SECONDARY METABOLITES PRODUCED BY MARINE ACTINOMYCETE *STREPTOMYCES* SP. G246

Do Thi Quynh[1, 2], Doan Thi Mai Huong[1, 2, *], Tran Van Hieu[1], Truong Bich Ngan[1], Le Thi Hong Minh[1], Vu Thi Quyen[1], Nguyen Thi Hoang Anh[3], Brian T. Murphy[4], Pham Van Cuong[1, 2, *]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang QuocViet, Cau Giay, Hanoi 10000, Viet Nam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi 10000, Viet Nam*

[3]*Thaibinh Medical College, 290 Phan Ba Vanh, Quang Trung, Thai Binh, Viet Nam*

[4]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA*

[*]Email: *huongdm@imbc.vast.vn*

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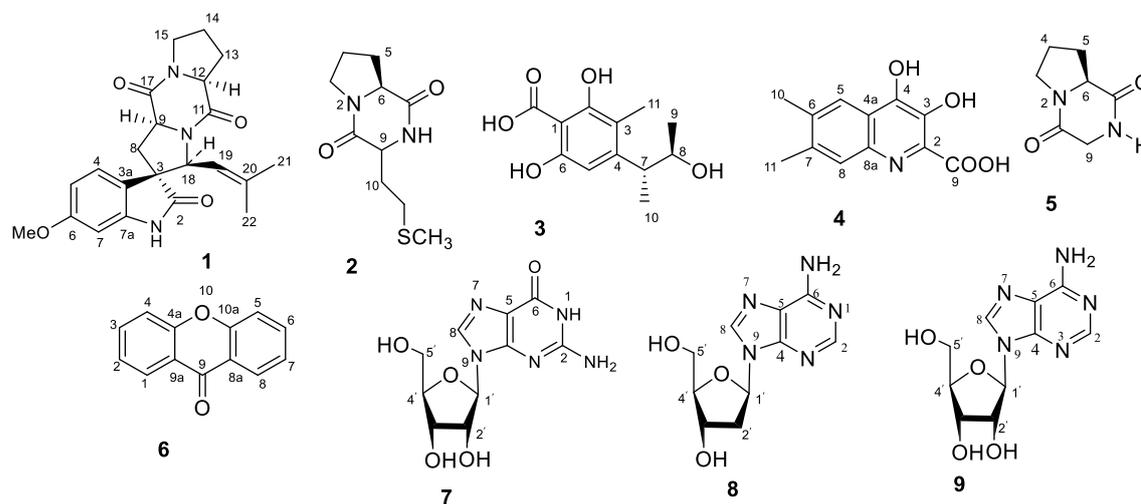
Figure 1. Nine compounds (**1-9**) from marine *Streptomyces* sp. G246 isolated by solid state fermentation technique.

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 δ_H 7.27 and 3.31 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak of at δ_C 77.1 (CDCl$_3$) and 49.0 (CD$_3$OD). For HMBC experiments the delay (1/2J) 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 ^{o}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, $CaCO_3$: 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 *Streptomyces*sp. 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 MG246after 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/H_2O gradient to provide eleven fractions (F1- F11). Fraction F4 (1.1 g) was separated 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 CH_2Cl_2/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 CH_2Cl_2/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 CH_2Cl_2/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 CH_2Cl_2/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, eluting with CH_2Cl_2/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 CH_2Cl_2/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 CH_2Cl_2/MeOH gradient to give compound **3** (4.7 mg).

