Components in Antineoplastic Actinomycete Strain (N2010-37) of Bottom Mud in Mangrove

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Abstract: **Objective** To study the antitumor components from an actinomycete strain (N2010-37) of bottom mud in Zhanjiang Mangrove, South China Sea. **Methods** The components were isolated and purified by chromatographic techniques and recrystallization, and the structures were identified by spectral methods together with physicochemical analyses. The antitumor effects of these components were tested *in vitro* by MTT method. **Results** Three compounds were identified including two anthrones and one novel lactone. They are (3*S*,4*R*,7*R*,8*R*,9*S*)-3,8-dihydroxy-4,7,9-trimethyl-2,6-cyclononanediolacetone (**1**), 2-hydroxy-1-methoxy-3-methylanthraquinone (**2**), and 1,6,8-thihydroxy-3-methyl-anthraquinone (**3**). **Conclusion** Compound **1** is a new compound, and compounds **1** and **3** show the favorable cytotoxic activities against human chronic granulocytic leukemia cell line K562 strain by MTT method *in vitro*.

Key words: actinomycete; antimycin; antitumor activities; mangrove; novel lactone

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Introduction

Marine microorganism has its special metabolism mechanism, since it lives in high-salt and high-pressure surroundings. Therefore, secondary metabolites of marine microorganism, one of the most important sources of antineoplastic drug, are valuable resources of novel compounds and active natural products. The active strains with antitumor effects were collected from bottom mud in Zhanjiang Mangrove and their secondary metabolites were tested *in vitro* by MTT method in this study. Marine actinomycete N2010-37 with a strong antitumor action was discovered by researchers in this study. In order to study antitumor components of marine actinomycete N2010-37, fermentations of actinomycete were studied. In the present paper, the isolation and structure elucidation of one new lactone, as well as two known compounds were described, and they are (3*S*,4*R*,7*R*,8*R*,9*S*)-3, 8-dihydroxy-4,7,9-trimethyl-2,6-cyclononanediolacetone (**1**), 2-hydroxy-1-methoxy-3-methylanthraquinone (**2**), and 1,6,8-thihydroxy-3-methylanthraquinone (**3**). Compounds **1** and **3** showed the favorable cytotoxic activities against human chronic granulocytic leukemia cell line K562 strain by MTT method *in vitro*.

Materials and methods

General

Melting points were determined on a Kofler Microscopic Melting Point Meter. The optical rotations were measured with a Jasco DIP—370 Digital Polarimeter in a 5 cm length cell. The ESI-MS (70 eV) was done on a Packard 1100 MSD Spectrometer. ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, and HMBC spectra were measured with a DRX—400 Spectrometer.

Actinomycetes materials

Antineoplastic actinomycete strain (N2010-37), collected from Zhanjiang Mangrove in 2010, was identified by Prof. YIN Wen-qing. The specimen was deposited in Chemistry Science and Technology School of Zhanjiang Normal University (Guangdong, China).

Cultivation conditions

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The required amount of bacterial strains from Gauze's medium No.1 which was cultivated at 28 ℃ for 4 d and then were inoculated to the seed medium (soluble starch 1%, glucose 2%, K_2HPO_4 0.05%, soybean powder 0.5%, proteose-peptone 1%, yeast extract 1%, beef extract 0.1%, and $CaCO_3$ 0.2% were prepared by sea water and the pH value was adjusted to 7.0), and then the seed culture solution was gained at 28 ℃ with shaking speed of 200 r/min for 3 d.

The optimal fermentation conditions were as follows: 500-mL Erlenmeyer flasks (150) with the seeds medium (100 mL, pH 7.0) separately, 10% inoculum size of the seed culture solution, temperature 28 ℃, shaking speed 200 r/min, and 6-day cultivation.

Extraction and isolation

Mycelium was soaked with 95% EtOH for three times. The extracted liquid was combined and concentrated in vacuum. A suspension of this crude extract (121 g) in distilled water was extracted with petroleum ether, EtOAc, and *n*-BuOH, respectively to yield petroleum ether extract (5.1 g), EtOAc extract (28.0 g), dried *n*-BuOH extract (19.2 g), and an aqueous residue. The EtOAc extract was subjected to silica gel column chromatography, eluted with petroleum ether-EtOAc (1:0→0:1) and $CHCl_3$-MeOH (1:30→1:1) to provide Frs. 1－5. Frs. 2 and 5 showed the favorable cytotoxic activity against human chronic granulocytic leukemia cell line K562 strain by MTT method *in vitro*. Fr. 2 (517 mg) was subjected to repeated column chromatography on silica gel, eluted with petroleum ether-EtOAc (1:10→1:1), then further separated by Sephadex LH-20 column chromatography with CH_3OH-$CHCl_3$ (1:1) to yield compounds **1** (14 mg), **2** (11 mg), and **3** (17 mg).

