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Chemomicin A, a New Angucyclinone Antibiotic Produced by *Nocardia mediterranei* subsp. *kanglensis* 1747-64

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Abstract A new angucyclinone antibiotic, chemomicin A was isolated from cultured broth of *Nocardia mediterranei* subsp. *kanglensis* 1747-64. Its chemical structure was determined to be 1,2,3,4a,5,6,6a,12a,12b-nonahydro-1,2,3,8,12,12b-hexahydroxy-3-methyl-6a,12a-epoxybenz-[a]anthracen-4,7(12*H*)-dione by a detailed spectroscopic analysis. Chemomicin A had antimicrobial activity against *Bacillus subtilis* and *Enterococcus faecium* with MIC values of 10.2 and 20.4 μM, respectively, and showed cytotoxicity against human colorectal cancer HCT116 cells and human esophageal carcinoma YES-2 cells with IC_{50} values of 127 and 153 μM, respectively.

Keywords angucyclinone, *Nocardia mediterranei* subsp. *kanglensis* 1747-64, chemomicin A, SF2315B, structural elucidation

Nocardia mediterranei subsp. *kanglensis* 1747-64 [1] (strain 1747-64), an angucyclinones producing strain, was isolated from soil sample collected from Kang-Le Area, Guangdong Province, P. R. China. Two new angucyclinones, kanglemycin C (**2**) [2] and kanglemycin M (**3**) [3] (Fig. 1), were found in the cultured broth of the strain. Aromatic protons (H-9, H-10, H-11) in D-ring of most angucyclinones [4], like PD116779 [5], rubiginones [6], EI-1507-1, EI-1507-2 [7] and ochracenomicins A, B and C [8], showed ABX coupling system in the downfield region (δ 7~8) in their ^{1}H-NMR spectra. Based on the hypothesis that the strain was a talented producer of angucyclinones like rubiginones producing strain [6], a project to use spectroscopic data mentioned above as a probe in HPLC-NMR to further screen new angucyclinones from the cultured broth of the strain 1747-64 was carried out. As a result, new members of angucyclinone group designated as chemomicins were early identified by HPLC-NMR. Among them, chemomicin A (**1**) was further isolated under the guidance of its retention time in HPLC. Structural studies showed **1** was a unique angucyclinone with six hydroxyl groups, a 4-carbonyl group and a 6a,12a-epoxide functional group (Fig. 1). In this paper, we wish to report the fermentation, isolation, physico-chemical properties, structure elucidation and biological activities of **1**.

A stock culture of the strain 1747-64 was maintained on Gause No. 1 agar slant consisting of KNO_3 0.1%, NaCl 0.05%, K_2HPO_4 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%, $MgSO_4 \cdot 7H_2O$ 0.05%, soluble starch (Beijing Qi Te Xin Chemical Co., Ltd., China) 2.0%, and agar 1.5% (pH 7.0) at 4°C. The stock culture was transferred into 250-ml Erlenmeyer flasks containing 50 ml of seed medium consisting of glucose 3.0%, yeast extract (Shanghai Yeast Manufactory, China) 0.5%, $(NH_4)_2SO_4$ 0.5% and $CaCO_3$ 0.5% (pH 6.5). The culture was incubated on a rotary shaker (220 rpm) at 28°C for 48 hours. Five milliliters of the seed culture was transferred to 500-ml Erlenmeyer flasks containing 100 ml

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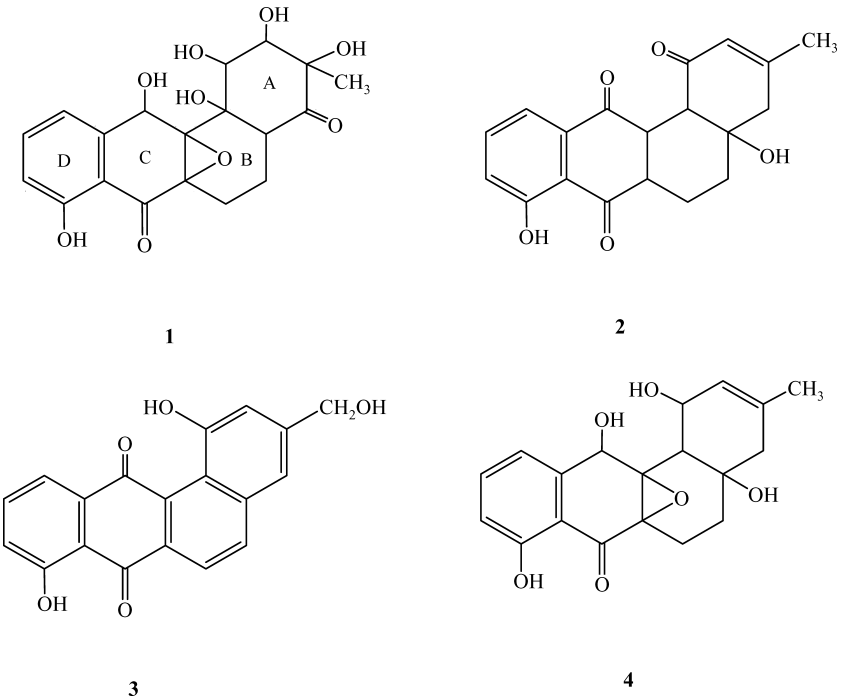