Spirotryprostatin A (1): White solid; $[\alpha]^{29}_D$ -29.5 (*c* 0.1 MeOH). ESI-MS (*m/z*): 396 [M+H]$^+$. ^{1}H-NMR (500 MHz, CD$_3$OD): δ_H 1.20 (3H,s, H-21); 1.67 (3H, s, H-22), 2.03 (1H, m, H$_a$-14), 2.14 (1H, m, H$_b$-14), 2.15 (1H, m, H$_a$-13), 2.32 (1H, m, H$_b$-13), 2.37 (1H, dd, *J* = 7.0,13.5 Hz, H$_a$-8), 2.61 (1H, dd, *J* = 10.5,13.5 Hz, H$_b$-8), 3.57 (2H, m, H-15), 3.80 (OCH$_3$), 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). ^{13}C-NMR (125 MHz, CD$_3$OD): δ_C 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 (OCH$_3$), 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]$^+$. ^{1}H-NMR (500 MHz, CDCl$_3$): δ_H (ppm) 1.90 (1H, m, H$_a$-4), 2.01 (2H, m, H$_b$-4,H$_a$-5), 2.11 (1H, m, H$_b$-5),2,12 (3H, s, SCH$_3$), 2.37 (2H, m, CH$_2$-10), 2.68 (2H, t, *J* = 7.0 Hz, CH$_2$-11), 3.54 (1H, m, H$_a$-3), 3.60 (1H, m, H$_b$-3), 4.10 (1H, t, *J* = 8.0 Hz,, H-6), 4.20 (1H, m, H-9), 6.77 (1H, br. s, NH). ^{13}C-NMR (125 MHz, CDCl$_3$): δ_C (ppm) 15.30 (SCH$_3$), 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 A acid (3): White solid, [α]$^{29}_D$ -45.5 (*c* 0.1 MeOH). HR-ESI-MS (*m/z*): 503.1881 [2M+Na]$^+$(Calcd. for C$_{24}$H$_{32}$O$_{10}$Na, *m/z* 503.1893). ^{1}H-NMR (500 MHz, CD$_3$OD): δ_H1.59 (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). ^{13}C-NMR (125 MHz, CD$_3$OD): δ_C10.60 (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 ^{o}C. ESI-MS (*m/z*): 234 [M+H]$^+$. ^{1}H NMR (500 MHz, DMSO-d$_6$): δ_H2.46 (3H, s, Me-10), 2.48 (3H, s, Me-11), 7.69 (1H, s, H-8), 7.89 (1H, s, H-5), 11.54 (1H, br, s, OH). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ_C 19.43 (C-10), 20.08 (C-11), 125.89 (C-8), 128.70 (C-5),129.83 (C-4a), 138.47 (C-8a), 139.10 (C-6), 141.69 (C-4), 144.92 (C-7), 146.28 (C-2), 149.89 (C-3), 160.52 (C-9).

Cyclo-(Pro-Gly) (5): White solid, mp. 210 – 211 ^{o}C, [α]$^{25}_D$ -142,5^o (*c* 0.40, MeOH). ESI-MS (*m/z*): 155 [M+H]$^+$. ^{1}H-NMR (500 MHz, CDCl$_3$): δ_H 2.01 (3H, m, CH$_2$-4, H-5a),2.34 (1H, m, H-5b), 3.56 (2H, m, CH$_2$-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* = 2.0,8.0 Hz, H-6),^{13}C-NMR (125 MHz, CDCl$_3$): δ_C 23.28 (C-4), 29.36 (C-5), 46.30 (C-3),47.00 (C-9), 59.84 (C-6), 166.44 (C-1), 171.98 (C-7).

Xanthone (6): ESI-MS (*m/z*): 195 [M-H]$^-$. ^{1}H-NMR (500 MHz, CDCl$_3$): δ_H (ppm) 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). ^{13}C-NMR (125 MHz, CDCl$_3$): δ_C (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]$^+$. ^{1}H-NMR (500 MHz, DMSO-*d$_6$*): δ_H 3.52 (1H, dt, *J* = 4.0, 12.0 Hz, H$_a$-5′), 3.62 (1H, dt, *J* = 4.5, 12.0 Hz, H$_b$-5′), 3.87 (1H, dt, *J* = 4.0, 3.5 Hz, H-4′), 4.08 (1H, br s, H-3′), 4.39 (1H, m, H-2′), 5.69 (1H, d, *J* = 6.0 Hz, H-1′), 7.92 (1H, s, H-8). ^{13}C-NMR (125 MHz, DMSO-*d$_6$*): δ_C 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]$^+$. ^{1}H-NMR (500 MHz, CD$_3$OD): δ_H 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$_3$OD): δ_C (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]$^+$, ^{1}H-NMR (500 MHz, CD$_3$OD):δ_H3.77 (1H, dd, *J* = 3.0, 12.5 Hz, H$_a$-5′), 3.91 (1H, dd, *J* = 2.5, 12.5 Hz, 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).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white solid with an [α]$^{29}_D$ 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

