SECONDARY METABOLITES PRODUCED BY MARINE ACTINOMYCETESTREPTOMYCESSP. G246

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Abstract. In a recent study, we described two new lavandulylated flavonoids, along with eight known compounds from the culture broth of a Streptomyces sp. (strain G246), isolated from the sponge Halichondria panicea, collected in the sea of Son Tra peninsula (Da Nang). A comparison study was conducted to differentiate between solid and liquid fermentation technique for secondary metabolites production of strain G246. In this paper, we report the isolation and structural characterization of nine secondary metabolites (1-9) from strain G246 by solid state fermentation. Compound 2 was the only one similarity between these fermentation techniques.

Keywords: Streptomyces, marine microorganism, actinomycete, solid state fermentation, secondary metabolites.

Classification numbers: 1.1.1, 1.5.3.

1. INTRODUCTION

Fermentation of micro-organisms has been widely used for the production of antibiotics and other bioactive compounds. The development of techniques such as solid state fermentation and liquid fermentation led to industrial-level for production of biological active compounds [1, 2]. At laboratory scale, in general, liquid fermentation is used more often for researches on secondary metabolite productions [3, 4]. Recently, we described two new lavandumulated flavonoids, along with eight known compounds from the culture broth of a Streptomyces sp. (strain G246) isolated from sponge Halichondria panicea, collected in the sea of Son Tra peninsula (Da Nang) [4]. In this paper, we report the isolation and structural characterization of 9 compounds including spirotry prostatins A (1), cyclo-(Pro-Met) (2), phenol A acid (3), 3,4-
dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (4), cyclo-(Pro-Gly) (5), xanthone (6), guanosine (7), 2′-deoxyadenosine (8), adenosine (9) from *Streptomyces* sp. (strain G246) by solid state fermentation technique (Figure 1). The study aim was to delineate the differences between solid state and liquid fermentation for secondary metabolites production of the strain G246. Indeed, there was only one similarity, compound (2), found from these fermentation techniques.

![Figure 1. Nine compounds (1-9) from marine *Streptomyces* sp. G246 isolated by solid state fermentation technique.](image)

2. MATERIAL AND METHODS

2.1. General experimental procedures

The ESI-MS were recorded on an Agilent 1100 LC-MSD Trap spectrometer. NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 125.76 MHz for $^{13}$C NMR, and at 500.13 MHz for $^1$H-NMR. The $^1$H chemical shifts were referenced to CDCl$_3$, and CD$_3$OD at $\delta^H$ 7.27 and 3.31 ppm, respectively, while the $^{13}$C chemical shifts were referenced to the central peak of at $\delta^C$ 77.1 (CDCl$_3$) and 49.0 (CD$_3$OD). For HMBC experiments the delay ($1/2$J) was 70 ms. TLC silica gel Merck 60 F$_{254}$ was used as thin layer chromatography. Column chromatography (CC) was carried out using silica gel 40 - 63 µm, YMC RP-18 (30-50 µm) or Sephadex LH-20.

2.2. Marine materials

The strain G246 was isolated from a sample of sponge *Halichondria panicea* collected in the sea of Son Tra island (Da Nang, Viet Nam), August 2016. Marine sample was identified by Prof. Do Cong Thung of Institute of Marine Environment and Resources - Vietnam Academy of Science and Technology (VAST). Voucher specimens were deposited at the Institute of Marine Environment and Resources, Hai Phong, Viet Nam. Strain G246 was later identified belonging to *Streptomyces* genus based on its 16S rRNA gene sequence (GeneBank Access Code: MG917689) [4].
2.3. Solid state fermentation

Strain G246 was firstly activated and inoculated into 1 L of A1 medium, pH 7.0, comprising: soluble starch: 5 g, yeast extract: 2 g, peptone: 1 g and instant ocean: 30 g in 1 L of distilled water. After 7 days of incubation at 28°C with agitation of 200 rpm, the culture broth was spread on 50 flasks containing 1L of high-nutrient solid medium A1+ (soluble starch: 10 g/L, yeast extract: 4 g/L, peptone: 2 g/L, instant ocean: 30 g/L, CaCO3: 1 g/L, agar: 15 g/L, and water: 1 L). The fermentation was incubated in an incubator at 28°C and harvested on the tenth day.

