J. Antibiot. 60(3): 211–215, 2007

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THE JOURNAL OF ANTIBIOTICS

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

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Received: September 8, 2006 / Accepted: March 7, 2007
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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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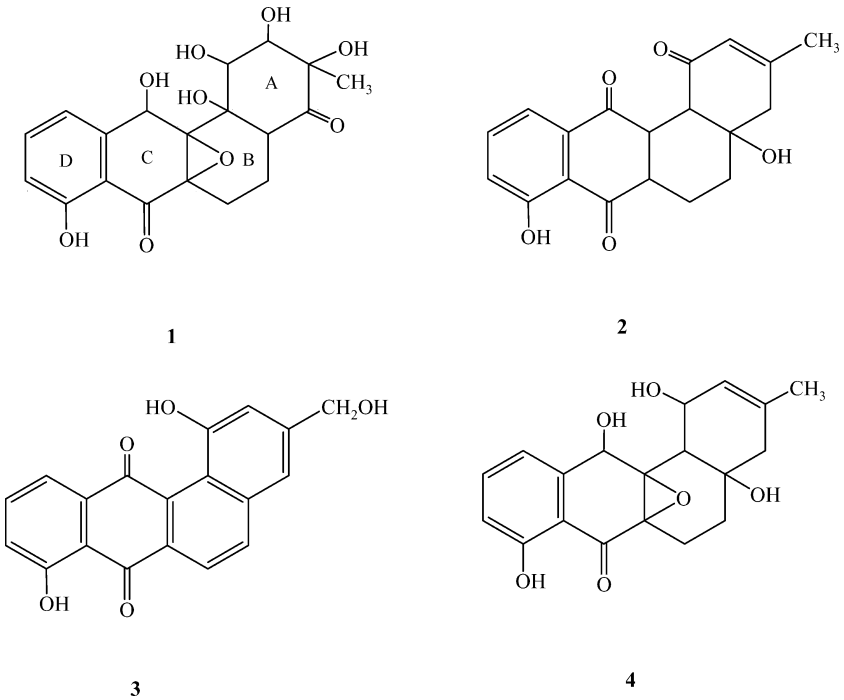

Fig. 1 Structures of chemomicin A (**1**), kanglemycin C (**2**), kanglemycin M (**3**) and SF2315B (**4**).

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.

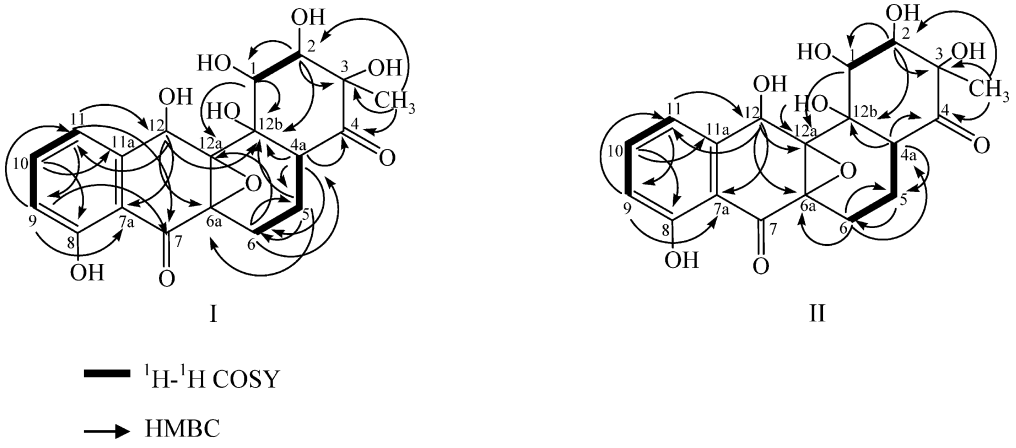

Fig. 2 Summary of ^{1}H-^{1}H COSY and HMBC experiments of **1** in CD_3OD (I) and in DMSO-d_6 (II).

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.

Acknowledgments This work was supported in part by Social Commonwealth Research Project (Project No. 2005DIB1J090), Mega-projects of Science Research for the 10th Five-Year Plan (Project No. 2004AA2Z3784) and National Basic Research Program of China (973, Project No.2004CB518908) funded by The Ministry of Science and Technology of the People's Republic of China. The authors are grateful to Drs. Bin Xin, Wenyi He, Yan Wu, and Jinhua Ni for MS, NMR and IR data. Highly appreciated to Prof. Hiroshi Tomoda in Kitasato University for his valuable scientific discussion and inspiring

References

1. Li Q, Yao ZY, Yao NT, Li YY. Taxonomic study on the kanglemycin producing microorganism, *Nocardia mediterranei kanglesis* 1747-64. Chin J Antibiot 20: 246–248 (1995)
2. Wang NJ, Yang XS, He CH. The structural elucidation of kanglemycin C. Chin J Antibiot 19: 86–90 (1994)
3. Sun CH, Wang NJ, Han BL. Isolation and structure determination of kanglemycin M, a new benz[a]anthraquinone antibiotic. Chin J Antibiot 21: 81–83 (1996)
4. Rohr J, Thiericke R. Angucycline group antibiotics. Nat Prod Rep 9: 103–137 (1992)
5. Kern DL, Schaumberg JP, Hokanson GC, French JC. PD 116,779, a new antitumor antibiotic of the benz[a]anthraquinone class. J Antibiot 39: 469–470 (1986)
6. Puder C, Zeeck A, Beil W. New biologically active rubiginones from *Streptomyces* sp. J Antibiot 53: 329–336 (2000)
7. Tsukuda E, Tanaka T, Ochiai K, Kondo H, Yoshida M, Agatsuma T, Saitoh Y, Teshiba S, Matsuda Y. EI-1507-1 and -2, novel interleukin-1 beta converting enzyme inhibitors produced by *Streptomyces* sp. E-1507. J Antibiot 49: 333–339 (1996)
8. Igarashi M, Sasao C, Yoshida A, Naganawa H, Hamada M, Takeuchi T. Ochracenomicins A, B and C, new benz[a]anthraquinone antibiotics from *Amicolatopsis* sp. J Antibiot 48: 335–337 (1995)
9. Sasaki T, Gomi S, Sezaki M, Takeuchi Y, Kodama Y, Kawamura K. New antibiotics SF2315A and B produced by an *Excellospora* sp. II. The structural elucidation. J Antibiot 41: 843–848 (1988)
10. Hayakawa Y, Ha SC, Kim YJ, Furihata K, Seto H. Studies on the isotetracenone antibiotics. IV. Hatomarubigins A, B, C and D, new isotetracenone antibiotics effective against multidrug-resistant tumor cells. J Antibiot 44: 1179–1186 (1991)
11. Bringmann G, Lang G, Maksimenka K, Hamm A, Gulder TA, Dieter A, Bull AT, Stach JE, Kocher N, Muller WE, Fiedler HP. Gephyromycin, the first bridged angucyclinone, from *Streptomyces griseus* strain NTK 14. Phytochemistry 66: 1366–1373 (2005)
12. Sasaki T, Yoshida J, Itoh M, Gomi S, Shomura T, Sezaki M. New antibiotics SF2315A and B produced by an *Excellospora* sp. I. Taxonomy of the strain, isolation and characterization of antibiotics. J Antibiot 41: 835–842 (1988)