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NOTE

A new anthracycline-type metabolite from *Streptomyces* sp. NEAU-L3

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A new anthracycline-type metabolite, designated as tetracenoquinocin A (1), was isolated from the fermentation broth of *Streptomyces* sp. NEAU-L3. Its structure was determined on the basis of spectroscopic analysis, including 1D and 2D NMR techniques as well as ESI-MS and comparison with data from the literature. Compound 1 showed potent cytotoxic activity against three cancer cell lines (HepG2, A549, HCT-116) with IC_{50} values of 5.57, 24.30 and 20.82 μM, respectively.
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Microorganisms, in particular the genus *Streptomyces*, are well known to yield a great number of bioactive substances. Many well-known antibiotics, such as bleomycin, mitomycin, anthracyclines (doxorubicin, epirubicin, idarubicin), are derived from *Streptomyces*.[1,2] Among them, anthracyclines are one class of the typical antitumor antibiotics that are widely used in the treatment of cancer chemotherapy.[3,4] Due to their adverse effects of cardiotoxicity and vomiting,[5–7] much effort has been made to develop novel anthracycline compounds expecting for more potent antitumor drugs.[8–10] During the course of screening for more microbial-derived metabolites, we investigated the bioactive constituents of the strain *Streptomyces* sp. NEAU-L3 and this led to the isolation of an anthracycline compound, designated as tetracenoquinocin A (**1**). In this paper, we report the fermentation, isolation, structural characterization and the bioactivity of compound **1**.

Strain *Streptomyces* sp. NEAU-L3 was isolated from a soil sample collected from the peak of Dayao Mountain of Guangxi province, China. The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: HQ916737 in the GenBank) exhibited a high-sequence similarity of 100% with that of *Streptomyces* sp. ATCC 19428 (T) (accession no: X75374).

This strain was incubated for 6–7 days at 28 °C on the YMS medium containing malt extract 10.0 g, yeast extract 4.0 g, glucose 4.0 g, $CoCl_2{\cdot}6H_2O$ 0.005 g and agar 20.0 g in 1.0 l tap water at pH 7.0–7.2. The strain of stock culture was transferred into 1 l Erlenmeyer flasks containing 25% volume of the seed medium and incubated at 28 °C for 24 h, shaken at 250 r.p.m. The seed medium consisted of glucose 4.0 g, malt extract 10.0 g and yeast extract 4.0 g in 1.0 l tap water, pH 7.0–7.2. All of the media were sterilized at 121 °C for 30 min. The seed culture (8%) was transferred into 1000 ml Erlenmeyer flasks containing 250 ml of production medium. The production medium was composed of glucose 1%, soluble starch 4%, yeast extract 0.4%, malt extract 1%, $CaCO_3$ 0.2%, $FeSO_4{\cdot}7H_2O$ 0.1%, $ZnSO_4{\cdot}7H_2O$ 0.1%, and $MnCl_2{\cdot}4H_2O$ 0.1% at pH 7.2–7.4 before sterilization. The flasks were incubated at 28 °C for 7 days on a rotary shaker at 250 r.p.m.

The final 15 l fermentation liquor was filtered to separate mycelial cake and supernatant. The mycelial cake was washed with water (3 l) and subsequently extracted with MeOH (3 l), and the supernatant was subjected to a Diaion HP-20 resin (Mitsubushi Chemical, Tokyo, Japan) column eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure to yield a mixture (23 g) at 55 °C. The crude extract was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–60:40, v/v) to give four fractions (Fr.1-Fr.4) based on the TLC profiles. TLC was performed on silica-gel plates (HSGF254, Yantai Chemical Industry Research Institute, Yantai, China) with solvent system of $CHCl_3$/MeOH (9:1, v/v). The Fr.2 eluted with $CHCl_3$/MeOH (90:10, v/v) was subjected to a Sephadex LH-20 (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected by TLC to give two subfractions (Fr.2-1-Fr.2-2). The Fr.2-2 was further purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 ml min^{-1}; 254 nm; Agilent, PaloAlto, CA, USA) eluting with 85% aqueous MeOH containing 0.1% acetic acid to give compound **1** (t_R 16.59 min, 11.6 mg). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The ESIMS and HRESIMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA).

