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SECONDARY METABOLITES PRODUCED BY MARINE BACTERIRUM *MICROMONOSPORA* sp. G019

Vu Thi Quyen[1], Tran Van Hieu[1], Le Cong Vinh[1], Doan Thi Mai Huong[1], Le Thi Hong Minh[1], Brian Murphy[2], Chau Van Minh[1], Pham Van Cuong[1*]

[1]*Institue of Marine Biochemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[2]*University of Illinois at Chicago, Chicago, Illinois, USA*

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Abstract

Nine compounds were isolated and characterized from the culture broth of the marine bacteria *Micromonospora* sp.G019 strain, which was isolated from sediment collecting at Ha Long Bay. Their structures were determined by spectroscopic analysis including MS and 2D NMR, as well as by comparison with reported data in the literature.

Keywords. *Micromonospora* sp., culture broth nitrogen-containing constituents, marine microorganisms.

1. INTRODUCTION

Marine microorganisms have been the important study in recent years because of production of novel metabolites which represent various biological properties such as antiviral, antitumor or antimicrobial activities. These secondary metabolites serve as model systems in discovery of new drugs [1, 2]. Metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms [3].

During our biological screening program, the extract of *Micromonospora* sp. G019 had inhibition activity against *Mycobacterium tuberculosis*. The present paper deals with the fractionation of the EtOAc extract of *Micromonospora* sp. G019 isolated from sediment of the Halong Bay. The purification led to the isolation and structural characterization of nine compounds **1**-**9**.

2. EXPERIMENTAL

2.1. General Experiment procedures

ESIMS were recorded on an Agilent 1100 LC-MSD Trap spectrometer. The ^{13}C NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 125.76 MHz, and ^{1}H and 2D NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 500.13 MHz. ^{1}H chemical shifts were referenced to $CHCl_3$ and CD_3OD at 7.26 and 3.33 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak of $CDCl_3$ at 77.0 and 49.0 ppm for CD_3OD.

Thin-layer chromatography (TLC) used TLC silica gel Merk 60 F_{254}. Column chromatography (CC) were carried out on silica gel 40-63 μm or Sephadex LH-20.

2.2. Bacteria isolation and fermentation

The marine sediment was collected in Ha Long Bay – Quang Ninh on July 2012. The sediment sample (1 g) was added to the 10 mL of sterile sea water in a conical flask. The flask was agitated for about one hour. The marine sediment was filtered and the filtrate was serially diluted to obtain 10^{-1} to 10^{-7} dilutions using the sterilized sea water. An aliquot of 100 μL of each dilution was spread on the media. Different media like Starch Casein Agar (SCA), Glycerol Asparagine Agar (GA Agar), Humic acid-B vitamin agar (HV Agar) and Glucose yeast malt extract agar (GYM) were used for isolation of actinomycetes. The media containing 50 % of sterile sea water were supplemented with rifampicin (5 μg/mL) and nystatin (25 μg/mL) (Himedia Mumbai) to inhibit bacterial and fungal contamination, respectively. The petriplates were incubated upto 3 weeks at 28 °C. The isolated discrete colonies were observed and used for identification. The fermentation was carried out in 3 L flask using a modification of the published method [4].

