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NOTE

Isolation and characterization of LS1924A, a new analog of emycins

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In the course of our screening program for new bioactive compounds from our natural product library,[1] a number of 'talented' strains were discovered based on their bioactivity spectrum or novel peaks on liquid chromatography-diode array detection-mass spectrometry (LC-DAD-MS) analysis. A *Streptomyces* sp. LS1924 strain was found to have the ability to produce bioactive metabolites and was selected to be studied further leading to the discovery of LS1924A (**1**), a new analog of emycins, together with three known compounds of angucyclinone family. In this paper, we report the fermentation, isolation, chemical characterization of these compounds and the bioactivity of the new compound.

The producing strain *Streptomyces* sp. LS1924 was isolated from a soil sample collected from Yaoli Virgin Forest (elevation 850 m) of Jiangxi Province, China. Strain LS1924 was deposited at the China General Microbiological Culture Collection Center (CGMCC) with the accession number CGMCC No.5240. The strain was grown and maintained on a GT-medium agar slant consisting of soluble starch 2.0%, L-asparagine 0.05%, KNO_3 0.1%, $K_2HPO_4 \cdot H_2O$ 0.05%, NaCl 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 7.5) at 28 °C. The stock culture was transferred into 250-ml Erlenmeyer flasks containing 40 ml of the seed medium with the same components as the agar slant medium. The culture was incubated on a rotary shaker (220 r.p.m.) at 28 °C for 4 days. Ten milliliters of the seed culture was transferred to 1000-ml Erlenmeyer flasks containing 250 ml of the producing medium, which contains glucose 1.0%, millet meal 2.0%, cottonseed protein powder 2.0% and MOPS 2.0% (pH 7.0). The fermentation broth was incubated at 28 °C for 8 days on a rotary shaker at 220 r.p.m.

The fermentation broth (10 l) was fractionated by centrifugation. The mycelium was extracted with methanol and the supernatant was applied on a Diaion HP-20 resin (Mitsubishi Chem. Ind., Co., Ltd., Tokyo, Japan) column (1 l), which was then washed with deionized water (3 l), and the bioactive components were subsequently eluted with ethanol. The organic phases from the mycelium and the HP-20 resins were combined and then evaporated to give a general extract (15 g). The general extract was then chromatographed on a column of silica gel (300 ml, Qingdao Silica Manufactory, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) first with petroleum ether/acetone gradient from 90:10 to 50:50 and later with chloroform/methanol gradient from 90:10 to 50:50 and two bioactive fractions (petroleum ether/acetone 80:20 and chloroform/methanol 90:10) were obtained. Fraction 1 was chromatographed on a silica gel column (300 ml, Qingdao Silica Manufactory) again with petroleum ether/acetone gradient elution from 98:2 to 80:20, resulting in three sub-fractions. The purification of sub-fraction 1 on a column of sephadex LH-20 (1.5 × 100 cm, Pharmacia. Uppsala, Sweden) with chloroform/methanol (1:1) elution afforded pure **2** (11.5 mg). Sub-fraction 2 was chromatographed on a column of sephadex LH-20 (1.5 × 100 cm, Pharmacia) with chloroform/methanol (1:1), followed by preparative high-performance liquid chromatography on a SB-C18 column (9.4 × 250 mm, 5 μm, Agilent, Santa Clara, CA, USA) with methanol/water (95:5) as mobile phase at 2 ml min^{-1} to give pure **3** (6.0 mg). Subfraction 3 was chromatographed on a column of LH-20 (1.5 × 100 cm, Pharmacia), developed with chloroform/methanol (1:1) and the active fraction was finally purified by preparative high-performance liquid chromatography on a SB-C18 column (9.4 × 250 mm, 5 μm, Agilent) with methanol/water (65:35) as mobile phase at 2 ml min^{-1} to yield pure **1** (3.2 mg). Fraction 2 was applied on a silica gel column with petroleum ether/acetone gradient elution from 90:10 to 50:50 and a subsequent purification for the bioactive fraction by preparative high-performance liquid chromatography on a SB-C18 column (9.4 × 250 mm, 5 μm, Agilent) with methanol/water (30:70) at 2 ml min^{-1} to give pure **4** (6.7 mg).

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The structures of **1**, **2**, **3** and **4** were determined as shown in Figure 1 and **1** was found to be a new compound after a SciFinder database search.