2.4. Extraction and isolation

The culture agar of Streptomycesp. G246 strain was minced, and extracted successively with ethyl acetate and methanol (three times × 30 minute each) at 40°C over sonication, and concentrated under reduced pressure to obtain corresponding extracts: ethyl acetate (EG246, 17.2 g) and methanol (MG246, 20.2 g). There were no differences between EG246 and MG246 after checking with TLC, so they were combined to obtain a single extract (S246). The S246 was separated by a reversed phase (RP) C18 column chromatography (CC), eluting with MeOH/H2O gradient to provide eleven fractions (F1-F11). Fraction F4 (1.1 g) was further divided into four sub-fractions (F4.1–F4.4) by Sephadex LH-20 column using MeOH as eluent.

Purification of the sub-fraction F4.4 (100 mg) by Sephadex LH-20 column using MeOH to give compound 7 (10 mg). Separation of fraction F10 (0.61 g) by Sephadex LH-20 column using MeOH to obtain six sub-fractions (F10.1–F10.6). The sub-fraction F10.4 (100 mg) was subjected to Sephadex LH-20 column using MeOH as mobile phase to give compound 8 (15 mg). Sub-fraction F10.5 (200 mg) was separated on Sephadex LH-20 column, eluting by MeOH to give compound 9 (11 mg). Fraction F11 (6.2 g) was submitted to a silica gel CC, eluted with CH2Cl2/MeOH gradient to yield four sub-fractions (F11.1-F11.4). Sub-fraction F11.2 (1.2 g) was further purified by silica gel CC, eluted with CH2Cl2/EtOAc gradient to give five sub-fractions (F11.2.1-F11.2.5). Sub-fraction F11.2.3 (400 mg) was further separated on Sephadex LH-20 column using MeOH as eluent to give compound 5 (17 mg). Sub-fraction F11.2.4 (200 mg) was purified on silica gel CC, eluted with CH2Cl2/EtOAc gradient to give compound 4 (2.7 mg). Sub-fraction F11.2.5 (100 mg) was subjected to CC on silica gel, eluted with CH2Cl2/acetone gradient to provide compound 1 (8.5 mg) and compound 6 (5 mg). Sub-fraction F11.3 (2.0 g) was chromatographed on silica gel column, eluted with CH2Cl2/MeOH gradient (0-50% MeOH) to give five sub-fractions (F11.3.1.1-F11.3.5). Purification of the sub-fraction F11.3.4 (500 mg) by silica gel CC, eluted with CH2Cl2/acetone gradient to give compound 2 (7.0 mg). Sub-fraction F11.3.5 (200 mg) was further separated on silica gel CC, eluted with CH2Cl2/MeOH gradient to give compound 3 (4.7 mg).