^{13}C NMR and DEPT spectrum of **1** revealed the presence of 22 carbons, including three carbonyl carbons at δ_C 168.94 (C-17), 169.45 (C-11), 183.20 (C-2), four sp^2 methines at δ_C 97.78(C-7), 107.77 (C-5), 122.67 (C-19), 128.20 (C-4), three sp^3 methines at δ_C59.81 (C-9), 61.83 (C-18), 62.26 (C-12), four sp^3methylenes at δ_C 24.44 (C-14), 28.52 (C-13), 35.33 (C-8), 46.18 (C-15), one methoxy group at δ_C 55.93, two methyls at δ_C 18.09 (C-22), 25.54 (C-21) and five quaternary carbons. The ^{1}H-NMR spectrum of **1** indicated the presence of a 1,2,4-trisubstituted benzene ring at δ_H 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 δ_H 5.09 (1H, d, J = 9.0 Hz, H-19), one methoxy group at δ_H 3,80 (3H, s, OCH$_3$), two singlet methyls at δ_H 1.20 (3H,s, H-21), 1.67 (3H, s, H-22) and a set of protons at the aliphatic region. In the ^{1}H-^{1}H 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 (δ_H 4.45)/H-13 (δ_H 2.15 and 2.32)/H-14 (δ_H 2.14 and 2.03)/H-15 (δ_H 3.57), ii) H-8 (δ_H 2.61 and 2.37)/H-9 (δ_H5.04), iii) H-18 (δ_H4.73)/H-19 (δ_H 5.09) (Figure 2). Analysis of the HMBC spectrum of **1** noted cross-peak of H-12 (δ_H 4.45) and H-13 (δ_H 2.15 and 2.32) with C-11 (δ_C169.45) and H-15 (δ_H 3.57) with C-17 (δ_C168.94). 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 (δ_C162.16) indicated the linkage of methoxy group at C-6 of ABX aromatic ring. HMBC correlations of H-21 (δ_H 1.20) and H-22 (δ_H 1.67) with C-19 (δ_C122.67), C-20 (δ_C 139.10) assigned the side chain as figure (Figure 2). Finally, cross-peak of H-8 with C-2 (δ_C183.20), C-3 (δ_C57.11), C-3a (δ_C120.13), C-17 (δ_C168.94), C-18 (δ_C61.83), cross-peak of H-18 (δ_H 4.73) with C-2 (δ_C 183,2), C-3 (δ_C 57,1),C-8 (δ_C 35,3), C-9 (δ_C 59,8), C-11 (δ_C169.45), cross-peak of H-9 with C-17 (δ_C168.94), C-11 (δ_C 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 $[\alpha]^{29}_D$ and comparison with reported literature [5], compound **1** was determined asspirotryprostatin A.
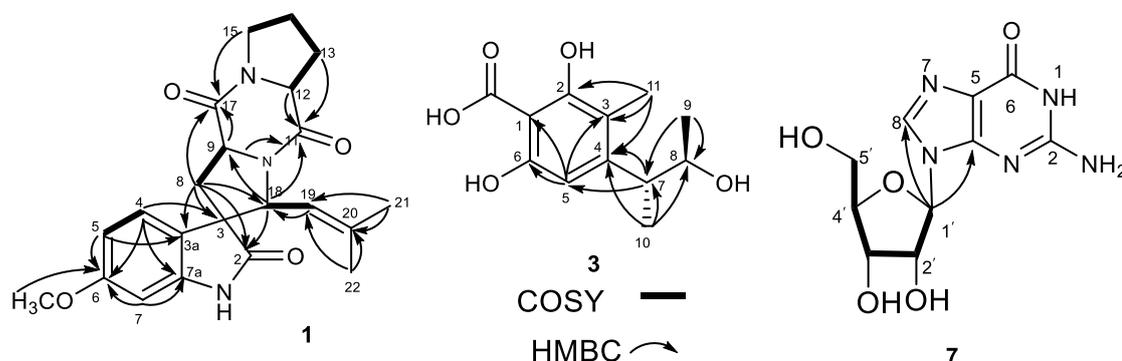