2.3. Extraction and isolation

Culture broth (30 L) of *Micromonospora sp.* G019 strain was extracted with EtOAc (5 x 15 L). The solvent was concentrated under reduced pressure. The residue (4.0 g) was subjected to a silica gel column chromatography (CC), eluted with CH_2Cl_2/MeOH mixture (0 to 100 % MeOH in CH_2Cl_2) to give 5 fractions. Fraction 2 (350 mg) was purified on silica gel CC, eluting with CH_2Cl_2/EtOAc gradient to obtain five subfractions. Subfraction 1 (36 mg) was separated by CC on Sephadex LH-20 to give compound **1** (6 mg). Subfraction 2 (43 mg) was subjected to CC on Sephadex LH-20 eluted with MeOH to yield compound **9** (5 mg). Subfraction 3 (54 mg) was chromatographed by on silica gel column, eluting with a mixture of CH_2Cl_2/acetone (9/1), followed by CC on Sephadex to obtain compound **2** (7 mg). Fraction 3 (800 mg) was purified by CC on silica gel using CH_2Cl_2/EtOH gradient affording four subfractions. Subfraction 1 (200 mg) was purified on a silica gel CC (CH_2Cl_2/EtOH gradient) following by CC on Sephadex (MeOH) to furnish compounds **3** (8 mg) and **6** (12 mg). Subfraction 2 (250 mg) was separated by CC on silica gel (CH_2Cl_2/EtOH gradient) to yield compounds **7** (4 mg) and **5** (3 mg). Fraction 4 (900 mg) was purified on a silica gel CC (CH_2Cl_2/MeOH gradient) yielding 4 subfractions. Subfraction 4 (200 mg) was subjected to a CC on silica gel (CH_2Cl_2/acetone gradient) to give compounds **8** (9 mg) and **4** (12 mg).

N-(4-hydroxyphenylethyl)propionamide (1): White solides; ESI-MS (*m/z*): 194 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 1.11 (3H, t, J = 7.5 Hz, CH_3), 2.18 (2H, q, J = 7.5 Hz, $\underline{CH_2}CH_3$), 2.70 (2H, t, J = 7.5 Hz, $\underline{CH_2}$-Ar), 3.32 (2H, m, $\underline{CH_2}$-N), 6.73 (2H, d, J = 7.0 Hz, H-3, 5), 7.04 (2H, d, J = 7.0 Hz, H-2, 6); ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 10.5 (CH_3), 30.2 ($\underline{CH_2}CH_3$), 35.7 ($\underline{CH_2}$-Ar), 42.2 ($\underline{CH_2}$-NH), 116.2 (CH-2, 6), 130.7 (CH-3,5), 131.3 (C-4), 156.9 (C-1), 177.0 (C=O).

N-(4-hydroxyphenylethyl)acetamide (2): White needles; ESI-MS (*m/z*): 178.1 $[M-H]^-$. ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 1.91 (3H, s, $\underline{CH_3}$C=O), 2.69 (2H, t, J = 7.5 Hz, $\underline{CH_2}$-Ar), 3.35 (2H, m, $\underline{CH_2}$-N), 6.75 (2H, d, J = 7.0 Hz, H-3, 5), 7.02 (2H, d, J = 7.0 Hz, H-2, 6); ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 21.9 (CH_3), 34.1 ($\underline{CH_2}$-Ar), 40.7 ($\underline{CH_2}$-NH), 114.9 (CH-3, 5), 129.2 (CH-2,6), 129.6 (C-1), 154.9 (C-4), 171.3 (C=O).

2'-deoxythymidine (3): Light yellow needles; ESI-MS (*m/z*): 243.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm) 1.90 (3H, s, CH_3-7), 2.27 (2H, m, H-2'), 3.75 (1H, dd, J = 3.5, 12.0 Hz, H_a-5'), 3.82 (1H, dd, J = 3.0, 12.0 Hz, H_b-5'), 3.93 (1H, m, H-4'), 4.42 (1H, m, H-3'), 6.30 (1H, t, J = 7.0 Hz, H-1'), 7.83 (1H, s, H-6); ^{13}C- NMR (125 MHz, CD_3OD) δ(ppm) 12.4 (C-7), 41.2 (C-2'), 62.8 (C-5'), 72.2 (C-3'), 86.3 (C-1'), 88.8 (C-4'), 111.5 (C-5), 138.2 (C-6), 152.4 (C-2), 166.4 (C-4).