**Spirotryprostatin A (1):** White solid; [α]D20 -29.5 (c 0.1 MeOH). ESI-MS (m/z): 396 [M+H]+. 1H-NMR (500 MHz, CD3OD): δH 1.20 (3H, s, H-21); 1.67 (3H, s, H-22); 2.03 (1H, m, Hα-14); 2.14 (1H, m, Hβ-14); 2.15 (1H, m, Hα-13); 2.32 (1H, m, Hβ-13); 2.57 (1H, dd, J = 7.0, 13.5 Hz, Hα-8); 2.61 (1H, dd, J = 10.5, 13.5 Hz, Hβ-8); 3.57 (2H, m, H-15); 3.80 (OCH3); 4.45 (1H, t, J = 8.0 Hz, H-12); 4.73 (1H, d, J = 9.0 Hz, H-18); 5.04 (1H, dd, J = 10.0, 7.5 Hz, H-9); 5.09 (1H, d, J = 9.0 Hz, H-19); 6.51 (1H, d, J = 2.0 Hz, H-7); 6.56 (1H, dd, J = 2.0, 7.5 Hz, H-5); 7.03 (1H, d, J = 7.5 Hz, H-4). 13C-NMR (125 MHz, CD3OD); δC 18.09 (C-22), 24.44 (C-14), 25.54 (C-21), 28.52 (C-13), 35.33 (C-8), 46.18 (C-15), 55.93 (OCH3), 57.11 (C-3), 59.81 (C-9), 61.83 (C-18), 62.26 (C-12), 97.78 (C-7), 107.77 (C-5), 120.13 (C-3a), 122.67 (C-19), 128.20 (C-4), 139.10 (C-20), 144.35 (C-7a), 162.16 (C-6), 168.94 (C-17), 169.45 (C-11), 183.20 (C-2).
Cyclo-(Pro-Met) (2): White solid, ESI-MS (m/z): 229 [M+H]+. 1H-NMR (500 MHz, CDCl3): δH (ppm) 1.90 (1H, m, H-4), 2.01 (2H, m, Hδ-4,Hδ-5), 2.11 (1H, m, Hδ-5), 2.12 (3H, s, SCH3), 2.37 (2H, m, CH-2), 2.68 (2H, t, J = 7.0 Hz, CH-11), 3.54 (1H, m, H-3), 3.60 (1H, m, Hδ-3), 4.10 (1H, t, J = 8.0 Hz, H-6), 4.20 (1H, m, H-9), 6.77 (1H, br, s, NH). 13C-NMR (125 MHz, CDCl3): δC (ppm) 15.30 (SCH3), 22.67 (C-4), 28.21 (C-5), 28.88 (C-11), 30.27 (C-10), 45.49 (C-3), 54.65 (C-9), 59.03 (C-6), 165.40 (C=O), 170.33 (C=O).

Phenol Acid (3): White solid, [α]20D = -45.5 (c 0.1 MeOH). HR-ESI-MS (m/z): 503.1881 [2M+Na]+ (Calcd. for C22H19O10Na, m/z 503.1893). 1H-NMR (500 MHz, CD3OD): δH 1.59 (3H, d, J = 6.5 Hz, Me-9), 1.67 (3H, d, J = 6.5 Hz, Me-10), 2.11 (3H, s, Me-11), 3.10 (1H, q, J = 7.0 Hz, H-7), 3.91 (1H, q, J = 7.0 Hz, H-8), 6.25 (1H, s, H-5). 13C-NMR (125 MHz, CD3OD): δC 10.60 (C-11), 16.37 (C-10), 19.81 (C-9), 43.41 (C-7), 71.87 (C-8), 103.51 (C-1), 104.69 (C-5), 114.15 (C-3), 149.72 (C-4), 160.32 (C-6), 160.80 (C-2), 173.04 (COOH).

3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (4): Yellow solid, mp. 153 – 154 °C. ESI-MS (m/z): 234 [M+H]+. 1H NMR (500 MHz, DMSO-d6): δH 2.46 (3H, s, Me-10), 2.48 (3H, s, Me-11), 1.79 (1H, s, H-8), 7.85 (1H, s, H-5), 11.54 (1H, br, s, OH). 13C-NMR (125 MHz, DMSO-d6): δC 19.43 (C-10), 20.08 (C-11), 125.89 (C-8), 128.70 (C-5), 129.83 (C-4a), 134.17 (C-8a), 139.10 (C-6), 141.69 (C-4), 144.92 (C-7), 146.28 (C-2), 148.89 (C-3), 160.52 (C-9).

Cyclo-(Pro-Gly) (5): White solid, mp. 210 – 211 °C, [α]25D = -142.5° (c 0.40, MeOH). ESI-MS (m/z): 155 [M+H]+. 1H-NMR (500 MHz, CDCl3): δH 2.01 (3H, m, CH2-4, H-5a), 2.34 (1H, m, H-5b), 3.56 (2H, m, CH2-3), 3.77 (1H, d, J = 17.0 Hz, H-9a), 4.13 (1H, d, J = 17.0 Hz, H-9b), 4.25 (1H, td, J = 2.0.8 Hz, H-6). 13C-NMR (125 MHz, CDCl3): δC 23.28 (C-4), 29.36 (C-5), 46.30 (C-3), 47.00 (C-9), 59.84 (C-8a), 141.64 (C-1), 171.98 (C-7).