Figure 2. Key COSY and HMBC correlations for compounds of **1, 3** and **7**.

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 ^{13}C NMR and DEPT spectra of **2** showed the presence of 10 carbons, includingtwo carbonyl carbons at δ_C 165.40 (C=O) and 170.33 (C=O), two sp^3 methines bearing nitrogen at δ_C 54.65 (C-9), 59.03 (C-6), five sp^3 methylenes and one singlet methyl at δ_C15.30 (SCH$_3$). The ^{1}H-NMR spectrum of **2** showed the presence of one amide NH at 6.77 (1H, br s, NH), one singlet methyl at δ_H 2,12 (3H, s, SCH$_3$), one triplet methylene at δ_H 2.68 (2H, t, J=7.0 Hz, H-11), two sp^3 methines at δ_H 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

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 solidand was optically active $[\alpha]^{29}_D$ -45.5 (*c* 0.1 MeOH). The molecular formula of **3** was determined to be $C_{12}H_{16}O_5$ on the basis of the *pseudo-molecular* ion $[2M+Na]^+$ at *m/z*503.1881(calcd. for $C_{24}H_{32}O_{10}Na$, *m/z* 503,1893) in HR-ESI-MS spectrum and ^{13}C-NMR datas. The ^{1}H-NMR spectrum of **3** revealed the signals of one singlet proton aromatic at δ_H 6.25, suggesting the presence of a 1,2,3,4,6-pentasubstituted benzene ring, one singlet methyl at δ_H2,11(3H, s, Me-11), two doublet methyls at δ_H 1.59 (3H, d, *J* = 6.5 Hz, Me-9), 1.67 (3H, d, *J* = 6.5 Hz, Me-10) and signals of two *sp*[3]methines at δ_H 3.10 (1H, q, *J* = 7.0 Hz, H-7), 3.91 (1H, q, *J* = 7.0 Hz, H-8). The ^{13}C-NMR and HSQC spectra of **3** showed the presence of 12 carbons including ones p^2 methine group at δ_C104.69 (C-5), one carboxyl group at δ_C 173.04 (COOH), three methyl groups at δ_C 10.60 (C-11), 16.37 (C-10), 19.81 (C-9), two sp^3methine which one was linked to the oxygen at δ_C43.41 (C-7), 71.87 (C-8), and five quaternary carbons. In the HMBC spectrum of **3**, cross-peaks ofthe proton of CH$_3$-11 (δ_H2.11) with C-2 (δ_C 160.80), C-3 (δ_C 114.15), C-4 (δ_C 149.72) indicated the linkage of this methyl group at C-3. Futhermore, HMBC corelation of the proton of CH$_3$-10 (δ_H 1.67) with C-7(δ_C 43.41)/C-4(δ_C 149.72), C-8 (δ_C 71.87) indicated the linkage of this methyl group at C-7. The cross peak of H-7 (δ_H 3.10) with C-4 (δ_C 149.72), C-3 (δ_C 114.2), C-5 (δ_C 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 pseudo-molecular ion $[M+H]^+$ at *m/z* 234. The ^{1}H NMR spectrum of **4** showed signals of two singlet aromatic protons at δ_H7.89 (1H, s, H-5) and 7.69 (1H, s, H-8), two singlet methyls at δ_H 2.46 (3H, s, Me-10) and 2.48 (3H, s, Me-11), and a signal of hydroxyl proton at δ_H11.54. Analyses of the ^{13}C NMR spectra with the aid of the HSQC of **4** indicated the presence of 12 carbons including a carbonyl carbon at δ_C 160.52 (C=O), two aromatic methine at δ_C125.89 (C-8), 128.70 (C-5), two methyl carbons at δ_C19.43 (C-10), 20.08 (C-11), and seven quaternary aromatic carbons. Based on the chemical shift of four quaternary carbons at δ_C141.69 (C-4), 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 masse and 1D-, 2D-NMR spectra and consulting the literature, compound **4** was confirmed to be 3,4-dihydroxy-6,7-dimethyl-quinoline-2 carboxylic [8].