2'-deoxyadenosine (4): Amorphous solid; ESI-MS (*m/z*): 252.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 2.43 (1H, m, H_a-2'), 2.84 (1H, m, H_b-2'), 3.76 (1H, dd, J = 3.5, 12.5 Hz, H_a-5'), 3.87 (1H, dd, J = 3.0, 12.5 Hz, H- H_b-5'), 4.09 (1H, m, H-3'), 4.60 (1H, m, H-4'), 6.45 (1H, dd, J = 6.0, 8.0 Hz, H-1'), 8.20 (1H, s, H-8), 8.34 (1H, m, H-2); ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 41.6 (C-2'), 63.6 (C-5'), 73.1 (C-3'), 87.1 (C-4'), 89.9 (C-1'), 120.8 (C-5), 141.5 (C-8), 149.9 (C-4), 153.6 (C-2), 157.5 (C-6).

Adenosine (**5**): White solid; ESI-MS (*m/z*): 268.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 3.77 (1H, dd, J = 3.0, 12.5 Hz, H_a-5'), 3.91 (1H, dd, J = 2.5, 12.5 Hz, H- H_b-5'), 4.19 (1H, m, H-4'), 4.35 (1H, dd, J = 3.0, 5.0 Hz, H-3'), 4.76 (1H, dd, J = 5.0, 6.5 Hz, H-2'), 5.99 (1H, d, J = 6.0 Hz, H-1'), 8.20 (1H, s, H-8), 8.32 (1H, s, H-2).

2'-deoxyuridine (6): Light yellow needle; ESI-MS (*m/z*): 229.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ(ppm) 2.23 (1H, m, H_a-2'), 2.30 (1H, m, H_b-2'), 3.74 (1H, dd, J = 3.5, 12.0 Hz, H_a-5'), 3.80 (1H, dd, J = 3.5, 12.0 Hz, H- H_b-5'), 3.95 (1H, m, H-4'), 4.41 (1H, m, H-3'), 5.72 (1H, d, J = 8.0 Hz, H-5), 6.28 (1H, t, J = 6.5 Hz, H-1'), 7.99 (1H, d, J = 8.0 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 41.3 (C-2'), 62.8 (C-5'), 72.2 (C-3'), 86.6 (C-4'), 88.9 (C-1'), 102.6 (C-5), 142.5 (C-6), 152.2 (C-2), 166.6 (C-4).

Uridine (7): White solids; ESI-MS (*m/z*): 245.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 3.75 (1H, dd, J = 3.0, 12.5 Hz, H_a-5'), 3.86 (1H, dd, J = 2.5, 12.5 Hz, H- H_b-5'), 4.03 (1H, m, H-4'), 4.17 (1H, m, H-3'), 4.20 (1H, m, H-2'), 5.72 (1H, d, J = 8.0 Hz, H-5), 5.92 (1H, d, J = 4.5 Hz, H-1'), 8.02 (1H, d, J = 8.0 Hz, H-6).

Adenine (8): White solids; ESI-MS (*m/z*): 136.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 8.13 (1H, s, H-8), 8.20 (1H, s, H-2).

Thymine (9): White needles; ESI-MS (*m/z*): 127.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 1.86 (3H, s, CH_3), 7.06 (1H, s, H-6).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white solid. The

ESI mass spectrum of **1** presented a base peak at *m/z* 194 $[M+H]^+$. The ^{1}H-NMR spectrum showed signals characteristic of an A_2B_2 aromatic system [δ_H 6.73 (2H, d, *J* = 7.0 Hz, H-3, H-5), 7.04 (2H, d, *J* = 7.0 Hz, H-2, H-6)], three methylenes at δ_H 2.18 (q, *J* = 7.5 Hz, $\underline{CH_2}CH_3$), 2.70 (t, *J* = 7.5 Hz, $\underline{CH_2}$-Ar), 3.32 (m, -$\underline{CH_2}$-N) and a methyl group at δ_H 1.11 (t, *J* = 7.5 Hz, $CH_2\underline{CH_3}$). The ^{13}C-NMR and DEPT spectra of **1** presented the groups observed in the ^{1}H-NMR spectrum with additional signals of a carbonyl at δ_C 177.0 and two sp^2 quaternary cacbons at δ_C 131.3 (C-1) and 156.9 (C-4). The chemical shift of C-1 at δ_C 156.9 indicated its linkage to oxygen while those of the methylene at δ_C 40.7 and δ_H 3.32 suggested its connection to nitrogen. Analyses of 2D NMR spectra established the structure of **1** as *N*-(4-hydroxyphenylethyl)propionamide. The presence of amide group was defined by the correlation of the carbonyl carbon at δ_C 177.0 (C-4') with protons of CH_2-2' (δ_H 3.32) in the HMBC spectrum. The NMR data of **1** were in agreement with those previously reported [5].