Xanthone (6): ESI-MS (m/z): 195 [M-H]-. 1H-NMR (500 MHz, CDCl3): δH (ppm) 6.67 (2H, m, H-2), 7.30 (1H, t, J = 7.5 Hz, H-3), 7.92 (1H, d, J = 7.5 Hz, H-1). 13C-NMR (125 MHz, CDCl3): δC (ppm) 109.78 (C-9a), 116.49 (C-2), 116.80 (C-4), 132.14 (C-1), 135.00 (C-3), 151.06 (C-4a), 173.0 (C-9).

Guanosine (7): White solid. ESI-MS (m/z): 284 [M+H]+. 1H-NMR (500 MHz, DMSO-d6): δH 3.52 (1H, dt, J = 4.0, 12.0 Hz, H5′), 3.62 (1H, dt, J = 4.5, 12.0 Hz, H6′), 3.87 (1H, dt, J = 4.0, 3.5 Hz, H4′), 4.08 (1H, br s, H3′), 4.39 (1H, m, H2′), 5.69 (1H, d, J = 6.0 Hz, H-1′), 7.92 (1H, s, H-8). 13C-NMR (125 MHz, DMSO-d6): δC 61.39 (C-5′), 70.35 (C-3′), 73.68 (C-2′), 85.18 (C-4′), 86.35 (C-1′), 116.68 (C-5), 135.54 (C-8), 151.28 (C-4), 153.65 (C-2), 156.73 (C-6).

2′-Deoxyadenosine (8): White solid, ESI-MS (m/z): 252 [M+H]+. 1H-NMR (500 MHz, CD3OD): δH 2.43 (1H, m, H2′), 2.84 (1H, m, H2′), 3.76 (1H, dd, J = 3.5, 12.5 Hz, H5′), 3.87 (1H, dd, J = 3.0, 12.5 Hz, H6′), 4.09 (1H, m, H-3′), 4.60 (1H, m, H-4′), 6.45 (1H, dd, J = 6.0, 8.0 Hz, H1′), 8.20 (1H, s, H-8), 8.34 (1H, m, H-2), 8.34 (1H, m, H-2). 13C-NMR (125 MHz, CD3OD): δC (ppm) 41.64 (C-2′), 63.58 (C-5′), 73.12 (C-3′), 87.11 (C-4′), 89.91 (C-1′), 120.81 (C-5′), 141.53 (C-8), 149.87 (C-4), 153.49 (C-2), 157.53 (C-6).

Adenosine (9): White solid, ESI-MS (m/z): 268 [M+H]+. 1H-NMR (500 MHz, CD3OD): δH 3.77 (1H, dd, J = 3.0, 12.5 Hz, H5′), 3.91 (1H, dd, J = 2.5, 12.5 Hz, H6′), 4.19 (1H, m, H-4′), 4.35 (1H, dd, J = 3.0, 5.0 Hz, H3′), 4.76 (1H, dd, J = 5.0, 6.5 Hz, H-2′), 5.99 (1H, d, J = 6.0 Hz, H1′), 8.20 (1H, s, H-8), 8.32 (1H, s, H-2).