Compound **1** was obtained as white amorphous powder and the physico-chemical properties of **1** are summarized in Table 1. The IR spectrum indicated the presence of C=O (1681 cm^{-1}), OH (3510 cm^{-1}), C-O (1082 and 1046 cm^{-1}), CH_3 (2964 cm^{-1}) and CH_2 groups (2928 and 849 cm^{-1}) and aromatic ring (1600–1430 cm^{-1}). high resolution electrospray ionization mass spectroscopy (HRESIMS) revealed a quasi-molecular ion peak of *m/z* 339.1228 for $C_{20}H_{19}O_5$ $[M+H]^+$ (calcd. 339.1227 for $C_{20}H_{19}O_5$) and suggested 338 as the molecular weight and $C_{20}H_{18}O_5$ as the molecular formula.

The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra in combination with ^1H–^{13}C heteronuclear single quantum coherence NMR data (Table 2) of **1** exhibited signals of one aliphatic methyl group 3-CH_3 (δ_H 1.40; δ_C 30.4), one methoxy group 12-OCH_3 (δ_H 3.89; δ_C 56.0), two aliphatic methylenes CH_2-2 (δ_H 2.94, 2.70; δ_C 54.1) and CH_2-4 (δ_H 3.15, 2.95; δ_C 43.9), two aliphatic methines CH-17 (δ_H 6.70; δ_C 78.8) and CH-10 (δ_H 6.75; δ_C 100.1), and five aromatic methines CH-13 (δ_H 6.91; δ_C 112.0), CH-15 (δ_H 7.19; δ_C 113.1), CH-7 (δ_H 6.84; δ_C 122.7), CH-6 (δ_H 7.09; δ_C 130.6) and CH-14 (δ_H 7.24; δ_C 132.0). In addition, eight quaternary carbon atoms comprising a carbonyl C-1 (δ_C 200.1), two oxygenated aromatic C-8 (δ_C 149.4) and C-12 (δ_C 154.9), five olefinic C-11 (δ_C 124.5), C-18 (δ_C 127.4), C-19 (δ_C 128.3), C-5 (δ_C 135.3) and C-16 (δ_C 149.7) and one aliphatic C-3 (δ_C 72.2) moieties were observed. One hydrogen resonance lacked correlations in the HSQC spectrum of **1** and was recognized as an aliphatic OH group (δ_H 2.06, s).

Two aromatic spin systems and substructures were established, ABX system for H-13/ H-14/H-15 and AB system for H-6/H-7, by interpretation of the gradient correlation spectroscopy (gCOSY) spectrum (Figure 2). The connection between these substructures was established using ^1H–^{13}C heteronuclear multiple bond correlation (HMBC) correlations (Figure 2). The HMBC correlations revealed that the methoxyl group 12-OCH_3 was attached to the aromatic carbon C-12. Observed correlations from H-14 to C-12 and C-16; H-13 to C-11 and C-15 together with the gCOSY information, supported the trisubstituted aromatic ring. Also, correlations from H_2-2 to C-4; H_2-4 to C-19; H-6 to C-8 and C-19; and H-7 to C-18 and C-5 supported the tetrasubstituted benzene ring fused with the substituted cyclohexane. Observed HMBC correlations from the methyl group 3-CH_3 to C-2 and C-4 indicated that the methyl group was attached to the oxygen-bearing quaternary carbon C-3. According to the molecular formula suggested by HRESIMS and the ^{13}C NMR shift of the unassigned carbon atoms, C-10 should share one oxygen atom with C-8 and one oxygen atom with C-17. These information, combined with HMBC correlations from H-17 to C-11 and H-10 to C-16 helped to establish the structure of **1**, which was further supported by NMR data comparison between **1** and

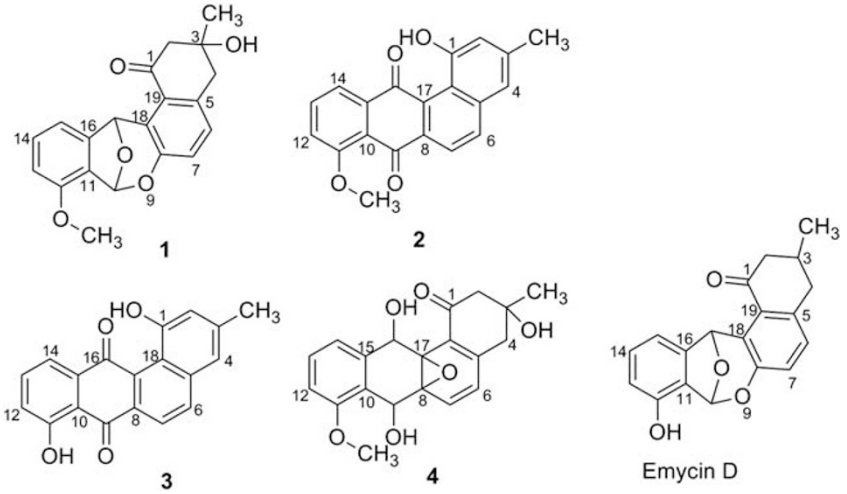


Figure 1 Structures of compounds **1**, **2**, **3** and **4**.

