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CHEMICAL CONSTITUENTS FROM A MARINE-DERIVED ACTINOMYCETE *MICROMONOSPORA AURANTIACA* G657

Phi Thi Dao[1], Nguyen Thuy Linh[1], Le Thi Hong Minh[1], Vu Thi Quyen[1], Doan Thi Mai Huong[1,2,*], Pham Van Cuong[1,2,*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam;*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam*

*Corresponding author: huongdm@imbc.vast.vn or phamvc@imbc.vast.vn

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Summary

Chemical Constituents from a Marine-Derived Actinomycete *Micromonospora aurantiaca* G657

Seven compounds astellolide A (**1**), cyclopiazonic acid (**2**), cyclopiamide F (**3**), 5,6-epoxyergosterol (**4**), 3-indolecarbadehyde (**5**), 2'-deoxythymidine (**6**) and uridine (**7**) were isolated and characterized from the culture broth of the marine-derived *Micromonospora aurantiaca* G657 strain, which was isolated from the sponge sample collected at Van Phong, Khanh Hoa, Vietnam. Their structures were determined by analyses of mass spectroscopy (MS), nuclear magnetic resonance spectroscopy (NMR) and comparison with the reference data. To the best of our knowledge, this is the first report of the isolation of astellolide A (**1**), cyclopiazonic acid (**2**), cyclopiamide F (**3**) from *Micromonospora aurantiaca.*

Keywords: *Micromonospora aurantiaca, Indole-tetramic Acid alkaloid, Drimane-type sesquiterpene, Pyrimidine nucleoside.*

1. Introduction

Marine actinomycetes have been known as an important source of biologically active natural products with many bioactive applications such as anticancer, antifungal, antiviral properties, etc [1]. Vietnam has the long coastline and is considered as one of the countries with the leading marine biodiversity in the world. However, the number of publications about investigations of bioactive compounds isolated from marine actinomycetes in Vietnam have been limited [2],[3],[4]. In the course of our screening program, the crude extract of *Micromonospora aurantiaca* G657 fermentation exhibited antimicrobial activity against three Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579), one Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 and one yeast strain *Candida albicans* ATCC 10231 with MIC values of 8, 32, 64, 128, 16 µg/mL, respectively. In this study, we reported the results of isolation of seven compounds (**1-7**) from the marine actinomycetes *Micromonospora aurantiaca* G657 strain, which was isolated from the sponge sample (*Clathria* sp.) collected at a depth of 4 m, from the coast of Van Phong, Khanh Hoa province (Vietnam). Their structures were described in Fig. 1.