3. RESULTS AND DISCUSSION

Compound 1 was obtained as white solid with an [α]20D value of -29.5 (c 0.1 MeOH). The ESI-MS spectra of 1 showed the pseudomolecular ion peak at m/z 396 [M+H]+. Analysis of the
13C NMR and DEPT spectrum of 1 revealed the presence of 22 carbons, including three carbonyl carbons at δC 168.94 (C-17), 169.45 (C-11), 183.20 (C-2), four sp2 methines at δC 97.78 (C-7), 107.77 (C-5), 122.67 (C-19), 128.20 (C-4), three sp2 methines at δC 59.81 (C-9), 61.83 (C-18), 62.26 (C-12), four sp2 methylenes at δC 24.44 (C-14), 28.52 (C-13), 35.33 (C-8), 46.18 (C-15), one methoxy group at δC 55.93, two methyls at δC 18.09 (C-22), 25.54 (C-21) and five quaternary carbons. The 1H-NMR spectrum of 1 indicated the presence of a 1,2,4-trisubstituted benzene ring at δH 6.51 (1H, d, J = 2.0 Hz, H-7), 6.56 (1H, dd, J = 2.0 and 7.5 Hz, H-5), 7.03 (1H, d, J = 7.5 Hz, H-4), one olefinic proton at δH 5.09 (1H, d, J = 9.0 Hz, H-19), one methoxy group at δH 3.80 (3H, s, OCH3), two singlet methyls at δH 1.20 (3H, s, H-21), 1.67 (3H, s, H-22) and a set of protons at the aliphatic region. In the 1H-1H COSY spectrum, in addition to the correlations of protons of ABX aromatic ring, three separated spin-spin coupling systems was observed as follows: i) H-12 (δH 4.45)/H-13 (δH 2.15 and 2.32)/H-14 (δH 2.14 and 2.03)/H-15 (δH 3.57), ii) H-8 (δH 2.61 and 2.37)/H-9 (δH 5.04), iii) H-18 (δH 4.73)/H-19 (δH 5.09) (Figure 2). Analysis of the HMBC spectrum of 1 noted cross-peak of H-12 (δH 4.45) and H-13 (δH 2.15 and 2.32) with C-11 (δC 169.45) and H-15 (δH 3.57) with C-17 (δC 168.94). This observation demonstrated the presence of proline moiety of a cyclodipeptide molecule. Similarly, HMBC correlations of proton of methoxy group, H-4, H-5 and H-7 with C-6 (δC 162.16) indicated the linkage of methoxy group at C-6 of ABX aromatic ring. HMBC correlations of H-21 (δH 1.20) and H-22 (δH 1.67) with C-19 (δC 122.67), C-20 (δC 139.10) assigned the side chain as figure (Figure 2). Finally, cross-peak of H-8 with C-2 (δC 183.20), C-3 (δC 57.11), C-3a (δC 120.13), C-17 (δC 168.94), C-18 (δC 61.83), cross-peak of H-18 (δH 4.73) with C-2 (δC 183.20), C-3 (δC 57.11), C-8 (δC 35.3), C-9 (δC 59.8), C-11 (δC 169.45), cross-peak of H-9 with C-17 (δC 168.94), C-11 (δC 169.45) established the second moiety of the cyclodipeptide molecule with spiro ring system as figure (Figure 2). On the basis of the above NMR, MS spectra, value of [α]D and comparison with reported literature [5], compound 1 was determined aspistrotryprostatin A.

Compound 2 was isolated as a white solid. Its positive ESI mass spectrum showed the proton adduct ion [M+H]+ at m/z 229 [M+H]+. The 13C NMR and DEPT spectra of 2 showed the presence of 10 carbons, including two carbonyl carbons at δC 165.40 (C=O) and 170.33 (C=O), two sp2 methines bearing nitrogen at δC 54.65 (C-9), 59.03 (C-6), five sp2 methylenes and one singlet methyl at δC 15.30 (SCH3). The 1H-NMR spectrum of 2 showed the presence of one amide NH at 6.77 (1H, br s, NH), one singlet methyl at δH 2.12 (3H, s, SCH3), one triplet methylene at δH 2.68 (2H, t, J=7.0 Hz, H-11), two sp3 methines at δH 4.10 (1H, t, J = 8.0 Hz., H-6), 4.20 (1H, m, H-9) and the 8 other protons at the aliphatic region. This observation suggested compound 2 is a cyclodipeptide with the presence of two amino acid moieties: proline and

Figure 2. Key COSY and HMBC correlations for compounds of 1, 3 and 7.
methionine. This analysis was further supported by comparison with reported data [6]. Thus, compound 2 was defined as cyclo-(Pro-Met).