Compound **1** was isolated as amorphous red powder with $[\alpha]_{25}^{D}$ −20 (*c* 0.06, EtOH) and UV (EtOH) λ_{max} nm (log ε): 262

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Table 1 ^{1}H and ^{13}C NMR data of compound 1 (in DMSO-d_6)

Position	δ_H *(J in Hz)*	δ_C *(p.p.m.)*	*Position*	δ_H *(J in Hz)*	δ_C *(p.p.m.)*
1	7.91 d (7.9)	121.0 (d)	12		182.0 (s)
2	7.84 t (7.9)	136.0 (d)	12a		136.2 (s)
3	7.69 d (7.9)	123.3 (d)	1′	5.63 br s	99.1 (d)
4		157.5 (s)	2′	4.02 m	70.5 (d)
4a		122.4 (s)	3′	4.02 m	70.6 (d)
5		187.7 (s)	4′	3.36 m	72.2 (d)
5a		109.8 (s)	5′	3.56 m	70.7 (d)
6		166.1 (s)	6′	1.11 d (5.8)	18.3 (q)
6a		117.1 (s)	1″	5.68 br s	99.1 (d)
7		155.8 (s)	2″	4.02 m	70.7 (d)
8	7.21 br s	116.5 (d)	3″	3.97 m	70.6 (d)
9		143.0 (s)	4″	3.36 m	72.3 (d)
10	7.56 br s	124.1 (d)	5″	3.36 m	70.6 (d)
10a		138.6 (s)	6″	1.13 d (5.8)	18.4 (q)
11	8.00 s	120.3 (d)	9-CH_3	2.46 s	22.0 (q)
11a		128.4 (s)	6-OH	16.0 s	

(4.17), 483 (3.52), 581 (2.97). Its molecular formula was determined to be $C_{31}H_{32}O_{13}$ (16 double-bond equivalents) by HRESIMS at *m/z* 611.1749 $[M\text{-}H]^-$ (calcd as 611.1770 for $C_{31}H_{31}O_{13}$) and the NMR data (Table 1). The IR spectrum indicated the presence of hydroxyl (3490 cm^{-1}) and carbonyl (1712 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** (Table 1) displayed a hydrogen-bonded phenolic hydroxy proton at δ_H 16.0 (1H, s), six aromatic protons from δ_H 7.21 to δ_H 8.00, one aromatic methyl at δ_H 2.46 (3H, s), two aliphatic doublet methyls at δ_H 1.11 (3H, d, $J=5.8$ Hz), 1.13 (3H, d, $J=5.8$ Hz), two singlet protons at δ_H 5.63 (1H, br s), 5.68 (1H, br s) in addition to 8 oxygenated methine protons from δ_H 3.36 to δ_H 4.02. The ^{13}C NMR and HMQC spectra (Table 1) showed 31 resonance lines, which were grouped into two carbonyl carbons (δ_C 182.0, 187.7), 16 aromatic carbons between 109.8 and 166.1 p.p.m, two anomeric carbons (δ_C 99.1, 99.1), three methyl carbons (δ_C 18.3, 18.4, 22.0), and 8 oxygen-bearing aliphatic carbons between 70.6 and 72.3 p.p.m. as edited by the DEPT135 and HMQC experiments. The complete assignment of all ^{1}H and ^{13}C NMR spectral data of **1** was subsequently accomplished by the ^{1}H–^{1}H COSY, HMQC and HMBC spectra. In this molecule, a 1,2,3-trisubstituted benzene ring was indicated by a group of signals at δ_H 7.69 (1H, d, $J=7.9$ Hz), 7.84 (1H, t, $J=7.9$ Hz), 7.91 (1H, d, $J=7.9$ Hz), which was also supported by the ^{1}H–^{1}H COSY experiment (Figure 1) and a 1,2,3,5-tetrasubstituld benzene ring was established by the correlation of δ_H 7.21 and δ_H 7.56 in the ^{1}H–^{1}H COSY spectrum, which was confirmed by the HMBC correlated signals (Figure 1) from δ_H 2.46 to C-8, C-9, C-10. Furthermore, the aromatic methine proton H-11 (δ_H 8.00) was coupled to C-5a, C-6a, C-10, C-10a and the aromatic methine proton H-8 (δ_H 7.21) was coupled to C-6a, C-7, CH_3-9 and C-10. Additionally, the aromatic methine proton H-10 (δ_H 7.56) was coupled to C-6a, CH_3-9 and C-10a. These results indicated a 3-methyl naphthalene-1,8-diol moiety. The 1,2,3-trisubstituted benzene ring and 3-methyl naphthalene-1,8-diol moieties were incorporated with two ketonic carbons (δ_C 182.0, 187.7) to form the most probable structure of **1** according to the key HMBC correlations of H-1 (δ_H 7.91) with C-12 (δ_C 182.0) and C-4a (δ_C 122.4), H-3 (δ_H 7.69) with C-1 (δ_C 121.0) and C-4a, H-2 (δ_H 7.84) with C-12a (δ_C 136.2) and C-4 (δ_C 157.5), and of the H-11 with C-12. In addition to these resonances ascribed to the tetracene chromophore moiety, **1** showed 12 extra ^{13}C resonances. Due to the heavily signal overlapped, the 12 extra ^{13}C resonances were postulated to include two rhamnose moieties according to two singlet anomeric protons (δ_H 5.63, 5.68), two doublet methyls (δ_C 1.11 1.13) and eight oxygenated methine protons. Furthermore, the presence of L-rhamnose was evidenced by the hydrolysis experiment. Compound **1** (2 mg) heating in 2 M HCl (4 ml) at 80–90 °C for 1 h, and then extracted two times using an equal volume of EtOAc. The aqueous layer was concentrated to dry *in vacuo* to obtain the residue. The residue was dissolved in pyridine (1 ml) containing L-cysteine methyl ester hydrochloride (1 mg) and heated at 60 °C for 1 h. Then 0.2 ml solution of *o*-torylisothiocyanate (1 mg) in pyridine was added to the mixture, which was heated at 60 °C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC (Amethyst C18-H, 5 μm, 250×4.6 mm inner diameter; 0.8 ml min^{-1}; 250 nm; Amethyst, Suzhou, China) at 35 °C eluting with CH_3CN/H_2O (25:75, v/v). The peak of the reaction mixture was detected at 29.343 min (L-rhamnose). Treated in the same manner, standard L-rhamnose (Sigma) gave a peak at 29.607 min. The chemical shifts of the two anomeric protons in **1** were very similar to those reported in tetracenouinocin, actinosporin A and actinosporin B[11,12] and the configurations of the C-1′ and C-1″ of **1** were attempted to assign α. On the basis of the above evidences, the structure of **1** was established as shown in Figure 1, named tetracenouinocin A by the similar to tetracenouinocin.