Results and discussion

Compound **1**: white crystal; mp 113－116 ℃; $[\alpha]_D^{20}$ +79.4° (*c* 0.5, $CHCl_3$). The molecular formula is $C_{10}H_{16}O_6$, determined by ESI-MS (231 $[M-H]^+$), HR-ESI-MS *m/z*: 233.2126 $[M+H]^+$ (cal. 233.2129), and confirmed by ^{1}H-NMR and ^{13}C-NMR data.

The ^{1}H-NMR data (Table 1) indicated four oxymethylidyne protons at δ 4.78 (1H, d, J = 5.2 Hz), 5.09 (1H, m, J = 5.2, 7.0 Hz), 4.45 (1H, t, J = 10.3 Hz), and 4.98 (1H, m, J = 7.0, 10.3 Hz), and three methyl groups at δ 1.39 (3H, d, J = 7.0 Hz), 1.31 (3H, d, J = 7.0 Hz), and 1.43 (3H, d, J = 7.1 Hz). The 10 signals of ^{13}C-NMR spectrum and a DEPT experiment of compound **1** showed the corresponding carbon signals as well as two carbonyls at δ 172.51 and 176.60, three methyl carbons at δ 13.79, 13.31, and 15.48, and four oxygen-bearing tertiary carbons at δ 71.67, 73.84, 71.34, and 77.27. According to the degree of unsaturation of compound **1**, this compound contains a lactone ring.

Table 1 NMR data of compound 1 and antimycin A_7 (in CD_3Cl, 400/100 MHz)

Position	Compound **1**		Antimycin A_7 δ_C
	δ_H	δ_C	
1			
2		172.51	170.10
3	4.78 (1H, d, J = 5.2 Hz)	71.67	53.82
4	5.09 (1H, m, J = 5.2, 7.0 Hz)	73.84	70.94
5			
6		176.60	172.93
7	3.07 (1H, m, J = 7.1, 10.0 Hz)	44.92	50.35
8	4.45 (1H, t, J = 10.3 Hz)	71.34	75.39
9	4.98 (1H, m, J = 7.0, 10.3 Hz)	77.27	74.92
10	1.39 (3H, d, J = 7.0 Hz)	13.79	14.97
11	1.31 (3H, d, J = 7.0 Hz)	13.31	
12	1.43 (3H, d, J = 7.1 Hz)	15.48	17.82

Two fragments (A and B) were confirmed by ^{1}H-^{1}H COSY correlations (Figs. 1 and 2). The ^{1}H-NMR and ^{13}C-NMR data of compound **1** were very similar to those of antimycin A_7 (Barrow *et al*, 1997) except for the substituents at C-3, C-7, and C-8 positions of compound **1**. In HMBC spectrum, the correlations between the proton of δ 4.78 (1H, d, J = 5.2 Hz) and carbon signals at δ 73.84, 15.48, and 172.51 and the correlations between the proton of δ 5.09 (1H, m, J = 5.2, 7.0 Hz) and carbon signal at δ 71.67, 15.48, 172.1, and 176.60 together could indicate that the carbonyl carbon (δ 172.51) linked to the carbon atom (δ 71.67). The correlations between the proton of δ 4.98 (1H, m, J = 7.0, 10.3 Hz) and carbon signals at δ 172.51, 13.79, 71.34, and 44.92, the correlations between the proton of δ 3.07 (1H, m, J = 7.1, 10.0 Hz) and carbon signals at δ 176.60, 13.31, 71.34, and 77.27, and the correlations between the proton of δ 4.45 (1H, t, J = 10.3 Hz) and

carbon signals at δ 44.92, 176.60, 13.31, 77.27, and 13.79 together could indicate that the carbonyl carbon (δ 176.60) linked to the carbon atom (δ 44.92). So far, fragments A and B have been joined and the Key HMBC and ^{1}H-^{1}HCOSY correlations of compound **1** has been determined (Fig. 2).