Compound 3 was isolated as a white solid and was optically active [α]D20 −45.5 (c 0.1 MeOH). The molecular formula of 3 was determined to be C12H10O5 on the basis of the pseudomolecular ion [2M+Na]+ at m/z 503.1881 (calcd. for C24H18O6Na, m/z 503.1893) in HR-ESI-MS spectrum and 13C-NMR data. The 1H-NMR spectrum of 3 revealed the signals of one singlet proton aromatic at δH 6.25, suggesting the presence of a 1,2,3,4,6-pentasubstituted benzene ring, one singlet methyl at δH 2.11 (3H, s, Me-11), two doublet methyls at δH 1.59 (3H, d, J = 6.5 Hz, Me-9), 1.67 (3H, d, J = 6.5 Hz, Me-10) and signals of two sp2 methines at δH 3.10 (1H, q, J = 7.0 Hz, H-7, 3.91 (1H, q, J = 7.0 Hz, H-8). The 13C-NMR and HSQC spectra of 3 showed the presence of 12 carbons including one sp2 methine group at δC 104.69 (C-5), one carboxyl group at δC 173.04 (COOH), three methyl groups at δC 10.60 (C-11), 16.37 (C-10), 19.81 (C-9), two sp2 methine which one was linked to the oxygen at δC 43.41 (C-7), 71.87 (C-8), and five quaternary carbons. In the HMBC spectrum of 3, cross-peaks of the proton of CH3-11 (δH 2.11) with C-2 (δC 160.80), C-3 (δC 114.15), C-4 (δC 149.72) indicated the linkage of this methyl group at C-3. Futhermore, HMBC correlation of the proton of CH3-10 (δH 1.67) with C-7 (δC 43.41)/C-4 (δC 149.72), C-8 (δC 71.87) indicated the linkage of this methyl group at C-7. The cross peak of H-7 (δH 3.10) with C-4 (δC 149.72), C-3 (δC 114.2), C-5 (δC 104.69) determined the linkage of the C-7 with C-4 of aromatic ring (Figure 2). Thus, complete analyses of the 2D-NMR spectra, and comparison with the reported data allowed determining the structure of 3 to be phenol A acid [7].

Compound 4 was isolated as yellow solid. Its ESI mass spectrum showed the pseudomolecular ion [M+H]+ at m/z 234. The 1H NMR spectrum of 4 showed signals of two singlet aromatic protons at δH 7.89 (1H, s, H-5) and 7.69 (1H, s, H-8), two singlet methyls at δH 2.46 (3H, s, Me-10) and 2.48 (3H, s, Me-11), and a signal of hydroxy proton at δH 11.54. Analyses of the 13C-NMR spectra with the aid of the HSQC of 4 indicated the presence of 12 carbons including a carbonyl carbon at δC 160.52 (C=O), two aromatic methine at δC 125.89 (C-8), 128.70 (C-5), two methyl carbons at δC 19.43 (C-10), 20.08 (C-11), and seven quaternary aromatic carbons. Based on the chemical shift of four quaternary carbons at δC 141.69 (C-9), 144.92 (C-7), 146.28 (C-2), 149.89 (C-3), it can be suggested that they linked to the nitrogen or oxygen atom. From the analysis of the mass and 1D-, 2D-NMR spectra and consulting the literature, compound 4 was confirmed to be 3,4-dihydroxy-6,7-dimethyl-quinoline-2 carboxylic acid [8].

The ESI-MS spectra of 5 showed the pseudo-molecular ion at m/z 155 [M+H]+. The 13C-NMR and DEPT spectra of 5 showed the presence of seven carbons including four sp2 methylene groups at δC 23.28 (C-4), 29.36 (C-5), 46.30 (C-3), 47.00 (C-9), one sp2 methine group at δC 59.84 (C-6), two carbonyl groups at δC 166.44 (C-1), 171.98 (C-7). The chemical shifts of C-3 (δC 46.30), C-6 (δC 59.84) and C-9 (δC 47.00) suggested their linkage to oxygen and nitrogen atoms. The 1H NMR spectrum of 5 showed signals of 9 protons at the aliphatic region δH 2.01-4.25. This observation suggested a cyclopetide compound for 5. Comparison of NMR data revealed the structure of 5 which was identical to cyclo-(Pro-Gly) [9].