The ESI-MS spectra of **5** showed the pseudo-molecular ion at *m/z* 155 $[M+H]^+$.The ^{13}C-NMR and DEPT spectra of **5** showed the presence of seven carbons including four sp^3methylene groups at δ_C23.28 (C-4), 29.36 (C-5), 46.30 (C-3), 47.00 (C-9), one sp^3methine group at δ_C 59.84 (C-6), two carbonyl groups at δ_C166.44 (C-1), 171.98 (C-7). The chemical shifts of C-3 (δ_C 46.30), C-6 (δ_C 59.84) and C-9 (δ_C 47.00) suggested their linkage to oxygen and nitrogen atoms. The ^{1}H NMR spectrum of **5** showed signals of 9 protons at at the aliphatic region δ_H2.01-4.25. This observation suggested a cyclodipeptide compound for **5**. Comparison of NMR data revealed the structure of **5**wich was identical tocyclo-(Pro-Gly) [9].

The ESI-MS spectra of **6** presented a base peak at *m/z* 195 $[M-H]^-$. The ^{1}H-NMR spectrum showed the signals corresponding to a 1,2-disubstituted benzene ring δ_H 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 ^{13}C-NMR and DEPT spectra of **6** indicated the presence of a carbonyl carbon at δ_C 173.0 (C = O), four aromatic methines at δ_C 135.00 (C-3), 132.14 (C-1), 116.80 (C-4), 116.49 (C-2) and two aromatic quaternary carbons at δ_C 109.78 (C-9a), 151.06 (C-4a). The chemical shifts of C-4a (δ_C151.06)

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 pseudo-molecular ion $[M+H]^+$ at *m/z* 284. The ^{1}H-NMR spectrum of **7** displayed signals of one singlet aromatic proton at δ_H 7.92(1H, s, H-8), a anomeric proton at δ_H 5.69 (1H, d, *J* = 6.0 Hz, H-1′) and a set of 5 protons of ribofuranose sugar moiety at ranging from δ_H3.52 to δ_H4.39. In the ^{1}H-^{1}H COSY spectrum, the spin-spin coupling system of ribofuranose sugar moiety was observed (Figure 2). The ^{13}C-NMR and DEPT spectra of **7** showed the presence of 10 carbon atoms, including one sp^2 methine at δ_C 135.54(C-8), four sp^3 oxymethine at δ_C 70.35 (C-3′), 73.68 (C-2′), 85.18 (C-4′), 86.35 (C-1′) and one sp^3 oxymethylene at δ_C 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′ (δ_H 5.69) with C-8 (δ_C 135.54), C-4 (δ_C 151.28), suggested that the guanine was attached to sugar unit via β-N_9 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 (^{1}H-NMR and ^{13}C-NMR) of **8** displayed signals of 2'-deoxyribofuranoside moiety including threesp^3 oxymethine, one sp^3 oxymethylene and one sp^3 methylene. However, in the aromatic region, there are signals corresponding to an adenine moiety [δ_H8.20 (1H, s, H-8), 8.34 (1H, m, H-2); δ_C120.81 (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 pseudo-molecular ion peak at *m/z* 268 $[M+H]^+$. The signals on the ^{1}H-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 ^{1}H NMR spectrum of **9**. Whereas, this proton displayed a doublet of doublet in the ^{1}H 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 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**) were isolated from the solid state fermentation. Results revealed that compound **2** was the only one similarity between these fermentation techniques.

