ℃ on YMG media (yeast extract 4.0 g, malt extract 10.0 g, glucose 4.0 g, agar 15.0 g, distilled water 1 L) and cultured until colony or mycelium appeared surrounding the segments. After culturing about one month, a strain named Ly221 appeared and was isolated from the sterilized branch. It was deposited in Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Identification of Ly221 by amplification of the 16S rRNA sequence

The mycelium of Ly221 were ground to a fine powder in liquid nitrogen, and lysed with 1% SDS (containing 500 mM Tris-HCl, pH 8.0; 20 mM EDTA; 10 mM NaCl) and a final concentration of 200 μg/mL of proteinase K (Merck) for 2 h. Then the samples were extracted by phenol-chloroform treatment and precipitated with sodium acetate-ethanol by standard methods [10]. PCR was performed in a total volume of 50 μL using Primers 27f (5′>AGA GTT TGA TCM TGG CTC AG <3′) and 1492r (5′>TAC GGY TAC CTT GTT ACG ACT T<3′). Amplification reaction mixture contains 100 ng DNA template, 0.5 μM primers, 0.2 mM dNTP, 1 ×Ex-Taq buffer (Takara), and 1.25 U of Ex-Taq (Takara). The reaction mixture was incubated in a thermal cycler (Eppendorf) as follows: 10 min of predenaturation; then 25 cycles of 1 min of denaturation at 95 ℃, annealing at 55 ℃ for 1 min, and elongation for 1.5 min; 10 min of additional extension at 72 ℃. The 1.5 Kb PCR products were recovered by gel purification using UNIQ-10 column DNA gel extraction kit (Shanghai Sangon Biotechnology Co., Ltd.) and ligated into pUCm-T vector (Sangon). The competent *E. coli* JM109 was prepared, and plasmids were transformed into it by standard method [10]. Three randomly picked clones were sequenced by ABI 3700 sequencer for insert fragment. The 16S rDNA partial sequence was assembled using Vector NTI software and blasted against the latest GenBank database using BLASTn.

Extraction and Isolation

The strain was fermented in 20 L YMG liquid media. After 14 d, the cultures were filtered and the filtrate was extracted exhaustively five times by ethyl acetate to obtained EA part (3.45 g). The EA part (3.45 g) was subjected on silica gel (100 g) eluting with petroleum ether/acetone (10∶1 to 7∶3) to chloroform/Methanol (10∶1 to 8∶2) to produce 5 fractions (EA-1 to EA-5). Fraction EA-2 (220 mg) was subjected on Sephadex LH-20 (30 g) and eluted with acetone to afford 4 fractions (EA-2-1 to EA-2-4). EA-2-1 (20 mg) was chromatographied on silica gel (silica gel H, 1.5 g) and eluted with petroleum ether/acetone (10∶1 to 9∶1) to afford compound **5** (5 mg). Fraction EA-3 (63 mg) was subjected Sephadex LH-20 and eluted with methanol to afford 3 fractions (EA-3-1 to EA-3-3). The EA-3-1 was subjected on a silica gel column (silica gel G, 50 g) and eluted with petroleum ether/acetone (9∶1 to 7∶3), and then purified on MPLC over reversed-phase (RP-18) silica gel (25 g) column eluting with H_2O/MeOH (40% MeOH to 70% MeOH) to obtain compound **1** (10 mg) and compound **2**/**3** (5 mg). Fraction EA-4 (600 mg) was subjected on the Sephadex LH-20 (30 g) eluted by acetone to afford fraction EA-4-1 and EA-4-2. EA-4-2 (124 mg) was chromatographied on silica gel (silica gel H, 1.5 g) and eluted with petroleum ether/acetone (9∶1 to 8∶2) to achieve compound **4** (10 mg).

Results and Discussion

The 16S rDNA partial sequence of Ly221 was submitted to GenBank and obtained its accession number as EF125928. The blast search results showed that the sequence of Ly221 was highly homologous to other *Streptomyces* species, indicating that this strain was a member of the genus *Streptomyces*.

Compound 1 Colorless amorphous powder. $[\alpha]_D^{23}$ + 23.7 (*c* 0.7, $CHCl_3$). Its molecular formula was established as $C_{13}H_{22}O_3$ on the basis of ESI-MS data (*m*/*z*: 227 $[M + H]^+$, 453 $[2M + H]^+$) and NMR data

(Table 1 and 2). It was identified as 4, 10-dihydroxy-10-methyl-dodec-2-en-1, 4-olide [11] by comparison with the data given in reference (Fig. 1).