The ESI-MS spectra of 6 presented a base peak at m/z 195 [M-H]. The 1H-NMR spectrum showed the signals corresponding to a 1,2-disubstituted benzene ring δH 6.67 (2H, m, H-2+ H-4), 7.30 (1H, t, J= 7.5 Hz, H-3), 7.92 (1H, d, J= 7.5 Hz, H-1). Analysis of the 13C-NMR and DEPT spectra of 6 indicated the presence of a carbonyl carbon at δC 173.0 (C = O), four aromatic methines at δC 135.00 (C-3), 132.14 (C-1), 116.80 (C-4), 116.49 (C-2) and two aromatic quaternary carbons at δC 109.78 (C-9a), 151.06 (C-4a). The chemical shifts of C-4a (δC 151.06)
suggested their linkages to oxygen atom. These observations suggested a structural symmetry in the structure of 6. Complete analysis of NMR, MS spectra and comparison with the reported data allowed determining the structure of 6 to be xanthone [10].

Compound 7 was isolated as a white solid. Its ESI mass spectrum showed the pseudomolecular ion [M+H]+ at m/z 284. The 1H-NMR spectrum of 7 displayed signals of one singlet aromatic proton at δH 7.92 (1H, s, H-8), a anomeric proton at δH 5.69 (1H, d, J = 6.0 Hz, H-1′) and a set of 5 protons of ribofuranose sugar moiety ranging from δH 3.52 to δH 4.39. In the 1H-1H COSY spectrum, the spin-spin coupling system of ribofuranose sugar moiety was observed (Figure 2). The 13C-NMR and DEPT spectra of 7 showed the presence of 10 carbon atoms, including one sp2 methine at δC 135.54 (C-8), four sp3 oxymethine at δC 70.35 (C-3′), 73.68 (C-2′), 85.18 (C-4′), 86.35 (C-1′) and one sp3 oxymethylene at δC 61.39 (C-5′), and four quaternary carbons. The NMR spectra suggested that 7 was a purine nucleoside compound, forming from a guanine and a ribofuranose sugar unit. In the HMBC spectrum, the correlation of H-1′ (δH 5.69) with C-8 (δC 135.54), C-4 (δC 151.28), suggested that the guanine was attached to sugar unit via β-N9 bond (Figure 2). Detailed analysis of 2D NMR spectra, especially HMBC spectrum allowed determining the structure of 7 as guanosine [11].

Compound 8 was isolated as a white solid. The ESI-MS mass spectrum of 8 presented a base peak at m/z 252 [M+H]+. 1D NMR spectra (1H-NMR and 13C-NMR) of 8 displayed signals of 2-deoxyribofuranoside moiety including three sp3 oxymethine, one sp3 oxymethylene and one sp2 methylene. However, in the aromatic region, there are signals corresponding to an adenine moiety [δH 8.20 (1H, s, H-8), 8.34 (1H, m, H-2); δC 120.81 (C-5), 149.87 (C-4), 157.53 (C-6), 141.53 (C-8), 153.49 (C-2)]. This analyses suggested 8 being a nucleoside compound. Thus, analysis of MS and 1D NMR spectra determined 8 being 2′-deoxyadenosine [11].

Compound 9 was obtained as a white solid. The ESI-MS spectrum indicated the pseudomolecular ion peak at m/z 268 [M+H]+. The signals on the 1H-NMR spectrum of 9 were similar to those of 8, except for the appearance of an oxymethine (CH-2′) in 9 instead of a methylene in 8, and proton H-1′ appears as a doublet on the 1H NMR spectrum of 9. Whereas, this proton displayed a doublet of doublet in the 1H NMR spectrum of 8. From the above analysis and reference, 9 has been identified as adenosine [12].

4. CONCLUSIONS

A comparison study was conducted to differentiate between solid and liquid fermentation technique for secondary metabolites production of a Vietnam marine actinobacteria (strain G246), in which, nine secondary metabolites including spirotrypsin A (1), cyclo-(Pro-Met) (2), phenol A acid (3), 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (4), cyclo-(Pro-Gly) (5), xanthone (6), guanosine (7), 2′-deoxyadenosine (8), adenosine (9) were isolated from the solid state fermentation. Results revealed that compound 2 was the only one similarity between these fermentation techniques.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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