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NOTE

A new okicenone analog from *Streptomyces* sp. NEAU-W13

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The search for novel antibiotics and other bioactive microbial metabolites for potential pharmaceutical, agricultural and industrial applications has been and still is important.[1–6] As part of our continuous effort to discover microorganism-derived anti-tumor secondary metabolites, we investigated the bioactive constituents of a strain *Streptomyces* sp. NEAU-W13. As a result, a new okicenone analog (**1**, Figure 1) was isolated from the fermentation broth of the strain. In this paper, the isolation, structure determination and cytotoxic activity of compound **1** are described.

Strain NEAU-W13 was isolated from a soil sample collected from a farmland located in Haicheng, Liaoning province, China. The organism was isolated using the standard dilution plate method and grown on humic acid-vitamin agar[7] supplemented with nystatin (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: HQ596202 in the GenBank, National Center for Biotechnology Information) exhibited a high-sequence similarity of 99% with that of *Streptomyces thermocarboxydus* AT37 (accession no: NR026072).

The strain was maintained on the YMS medium containing 1% soluble amylum, 0.2% yeast extract, 0.1% KNO_3 and 2% agar, pH 7.0. The seed medium consisted of 2% glucose, 1.5% soybean flour and 0.5% yeast autolysate, pH 7.0. All the media were sterilized at 121°C for 20 min. Slant culture was incubated for 6–8 days at 28°C. A total of 10 ml of sterile water was added to the slant of the YMS medium. The spores were scraped and transferred into a sterile tube containing glass beads; the spore suspension was then filtered through six layers of sterile filter cheesecloth and adjusted to 10^7–10^8 c.f.u ml^{-1}. A volume of 2.0 ml of the spore suspension was inoculated into a 250-ml flask containing 25 ml of seed medium and incubated at 28°C for 24 h, shaken at 250 r.p.m. Then, 8 ml of the culture was transferred into a 1-l Erlenmeyer flask containing 100 ml of the producing medium consisting of 0.5% glucose, 1.5% lactose, 2.0% cotton seed powder and 0.3% $CaCO_3$, pH 7.0, before sterilization. Fermentation was carried out at 28°C for 7 days on a rotary shaker at 250 r.p.m.

The fermentation broth (30 l) was centrifuged to separate mycelial cake and supernatant. The mycelial cake was extracted with MeOH (5.0 l) and the supernatant was applied to Diaion HP-20 (Mitsubishi Chemicals Company, Tokyo, Japan) and eluted with 95% EtOH. The combined MeOH soluble and the EtOH eluates were concentrated under reduced pressure to give 40 g of crude extract. The crude extract was subjected to a silica gel column (90×5 cm i.d.; Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) and eluted with $CHCl_3$/MeOH mixture (98:2–70:30, v/v). The active fraction showing cytotoxic activity against the tumor cell line A549 was collected and evaporated to give 830 mg of residue. The residue was chromatographed on Sephadex LH-20 column (GE Healthcare, Glies, UK) and eluted with EtOH. Then, the active fraction was further isolated by semi-preparative HPLC (Agilent1100, Agilent, Palo Alto, CA, USA; column: Zorbax SB-C18, 5 μm, 250×9.4 mm i.d.) using a solvent of CH_3CN/H_2O (75:25, v/v) with the flow rate of 1.5 ml min^{-1} at a room temperature to give compound **1** (t_R 9.3 min, 5.6 mg). The structure of compound **1** was elucidated by extensive spectroscopic methods. The UV spectra were obtained on a Varian CARY 300 BIO spectrophotometer (Agilent). IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer (ν_{max} in cm^{-1}) (Madison, WI, USA); ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Rheinstetten, Germany). Chemical shifts are reported in p.p.m. (δ), using residual CH_3COCH_3 (δ_H 2.05 p.p.m.; δ_C 29.0) as an internal standard, and coupling constants (J) in Hz. The HRESI-MS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA).

Compound **1** was isolated as a yellow amorphous powder with UV (EtOH) λ_{max} nm (log ε): 227 (4.53), 274 (4.97) and 379 (4.28) and m.p. 135–137°C. Its molecular formula was determined to be $C_{17}H_{18}O_4$ on the basis of HRESI-MS at m/z 287.1277 $[M+H]^+$ (calculated as 287.1278 for $C_{17}H_{19}O_4$) in conjunction with NMR data (Table 1). The IR spectrum of **1** showed a hydroxyl absorption at

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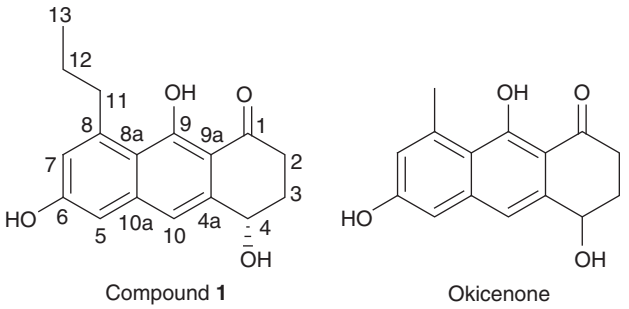

Figure 1 Structures of **1** and okicenone.