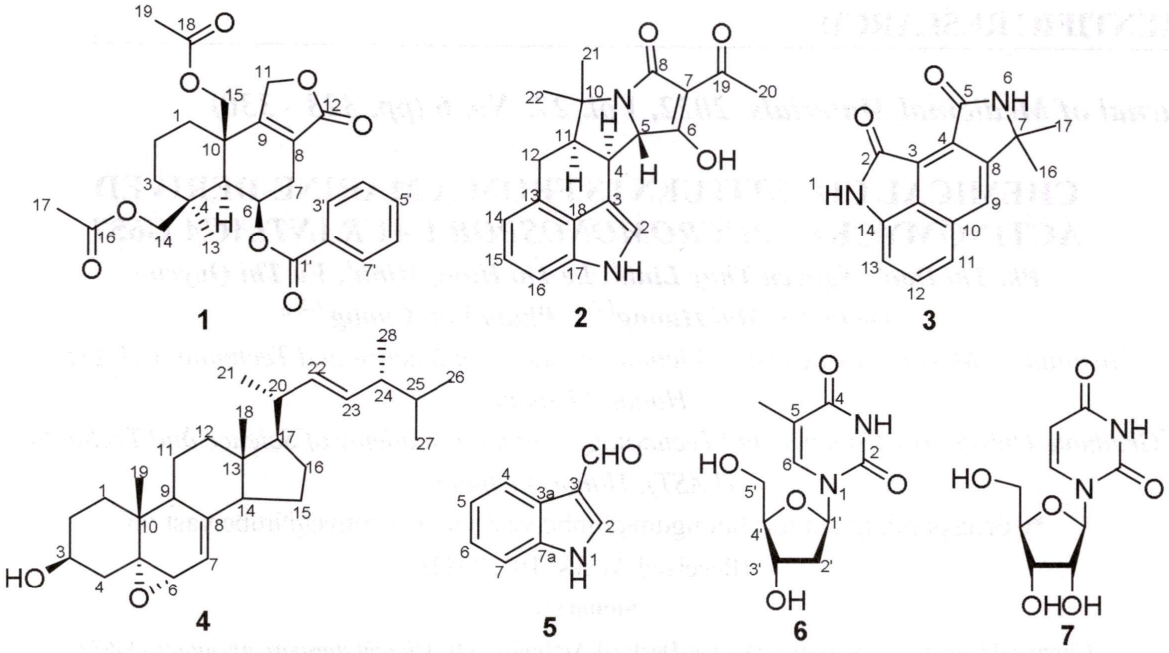

Fig. 1. Structures of compounds **1**-**7** isolated from *Micromonospora aurantiaca* G657

2. Material and methods

2.1. Marine materials

The *Micromonospora aurantiaca* G657 strain was isolated from the sponge sample (*Clathria* sp.) collected at a depth of 4 m, from the coast of Van Phong, Khanh Hoa province in May 2020, which was identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources - Vietnam Academy of Science and Technology (VAST). A voucher specimen (VP1-HM08) was deposited at the Institute of Marine Environment and Resources, VAST.

2.2. General experimental procedures

The ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on a Bruker 500 or 600 MHz spectrometer. The solvents for NMR spectra were $CDCl_3$, CD_3OD, and DMSO-d_6 using TMS as internal standard. TLC *silica gel* Merck 60 F_{254} was used for thin layer chromatography and preparative TLC. Column chromatography (CC) was carried out using *silica gel* (230-400 mesh, Merck), *silica gel* RP-18 (30-50 μm, YMC) or Sephadex LH-20 (Sigma). Fractions were monitored by thin layer chromatography. Spots were visualized by UV light (254 nm and 365 nm) and by heating *silica gel* plates sprayed with sulfuric acid 10% reagent. Medium pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.3. Isolation, identification, and fermentation of the actinomycete G657 strain

The sponge sample (0.5 g) was crushed by glass chopsticks in a falcon tube; 4.5 mL of sterile seawater was added, the mixture was homogenized by vortexing for 1 minute, and the suspension was treated using a wet-heat technique (60°C for 6 minutes). After that, 0.5 mL suspension was transferred to 4.5 mL sterile distilled water and vortexed for 1 minute to afford sample solution. Aliquot of 50 μL of the sample solution was spread on **A1** medium pH 7.0 (g/L): (5 g soluble starch, 2 g yeast extract, 1 g peptone, 30 g instant ocean salt, 15 g agar) supplemented with 50 μg/mL polymycin B and cycloheximide to inhibit Gram-negative bacterial and fungal contaminations. After 7 days of aerobic incubation at 30°C, the colony of the G657 actinomycete strain was transferred onto new petri dishes of **A1** medium for purification (Fig. 2) and identified as *Micromonospora aurantiaca* by using 16S rRNA gene sequence analysis.