Fig. 1 Structures of chemomicin A (**1**), kanglemycin C (**2**), kanglemycin M (**3**) and SF2315B (**4**).

of the producing medium consisting of glucose 4.0%, yeast extract 1.0%, peanut meal 0.5%, peptone (Shanghai Donghai Pharmaceutical Manufactory, China) 0.5% and $CaCO_3$ 0.1% (pH 6.5). The fermentation was carried out at 28°C for 96 hours on a rotary shaker (220 rpm).

The fermentation broth (25 liters) was adjusted to pH 5.0 with 2 N HCl and filtered. The filtrate was extracted with ethyl acetate (25 liters). The extract was concentrated to a small volume under reduced pressure to give a syrup. It was then chromatographed on a column of silica gel (300 ml, Qindao Silica Manufactory, China) and developed with $CHCl_3$ - MeOH, 19 : 1 (v/v 1500 ml). Thirty fractions (50 ml per fraction) were collected and 200 μl solutions from each fraction were dried in 1.5 ml eppendroff tubes with N_2 stream. Then, the resulting residues were dissolved in 50 μl methanol for analysis with HPLC on a Zorbax SB-C18 column (9.4×250 mm, 5 μm, Agilent), with MeOH - H_2O, 65 : 35 (v/v) at 1 ml/minute after filtered through 0.22 μm membrane. Fractions (No. 21 to 23) containing most of **1** were pooled to yield sample (166 mg). The sample (83 mg/ml, 2 ml) was further purified by HPLC on a shim-pack PRC-ODS column (250×20 mm, Shimadzu) with MeOH - H_2O, 55 : 45 (v/v) at 4 ml/minute to yield 40 mg of **1** as white powder.

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was established as $C_{19}H_{20}O_9$, on the basis of high-resolution SI-MS, NMR spectra in CD_3OD (I) and in DMSO-d_6 (II). The ^{1}H-NMR and ^{13}C-NMR spectral data of **1** are shown in Table 2.

Analysis of ^{1}H-NMR and ^{13}C-NMR together with DEPT and a heteronuclear single quantum coherence (HSQC) indicated 19 carbon signals of **1** could be attributed to two carbonyl carbons, six aromatic carbons (including three methine groups, two quaternary carbons, one oxygen-substituted quaternary carbon), seven other oxygen-substituted carbon signals (including three oxygenated methine groups and four oxygenated quaternary carbons), one methine group, two methylene groups, one methyl group. Six hydroxyl protons at δ 11.4 (H-bonded phenolic hydroxyl proton), 7.1, 6.9, 6.5, 5.4, and 5.1 appeared in ^{1}H-NMR spectrum in DMSO-d_6, but disappeared in CD_3OD.

Epoxide functional group existed in **1** was early deduced from calculating the oxygen atom number and the number of oxygen-substituted carbon signals, since only six oxygen atoms have to be assigned to seven oxygen-substituted carbon signals except that three oxygen atoms were ascribed to two carbonyl groups and one phenolic hydroxyl group.

The IR spectrum indicated the presence of hydroxyl groups (3410 cm^{-1}), a ketone carbonyl group (1714 cm^{-1}) and a chelated carbonyl group (1653 cm^{-1}), which were further confirmed by a ketone carbonyl carbon signal at δ 204.02 (C-4) and a chelated carbonyl carbon signal at δ 201.20 (C-7) in ^{13}C-NMR spectrum in DMSO-d_6, the same

Table 1 Physico-chemical properties of **1**

Appearance	White powder
Molecular weight	392
Molecular formula	$C_{19}H_{20}O_9$
HRSI-MS (*m*/*z*) Found:	391.1033 $(M-H)^-$
Calcd:	391.1034
UV λ_{max}^{MeOH} nm (ε)	217 (19,282), 260 (8,991), 335 (4,564)
IR ν_{max} (KBr) cm^{-1}	3410, 1714, 1653, 1616, 1456, 1250, 1053
Solubility	MeOH, DMSO, $CHCl_3$
TLC, Rf value[a]	0.24
HPLC, Rt (min)[b]	17.6

[a] Silica gel 60 F254 (Merck), $CHCl_3$ - MeOH, 19 : 1 (v/v).
[b] Zorbax SB-C18 (9.4×250 mm, 5 μm, Agilent), 65% MeOH, 1 ml/minute, 254 nm.