Compound **2** was obtained as a white needle. The ESI mass spectrum (negative) of **2** showed a pseudomolecular ion peak at *m/z* 178.1 $[M-H]^-$. The NMR data of compound **2** showed similar spectroscopic features as compound **1**. The differences between these two compounds were the presence of an acetyl instead of the propionyl group. The NMR data of **2** were consistent with those previously reported for N-(4-hydroxyphenylethyl)-acetamide [6, 7]. This compound was found to exhibit the aldose reductase inhibitory activity with an IC_{50} value of 1.6×10^{-4} M [7].

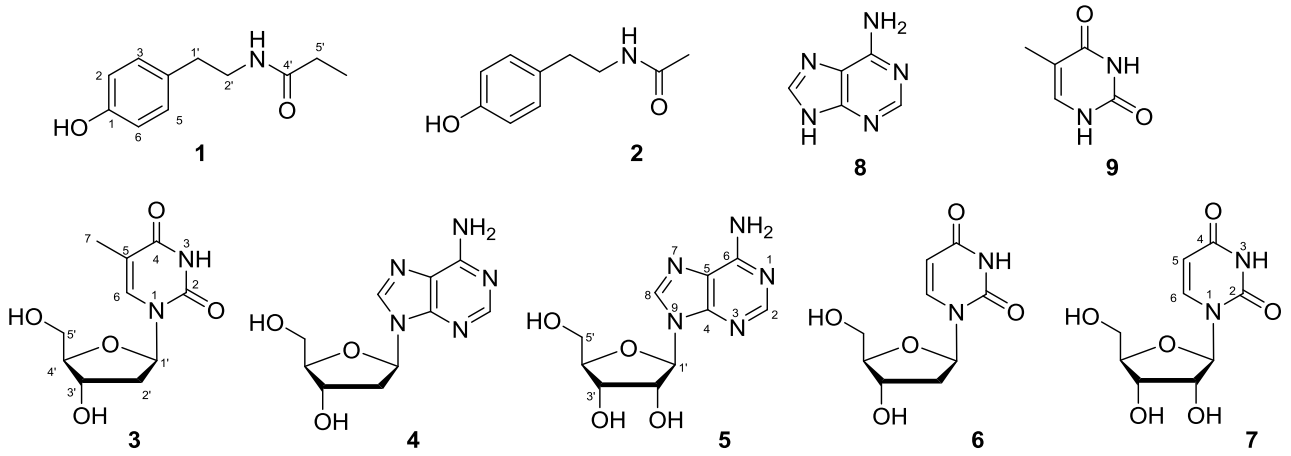

Figure 1: Compounds isolated from the broth culture of *Micromonospora* sp. G019

ESI mass spectrum of **3** presented a pseudomolecular ion peak at *m/z* 243.1 $[M+H]^+$. In the ^{1}H-NMR spectrum, compound **3** displayed signals of a olefin proton at δ_H 7.83 (s, H-6), a methyl group at δ_H 1.90 (s, CH_3-7) and a set of aliphatic protons at δ_H 2.27 (2H, m, H-2'), 3.75 (1H, dd, *J* = 3.5, 12.0 Hz, H_a-5'), 3.82 (1H, dd, *J* = 3.0, 12.0 Hz, H_b-5'), 3.93 (1H, m, H-4'), 4.42 (1H, m, H-3'), 6.30 (1H, t, *J* = 7.0 Hz, H-1'). The ^{13}C-NMR and DEPT spectra of **3** showed the presence of 10 carbon atoms, including two carbonyl groups at δ_C 152.4 (C-2), 166.4 (C-4), a sp^2 methine at δ_C 138.2 (C-6), a quartenary carbon at δ_C 111.5 (C-5), a methyl group at δ_C 12.4 (CH_3-7), two methylenes at δ_C 41.2 (C-2') and 62.8 (C-5'), and three methines at δ_C 73.1 (C-3'), 87.1 (C-4') and 89.9 (C-1'). The chemical shifts of CH-3', CH-4' and CH_2-5' indicated their bonding to oxygens, while those of CH-1' suggesting its connection to both nitrogen and oxygen. Complete analysis of NMR spectra and comparison with the data reported in the literature allowed determing the structure of **3** to be 2'-deoxy-thymidine [8].

Compound **4** was obtained as amorphous solid. The ESI mass spectrum of **4** showed a pseudomolecular ion peak at *m/z* 252.1 $[M+H]^+$. The 1D-NMR spectra (^{1}H and ^{13}C) of compound **4** displayed signals of the 2-deoxy-arabionoside moiety as in the structure of **3**. However, in the aromatic region, compound **4** demonstrated signals corresponding to an adenine moiety [δ_C 120.8 (C-5), δ_C 141.5, δ_H 8.20 (CH-8), δ_C 149.9 (C-4), δ_C 153.6 δ_H 8.34 (CH-2) and δ_C 157.5 (C-6)]. This observation suggesed **4** being a nucleoside. Detailed analyses of the NMR spectra, the structure of **4** was identified as 2'-deoxyadenosine [9].