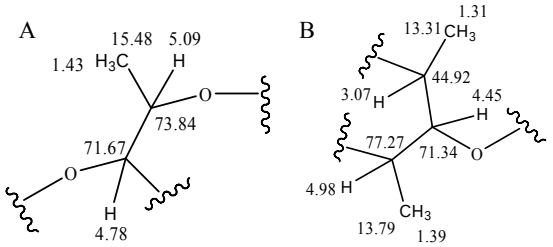

Fig. 1 Fragments A and B of compound 1

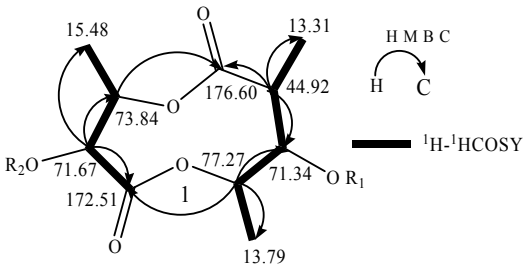

Fig. 2 Key HMBC and ^{1}H-^{1}HCOSY correlations of compound 1

In ROESY spectrum, the protons of δ 4.45 (1H, t, J = 10.3 Hz), 1.39 (3H, d, J = 7.0 Hz), 1.31 (3H, d, J = 7.0 Hz), 5.09 (1H, m, J = 5.2, 7.0 Hz), and 4.78 (1H, d, J = 5.2 Hz) have correlated signals. The correlations between the protons of δ 3.07 (1H, m, J = 7.1, 10.0 Hz) and 4.98 (1H, m, J = 7.0, 10.3 Hz) indicated two methyl groups (δ 13.7 and 13.3) were very close in space. The absolute stereochemistry of compound **1** was determined by comparing with antimycin A_7. ^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** were very similar to those of antimycin A_7. To be more specific, the specific rotation of compound **1** was $[\alpha]_D^{20}$ +79.4° (c 0.5, $CHCl_3$) and the specific rotation of the antimycin A_7 was $[\alpha]_D^{20}$ +72° (c 0.5, $CHCl_3$). Therefore, the structure of compound **1** was confirmed to be (3*S*,4*R*,7*R*,8*R*,9*S*)-3,8-dihydroxy-4,7,9-trimethyl-2,6-cyclononanediolacetone (Fig. 3).

Compound **2**, yellow crystal; mp 221－224 ℃; ESI-MS *m/z*: 268[M]$^+$; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ: 8.24 (1H, m, H-5), 8.18 (1H, m, H-8), 7.89 (1H, m, H-6), 7.80 (1H, m, H-7), 7.78 (1H, s, H-4), 3.86 (3H, s, OCH_3), 6.44 (1H, 2-OH), 2.31 (3H, s, CH_3); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ: 147.8 (C-1), 156.5 (C-2), 133.7 (C-3), 127.1 (C-4), 127.4 (C-5), 134.6 (C-6), 134.1 (C-7), 127.3 (C-8), 184.4 (C-9), 183.2 (C-10), 136.0 (C-8a), 132.9 (C-5a), 124.5 (C-4a), 121.0 (C-9b), 62.0 (-OCH_3), 17.1 (CH_3). These data were consistent with those of the reference (Li *et al*, 2010).

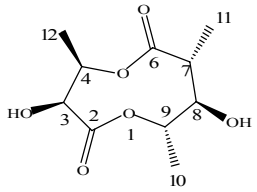

Fig. 3 Structure of compound 1

Compound **3**, yellow crystal; ESI-MS *m/z*: 270[M]$^+$; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ: 12.8 (1H, s, 1-OH), 12.2 (1H, s, 8-OH), 9.82 (1H, br s, 6-OH), 7.56 (1H, d, J = 2.0 Hz, H-4), 7.24 (1H, d, J = 2.0 Hz, H-5), 7.13 (1H, d, J = 1.5 Hz, H-2), 6.66 (1H, d, J = 1.5 Hz, H-7), 2.36 (3H, s); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ: 191.3 (C-9), 182.8 (C-10), 166.5 (C-1), 166.2 (C-8), 162.7 (C-6), 148.6 (C-3), 136.1 (C-5b), 134.3 (C-4a), 124.7 (C-2), 121.2 (C-4), 114.4 (C-1a), 110.6 (C-8b), 109.5 (C-7), 108.7 (C-5), 22.1 (CH_3). These data were consistent with those of the reference (Wang, Li, and Li, 2010).

MTT method was applied to screening the antitumor activities of actinomycetes and their secondary metabolites. Three compounds showed cytotoxicity activity against human chronic granulocytic leukemia cell line K562 strain *in vitro*. The IC_{50} values of the three compounds were 1.36, 19.73, and 6.14 μmol/L, respectively, and for C-DDP it is less than 0.1. Compound **1**, which is deserved to be further investigated, shows favorable cytotoxic activity against human chronic granulocytic leukemia cell line K562 strain.

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