analysis. L.T.H.M.: Methodology, Formal analysis. V.T.Q.: Investigation, Formal analysis. N.T.H.A.: Formal analysis. B.T.B.: Funding acquisition, Methodology. P.V.C.: Project administration, Supervision, Formal analysis.

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.

REFERENCES

1. Labeda D. P. - Multilocus sequence analysis of phytopathogenic species of the genus *Streptomyces*, International Journal of Systematic and Evolutionary Microbiology **61** (10) (2010) 2525-2531.

2. Manivasagan P., Kang K. H., Sivakumar K., Li-Chan E. C., Oh H. M., Kim S. K. - Marine actinobacteria - an important source of bioactive natural products, Environ. Toxicol. Pharmacol. **38** (1) (2014) 172-188.

3. Van Nam V., Huong D. T. M., Quynh D. T., Van Minh C., Quyen V. T., Hong Minh L. T., Cuong P. V. - Secondary metabolites from marine Actinomycete *Streptomyces* sp. A290, Vietnam J. Chem. **55** (4) (2018) 198-202.

4. Duc Danh Cao, Thi Quynh Do, Huong Doan Thi Mai, Quyen Vu Thi, Hong Minh Le Thi, Dang Thach Tran, Van Minh Chau, Cong Thung Do and Van Cuong Pham - Antimicrobial Lavandulylated Flavonoids from a Sponge-Derived Actinomycete, Natural Product Research **34** (3) (2020) 413-420.,

5. Cheng-Bin Cui, Hideaki Kakeya and Hiroyuki Osada - Novel Mammalian Cell cycle inhibitors, Spirotryprostatins A and B, produced by Aspergillus fumigatus, wich inhibit mamalian cell cycle at G2/M phase, Tetrahedron **52** (39) (1996) 12651-12666.

6. Jayatilake G. S., Thornton M. P., Leonard A. C., Grimwade J. E., Baker B. J. - Metabolites from an Antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*, J. Nat. Prod. **59** (1996) 293-296.

7. Rodel T., Gerlach H. - Enantioselective synthesis of the polyketide antibiotic (3*R*,4*S*)-(-)-citrinin, Liebigs Ann. **1995** (5) (1995) 885-888.

8. Vu T. Q., Tran V. H., Mai H. D. T., Le C. V., Le H. M., Murphy B. T., Chau V. M., and Pham V. C. - Antimicrobial Metabolites from a Marine-Derived Actinomycete in Vietnam's East sea, Natural Product Communications **11** (1) (2015) 45-49.

9. Pattalung P., Thongtheeraparp W., Wiriyachitra P., Taylor W. C. - Xanthones of *Garcinia cowa*, Planta Med. **60** (1994) 365-368.

10. Paul C. Kline and Anthony S. Serianni - (^{13}C)-Substituted erythronucleosides: Synthesis and Conformational analysis by ^{1}H and ^{13}C NMR spectroscopy, J. Org. Chem. **57** (1992) 1772-1777.

11. Huang R., Zhou X., Peng Y., Yang X., Xu T. and Liu Y. - Nucleosides from the marine sponge *Callyspongia* sp., Chem. Nat. Compd. **46** (6) (2011) 1010-1011.

12. Shi Z. D., Yang B. H. and Wu Y. L - Astereospecific synthesis of L-deoxyribose, L-ribose and L-ribosides,Tetrahedron **58** (2002) 3287-3296.