Table 2 NMR data of **1** in CD_3OD (I) and in DMSO-d_6 (II)

Position	I		II	
	δ_C[a]	δ_H[b] (mult, *J* Hz)	δ_C[a]	δ_H[b] (mult, *J* Hz)
1	66.52	4.5 (br s)	64.52	4.5 (br s)
1-OH				6.9 (br s)[c]
2	64.77	3.5 (d, 2)	62.45	3.5 (d, 2.5)
2-OH				5.1 (s)[c]
3	60.55		58.65	
3-OH				7.1 (br s)[c]
3-CH_3	15.16	1.4 (s)	15.29	1.3 (s)
4	205.15		204.02	
4a	50.75	2.7 (dd, 12, 3.5)	49.16	2.7 (dd, 12, 3.5)
5	16.37	α 1.8 (m)	14.89	α 1.7 (m)
		β 2.1 (m)		β 1.9 (m)
6	26.87	α 1.9 (ddd, 14.5, ~14.5[d], 4.5)	25.33	α 1.8 (ddd, 14.0, ~14.0[d], 4.0)
		β 2.1 (m)		β 2.0 (m)
6a	78.74		77.22	
7	202.22		201.20	
7a	114.81		113.42	
8	162.25		160.16	
8-OH				11.4 (s)
9	116.53	6.8 (d, 8)	115.15	6.8 (d, 8)
10	137.45	7.5 (dd, 8, 8)	136.37	7.6 (dd, 8, 8)
11	121.48	7.2 (d, 8)	120.09	7.2 (d, 8)
11a	145.31		144.76	
12	65.97	5.5 (s)	64.37	5.4 (s)
12-OH				5.4 (s)
12a	76.32		75.60	
12b	80.07		78.41	
12b-OH				6.5 (s)

[a] The ^{13}C-NMR was measured at 125 MHz. [b] The ^{1}H-NMR was measured at 500 MHz. [c] Hydroxyl proton assignment may be interchangeable. [d] $^3J_{HH}$ coupling constant between two vicinal protons: Hα-5 and Hα-6 was unresolved and was deduced from multiplicity of signal for Hα-6, which was similar as triplet doublet.

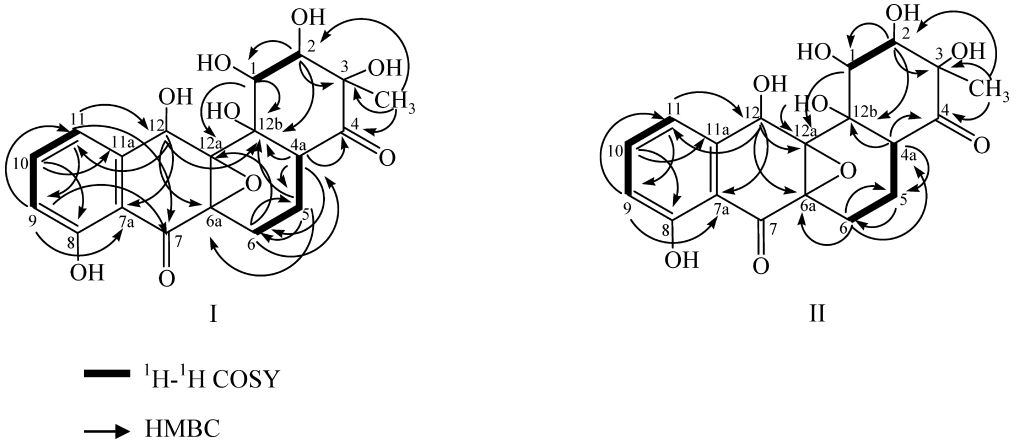

Fig. 2 Summary of ^{1}H-^{1}H COSY and HMBC experiments of **1** in CD_3OD (I) and in DMSO-d_6 (II).

carbon signals could be observed at δ 205.15 and δ 202.22 in CD_3OD. An ABX coupling system of three aromatic proton signals (H-9 to H-11), the probe mentioned above, was readily observed in ^{1}H-NMR. By tracing the cross peaks from H-9, H-10 and H-11 in HMBC in DMSO-d_6 and in CD_3OD, as shown in Fig. 2, C-7a, C-8, C-11a, and C-12 were assigned. The chemical shifts of C-8 at 160.16 in DMSO-d_6 and at 162.25 in CD_3OD suggested that phenolic hydroxyl proton at δ 11.4 in ^{1}H-NMR (DMSO-d_6) should be attached to C-8, the peri position of the chelated carbonyl carbon (C-7). It was further supported by HMBC (CD_3OD), in which, both 9-H and 11-H were long-range correlated with C-7. The chemical shifts of C-12 and H-12 together with the data mentioned above indicated the chromophore of **1** is an isosclerone moiety, the same with SF2315B (**4**) [9], as shown in Fig. 1, which has no absorption peak at wavelength longer than 400 nm, usually shown by quinone chromophore [10]. Hydroxyl proton at δ 5.68 (12-OH) of **4** suggested one of two protons at δ 5.4 of **1** should attributed to hydroxyl proton (12-OH).