Table 1 Physico-chemical properties of 1

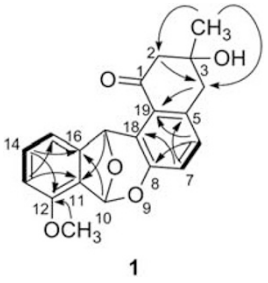
Appearance	White amorphous powder
Elemental composition reported by HRESIMS $[M+H]^+$	$C_{20}H_{19}O_5$
HRESIMS found	339.1228 $[M+H]^+$; 361.1072 $[M+Na]^+$
Calcd.	339.1227 $[M+H]^+$
Melting point (°C)	182–184
UV λ_{max}^{EtOH} (nm; log ε)	325 (3.46), 259 (3.80)
IR (KBr) ν_{max} (cm^{-1})	3510, 3347, 2964, 2928, 2849, 1681, 1659, 1596, 1485, 1468, 1437, 1281, 1242, 1199, 1170, 1082, 1046, 960, 933, 919, 896, 820
$[\alpha]_D^{25}$	+147.0 (EtOH, c 0.085)
Solubility	EtOH, $CHCl_3$, acetone, DMSO, acetonitrile

Abbreviations: DMSO, dimethyl sulfoxide; IR, infrared; UV, ultraviolet.

Table 2 ^{13}C and ^{1}H NMR assignments for **1** in acetone-d_6

	δ_C[a]		δ_H[b] *(mult, J Hz)*
Position	***1***	*Emycin D*[c]	***1***
1	200.1, C	200.0	
2	54.1, CH_2	48.2	2.94 (dd, 2.0, 16.4) 2.70 (dd, 2.0, 16.4),
3	72.2, C	30.9	2.95 (d, 15.4),
4	43.9, CH_2	37.5	3.15 (d, 15.4)
5	135.3, C	136.6	
6	130.6, CH	129.2	7.09 (d, 8.4)
7	122.7, CH	121.9	6.84 (d, 8.4)
8	149.4, C	148.6	
10	100.1, CH	99.1	6.75 (s)
11	124.5, C	122.3	
12	154.9, C	151.5	
13	112.0, CH	115.6	6.91 (d, 8.0)
14	132.0, CH	130.7	7.24 (t, 8.0)
15	113.1, CH	110.8	7.19 (d, 8.0)
16	149.7, C	148.9	
17	78.8, CH	77.8	6.70 (s)
18	127.4, C	127.0	
19	128.3, C	127.4	
12-OCH_3	56.0, CH_3		3.89 (s)
3-CH_3	30.4, CH_3	20.7	1.40 (s)
3-OH			2.06 (s)

Abbreviation: NMR, nuclear magnetic resonance.
[a]The ^{13}C-NMR was measured at 100 MHz.
[b]The ^{1}H-NMR was measured at 400 MHz.
[c]Reported data of emycin D in acetone-d_6 was measured at 125 MHz.[2]



1

Figure 2 Key HMBC correlations (H→C) and ^{1}H–^{1}H COSY correlations (bold lines) of **1.**

emycin D.[2] The ^{1}H and ^{13}C-NMR assignments were shown in Table 2. The new compound **1** is a ring-expanded angucyclinone with the same tetracyclic ring structure as emycin D, which was isolated from a mutated *Streptomyces cellulosae* ssp. *griseoincarnatus* (FH-S 1114-2) strain.[2] The relative stereochemistry of two chiral centers at C-3 and C-17 of compound **1** remained undefined.

Structures of compounds **2–4** were elucidated using 1D NMR, MS and by comparison with the previously reported data.[3–6] Compounds **2** and **3** were identified to be X-14881E[3,4] and tetrangulol,[4,5] respectively, and compound **4** was determined to be EI 1507-2 possessing an 8, 17-epoxide functional group.[6]

The cytotoxic activity of the new compound **1** was tested against two human cancer cell lines, HepG2 and A549, *in vitro* using MTT method.[7] The compound exhibited cytotoxic activity against these two cell lines with IC_{50} (the half-inhibitory concentration) values of 39.3 μM and >100 μM, respectively. Compound **1** showed no significant antibiotic activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus Calmette-Guerin* and *Candida albicans* with the MIC values all >100 μM. Compound **2** was reported to exhibit no significant antimicrobial activity *in vitro*.[3] Compound **4** was reported to inhibit the enzymic activity of human interleukin-1β covering enzyme with IC_{50} value of 0.42 μM, and have a weak antimicrobial activity against *Bacillus subtilis* with MIC value of 60 μM.[6]

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