Compound **5** was obtained as a white solid. The ESI mass spectrum of **5** showed a pseudomolecular ion peak at *m/z* 268.1 $[M+H]^+$. The 1D NMR spectra of **5** presented signals close to those of **4**, except for presence of an oxymethine in **5** instead of the methylene in **4**. Furthermore, the proton H-1'appeared as a doublet in the ^{1}H NMR spectrum of **5**. Whereas, this proton was a doublet of doublet in the ^{1}H NMR spectrum of **4**. This observation strongly suggested that **5** was adenosine. The NMR data were in agreement with the reported values for adenosine [10].

Compound **6** was obtained as a light yellow needle. The ESI mass spectrum of **5** showed a pseudomolecular ion peak at *m/z* 229.1 $[M+H]^+$. The 1D NMR spectra of **6** presented signals of the 2-deoxy-arabionoside moiety as in the structures of **3** and **4**. In the aromatic region of the ^{1}H-NMR of **6**, signals of two doublets were noted at 5.72 (1H, d, J = 8.0 Hz, H-5) and 7.99 (1H, d, J = 8.0 Hz, H-6). The DEPT spectrum of **6** showed additional signals of two carbonyl carbons at 152.2 (C-2) and 166.6 (C-4). This data suggested the presence of uracil moiety. Complete analyses of NMR data indicated that compound **6** was 2'-deoxyuridine [9].

ESI mass spectrum of **7** showed a base peak at *m/z* 245.1 $[M+H]^+$. The ^{1}H-NMR data of **7** showed similar spectroscopic features as **6**. The differences between these two compounds were the presence of the arabionoside sugar moiety instead of the 2-deoxy-arabionoside in **6** which was indicated by the absence of a methylene NMR signals and the appearance of an oxymethine group. Thus, compound **7** was identified as uridine [11].

Compounds **8** and **9** were also characterized as adenine [11] and thymine [13], respectively. Their NMR data were in agreement with those reported in the literature.

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Corresponding author: **Pham Van Cuong**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: phamvc@imbc.vast.vn.