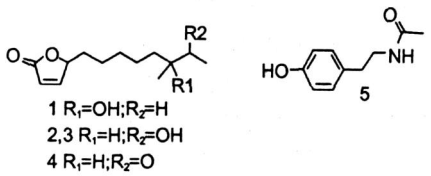

Fig. 1 Structures of compounds 1-5

Compound 2/3 Colorless amorphous powder. $[\alpha]_D^{23}$ +53.3 (*c* 0.3, $CHCl_3$). Its molecular formula was established as $C_{13}H_{22}O_3$ on the basis of ESI-MS data (*m/z*: 227 $[M+H]^+$, 453 $[2M+H]^+$ and NMR data (Table 1 and 2). It was identified as 4, 11-dihydroxy-10-methyl-dodec-2-en-1, 4-olides [11] by careful comparison with the reference data (Fig. 1).

Compound 4 Colorless amorphous powder. Its molecular formula was established as $C_{13}H_{20}O_3$ on the basis of ESI-MS data (*m/z*: 225 $[M+H]^+$) and NMR data (Table 1 and 2). It was identified as 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1, 4-olide by comparison with the literature data [11] (Fig. 1).

Table 1 ^{13}C NMR (100 MHz) for butenolides 1-4 in $CDCl_3$ (δ_{ppm})

Position	1	2	3	4
1	173.2 (s)	173.2 (s)	173.2 (s)	173.2 (s)
2	121.5 (d)	121.5 (d)	121.5 (d)	121.5 (d)
3	156.3 (d)	156.3 (d)	156.3 (d)	156.3 (d)
4	83.4 (d)	83.4 (d)	83.4 (d)	83.4 (d)
5	33.2 (t)	33.1 (t)	33.1 (t)	33.1 (t)
6	24.8 (t)	24.9 (t)	24.9 (t)	24.8 (t)
7	29.8 (t)	29.6 (t)	29.6 (t)	29.3 (t)
8	23.6 (t)	27.1 (t)	27.0 (t)	26.9 (t)
9	41.1 (t)	29.6 (t)	29.8 (t)	32.6 (t)
10	72.8 (s)	39.7 (d)	40.0 (d)	47.1 (t)
11	34.2 (t)	71.3 (d)	71.7 (d)	212.7 (s)
12	8.19 (q)	20.2 (q)	19.5 (q)	28.0 (q)
13	26.4 (q)	14.1 q)	14.5 (q)	16.3 (q)

Table 2 ^{1}H NMR (400 MHz) for butenolides 1-4 in $CDCl_3$ (δ_{ppm}, J_{Hz})

Position	1	2	3	4
1	-	-	-	-
2	6.12 (d, *J* = 5.7)	6.12 (d, *J* = 5.7)	6.12 (d, *J* = 5.7)	6.14 (d, *J* = 5.7)
3	7.46 (dd, *J* = 1.0, 5.7)	7.46 (dd, *J* = 1.0, 5.7)	7.46 (dd, *J* = 1.0, 5.7)	7.47 (d, *J* = 5.6)
4	5.08 (dt, *J* = 7.0, 1.5)	5.08 (dt, *J* = 7.0, 1.5)	5.08 (dt, *J* = 7.0, 1.5)	5.08 (m)
5	1.78 (m)	1.78 (m)	1.78 (m)	1.79 (m)
	1.70 (m)	1.70 (m)	1.70 (m)	1.69 (m)
6	1.50 (m)	1.50 (m)	1.50 (m)	1.45 (m)
7	1.69 (m)	1.69 (m)	1.69 (m)	1.69 (m)
8	1.38 (m)	1.42 (m)	1.42 (m)	1.30 (m)
		1.38 (m)	1.38 (m)	
9	1.50 (m)	1.38 (m)	1.38 (m)	1.69 (m)
	1.38 (m)	1.25 (m)	1.25 (m)	1.37 (m)
10	-	1.50 (m)	1.50 (m)	2.53 (m)
11	1.50 (m)	3.71 (m)	3.66 (m)	-
12	0.92 (t, *J* = 7.0)	1.15 (d, *J* = 6.6)	1.11 (d, *J* = 6.5)	2.16 (s)
13	1.15 (s)	0.90 (d, *J* = 7.0)	0.90 (d, *J* = 7.0)	1.11 (d, *J* = 7.0)

Compound 5 Colorless amorphous powder. Its molecular formula was established as $C_{10}H_{13}O_2N$ on the basis of ESI-MS data (*m/z*: 180 $[M+H]^+$). ^{1}H NMR (400 MHz, CD_3OD) δ: 6.71 (2H, d, *J* = 8.5, H-3, 5), 7.02 (2H, d, *J* = 9.2, H-2, 6), 3.33 (2H, t, *J* = 7.5, H-7), 2.69 (2H, t, *J* = 7.5, H-8), 1.89 (3H, s, Me); ^{13}C

NMR (100 MHz, CD_3OD) δ: 173.2 (C=O), 156.9 (C-4), 131.2 (C-1), 130.7 (C-2, 6), 116.2 (C-3, 5), 42.4 (C-7), 35.7 (C-8), 22.5 (Me). It was identified as N-acetyltyramine by comparison with the literature data [12] (Fig. 1).

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