H-12 was correlated with C-12a and C-6a in HMBC (DMSO-d_6) and cross peak between H-12 and C-6a could be observed as well in HMBC (CD_3OD). Thus, C-6a and C-12a were assigned. Considering their chemical shift and the reason oxygen atoms were unproportionate with oxygen-substituted carbon signals, as mentioned above, C-6a and C-12a should form an epoxide functional group. The substructure (rings C and D) of **1** was established as shown in Fig. 1 and was further supported by NMR data comparison between **1** and **4** and unambiguous elucidation of another substructure (rings A and B) of **1**.

In another substructure, the two structural fragments: $^{6}CH_2$–$^{5}CH_2$–^{4a}CH and ^{12b}C (OH)–^{1}CH (OH)–^{2}CH (OH)–(CH_3) ^{3}C (OH)–^{4}C (O) were readily identified by ^{1}H-^{1}H COSY and HMBC. Linkage between the two fragments was established by HMBC. The methine proton at δ 2.7 (H-4a) was coupled to ketone carbonyl carbon at δ 204.02 (C-4) and oxygenated quaternary carbon at δ 78.41 (C-12b) in HMBC (DMSO-d_6), meanwhile, methylene proton at δ 2.1 (5-H) and methine proton at δ 4.5 (1-H) were long range coupled to C-12b in HMBC (CD_3OD). The data above revealed the linkage of two structural fragments through C-4a with C-4 and C-12b.

Long range couplings observed between H-12 and C-12b; between H-6 and C-12a; between H-5 and C-6a in HMBC (CD_3OD) fused two substructures through C6–C6a and C12a–C12b, which were further supported by cross peaks between H-1 and C-12a; between H-6 and C-6a in HMBC (DMSO-d_6) as complementary evidence. Hydroxyl proton at δ 6.5 was assigned to 12b-OH by tracing cross peak from C-12a observed in HMBC (DMSO-d_6). Finally, the planar structure of **1** was established as 1,2,3,4a,5,6,6a,12a,12b-nonahydro-1,2,3,8,12,12b-hexahydroxy-3-methyl-6a,12a-epoxybenz[a]anthracen-4,7(12*H*)-dione.

In the ^{1}H-NMR (CD_3OD and DMSO-d_6) of **1**, coupling constants (2 and 2.5 Hz) between H-1 and H-2 showed the vicinal protons were *cis* configuration. Two coupling constants (12 and 3.5 Hz) between H-4a and H-5α; H-4a and H-5β revealed that H-4a and H-5α were axial protons in *trans* configuration and H-5β was an equatorial proton. The large $^{3}J_{HH}$ coupling constant (~14.5 Hz in CD_3OD and 14.0 Hz in DMSO-d_6) between two vicinal protons: Hα-5 and Hα-6 indicated anti-periplanar conformation of these protons and chair conformation of the B-ring [11]. In NOE experiments, irradiation of H-2 enhanced obviously the intensity of H-1 and 3-CH_3, suggesting that H-2 and 3-CH_3 should be *cis* configuration (data not shown). The relative

configuration of **1** remained to be studied in detail.

Within our knowledge, angucyclinones, including **4**, EI-1507-1 and EI-1507-2, rubiginone I, angucyclinone D and elmycin C [4], possess a 6a,12a-epoxide functional group. Most of them were weakly active against Gram-positive and Gram-negative bacteria. For example, EI-1507-1 had weak antimicrobial activities against *Enterococcus faecium*, *Bacillus subtilis* and *Proteus vulgaris* with MIC values of 120, 60 and 60 μM, respectively. On the other hand, all of them have different bioactivities. For example, **4** showed inhibitory activity against reverse transcriptase of avian myeloblastosis virus [12], and both EI-1507-1 and EI-1507-2 inhibited mature interleukin-1β secretion from THP-1 cell with IC_{50} values of 1.1 and 1.4 μM, respectively. Preliminary bioactive studies demonstrated **1** had moderate antimicrobial activities against *B. subtilis* and *E. faecium* with MIC values of 10.2 and 20.4 μM, respectively, and showed cytotoxicity against human colorectal cancer HCT116 cells and human esophageal carcinoma YES-2 cells with IC_{50} values of 127 and 153 μM, respectively. Further studies on detailed biological activities of **1** are in progress.

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