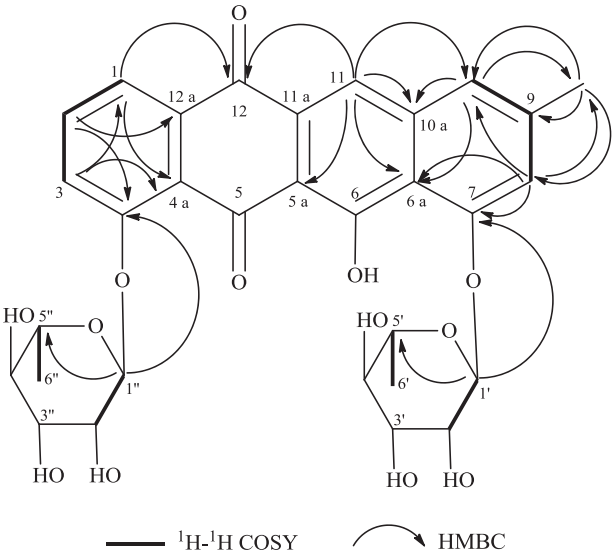

Figure 1 Structure and key ^{1}H-^{1}H COSY and HMBC correlations of tetracenoquinocin A (**1**).

Table 2 Cytotoxic activity of 1 and doxorubicin against selected human tumor cell lines

	IC_{50} (μM)	
Compounds	***1***	*doxorubicin*
HepG2	5.57	1.09
A549	24.30	0.40
HCT-116	20.82	0.65

The cytotoxicity of **1** was assayed for growth-inhibition activity *in vitro* against three human tumor cell lines, human hepatocellular liver carcinoma cells HepG2, human lung tumor cells A549 and human colon carcinoma cells HCT-116 according to the CCK8 colorimetric method as reported in our previous papers[13,14] using

doxorubicin as positive control. As a result, the bioassay showed that **1** has weaker cytotoxicity against the three tumor cell lines than doxorubicin (Table 2).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)