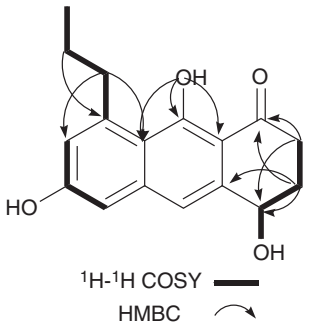

Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

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

No.	*^{13}C (mult)*	*^{1}H*	*No.*	*^{13}C (mult)*	*^{1}H*
1	204.0 (s)		8a	116.7 (s)	
2a	34.3 (t)	2.72 (1H, m)	9	166.4 (s)	
2b		2.93 (1H, m)	9a	108.7 (s)	
3a	31.3 (t)	2.14 (1H, m)	10	115.3 (d)	7.20 (1H, s)
3b		2.29 (1H, m)	10a	142.3 (s)	
4	67.2 (d)	4.91 (1H, m)	11	39.0 (t)	3.26 (2H, t, *J*=7.6 Hz)
4a	141.1 (s)		12	25.2 (t)	1.72 (2H, m)
5	108.7 (d)	7.00 (1H, d, *J*=2.5 Hz)	13	13.5 (q)	1.01 (3H, t, *J*=7.4 Hz)
6	158.7 (s)		4-OH		4.56 (1H, d, *J*=4.9 Hz)
7	119.8 (d)	6.91 (1H, d, *J*=2.5 Hz)	6-OH		9.11 (1H, s)
8	145.9 (s)		9-OH		15.5 (1H, s)

3375 cm^{-1}. ^{1}H NMR data of **1** revealed two aromatic meta-coupled doublet protons at δ_H 7.00 (1H, d, *J*=2.5 Hz) and 6.91 (1H, d, *J*=2.5 Hz) and one aromatic singlet signal at δ_H 7.20 (1H, s), one methyl triplet at δ_H 1.01 (3H, t, *J*=7.4 Hz), two phenolic hydroxyl singlets at δ_H 15.5 (1H, s) and δ_H 9.11 (1H, s), one hydroxyl doublet at δ_H 4.56 (1H, d, *J*=4.9 Hz), one oxymethine multiplet signal at δ_H 4.91 (1H, m), as well as eight protons at δ_H 1.72 (2H, m), 3.26 (2H, t), 2.14 (1H, m), 2.29 (1H, m), 2.72 (1H, m) and 2.93 (1H, m). The ^{13}C NMR and DEPT135 data (Table 1) of **1** exhibited one carbonyl carbon, three sp^2 methines, seven sp^2 quaternary carbons, one aliphatic oxymethine, four aliphatic methylenes and one methyl group. The sequence from δ_H 1.01 (H-13) to δ_H 3.26 (H-11) through δ_H 1.72 (H-12) in ^{1}H-^{1}H COSY spectrum indicated the presence of a *n*-propyl moiety. By detailed analysis of the NMR data of **1**, it was showed that **1** belongs to the 1,2,3,4-tretrahydro anthracene structure class with substituents of three hydroxyl groups, one *n*-propyl group and one carbonyl group (Figure 2). The correlated signals of δ_H 4.91 (H-4) and δ_H 2.14/2.29 (H-3a, 3b), δ_H 2.14/2.29 (H-3a, 3b) and δ_H 2.72/2.93 (H-2a, 2b) in the ^{1}H-^{1}H COSY spectrum further confirmed the above conclusion. The whole structure assignment was supported by the ^{1}H-^{1}H COSY, heteronuclear multiple quantum coherence and HMBC experiments. In the HMBC spectrum (Figure 2), the correlations from H_2-2 to a carbonyl carbon C-1 and a hydroxyl carbon C-4, and from H_2-3 to C-1, C-4 and C-4a were established. A downfield hydroxyl signal at δ_H 15.5 (9-OH) and the HMBC correlations between δ_H 15.5 (9-OH) and C-9, C-9a and C-8a showed the hydroxyl group substituted at C-9. The linkage of C-11 and C-8 was evident from the HMBC correlations from H_2-12 to C-8, and from H_2-11 to C-7, C-8 and C-8a. The meta-coupling protons of H-5 and H-7 suggested that the remaining hydroxyl was located at C-6. The structure of **1** was very similar to that of okicenone[8] and was replaced a methyl at C-8 in okicenone with a *n*-propyl group. Furthermore, compound **1** and aloesaponol III 8-methyl ether[9] have the same skeleton and one chiral center at C-4. The absolute stereochemistry of aloesaponol III 8-methyl ether has been solved by the extended benzoate chirality method as (*S*).[9] The attempt to differentiate the coupling constants between H-4 and H_2-3 was incomplete because of the multiplet of H-4 in the ^{1}H NMR spectrum. In further NMR experiment, the proton signal of 4-OH disappeared with the addition of D_2O and the peak of H-4 was displayed as a double doublet with two coupling constants (J_{aa}=8.1 Hz and J_{ae}=3.3 Hz). The two coupling constants between H-4 and H_2-3 revealed that the hydroxyl group at C-4 is quasi-equatorial, which is identical with that of aloesaponol III 8-methyl ether[9]. The optical rotation $[\alpha]_D^{25}$+23.1 (c 0.039, EtOH) of **1** was also similar to that of aloesaponol III 8-methyl ether. This finalizes the study and allows us to report the structure of **1** as 1-*oxo*-4(*S*),6,9-trihydroxy-8-*n*-propyl-1,2,3,4-tetrahydroanthracene.

We tested the inhibitory activity of compound **1** against the growth of human lung adenocarcinoma cell line A549 using the CCK-8 colorimetric method, as described in our previous paper.[10] The result showed that compound **1** dose-dependently inhibited the growth of A549 cells with an IC_{50} value of 12.5 μg ml^{-1}.

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