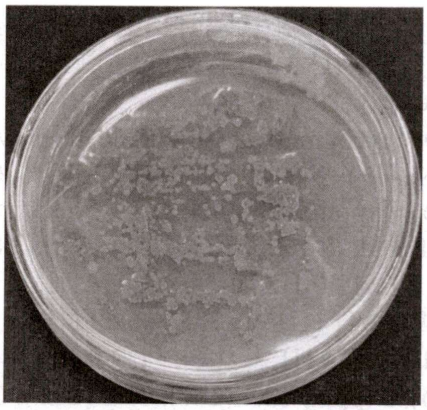

Fig. 2. Morphological appearance of G657 strain's colonies

The *M. aurantiaca* G657 strain was activated and inoculated into 1 L of A1 broth medium. After 7 days of incubation at 30°C with shaking of 150 rpm, the culture broth was used to spread on the medium surface of 50 flasks containing 1 L of rich-nutrient solid medium $A1^+$ (g/L) (soluble starch: 10 g, yeast extract: 4 g, peptone: 2 g, instant ocean salt: 30 g, KBr (20 mg/mL): 5 mL, $FeSO_4$ (8 mg/mL): 5 mL, $CaCO_3$: 1 g, agar: 15 g). The fermentation was incubated in an incubator at 30°C and harvested on the fifteen day.

2.4. Isolation of chemical constituents from the ethyl acetate extract of M. aurantiaca G657

The culture broth (50 kg) of *Micromonospora aurantiaca* G657 was cultured on the agar-based media for fifteen days and the agar was cut into small pieces and extracted with ethyl acetate (4 times x 5 liters x 30 minute each) at 40°C in an ultrasonic bath. These solutions were concentrated *in vacuo* to give 15.0 g ethyl acetate extract. This extract was subjected to *silica gel* column chromatography on a Medium-Performance Liquid Chromatography equipment using a gradient of CH_2Cl_2/MeOH (0-100% MeOH) to yield 9 fractions F1-F9. Fraction F2 (3.7 g) was separated by Sephadex LH-20 column chromatography with MeOH to give 6 fractions F2.1-F2.6. Fraction F2.3 (0.51 g) was continuously separated by *silica gel* column chromatography, eluted with CH_2Cl_2/MeOH (95/5) to yield **1** (2 mg) and **2** (3 mg). Fraction F5 (2.1 g) was separated by Sephadex LH-20 column chromatography, eluted with MeOH to give 5 fractions F5.1-F5.5. Fraction F5.2 (0.5 g) was continuously separated by *silica gel* column chromatography with a mixture of CH_2Cl_2/MeOH gradient as mobile phase to yield **6** (2.5 mg). Fraction F6 (0.75 g) was purified over Sephadex LH-20 column chromatography with MeOH as eluent to give 4 fractions F6.1-F6.4. Fraction F6.3 (0.16 g) was separated by *silica gel* column chromatography with CH_2Cl_2/MeOH gradient (0-100% MeOH) as mobile phase to yield **5** (1.5 mg). Fraction F6.2 (0.21 g) was further purified by *silica gel* column chromatography with CH_2Cl_2/acetone gradient (0-100% acetone) to give 6 fractions F6.2.1-F6.2.5. Fraction F6.2.3 (12 mg) was separated by preparative TLC with CH_2Cl_2/acetone (8/2) to give **7** (3.0 mg). Fraction 7 (2.36 g) was subjected to Sephadex LH-20 column chromatography with MeOH as eluent to give 5 fractions F7.1-F7.5. Fraction F7.3 (0.8 g) was continuously separated by *silica gel* column chromatography with a mixture of CH_2Cl_2/MeOH gradient as mobile phase to yield **3** (2.5 mg) and **4** (1.5 mg).

Astellolide A (1): White solid, $[\alpha]_D^{25}$ = -11.2° (*c*. 0.2, MeOH), ESI-MS (*m/z*): 493 $[M+Na]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 1.15 (3H, s, H-13), 1.21 (1H, m, H_a-3), 1.35 (1H, m, H_a-1), 1.65 (1H, m, H_a-2), 1.75 (1H, m, H_b-2), 1.82 (1H, br s, H-5), 1.89 (3H, m, H-17), 1.92 (1H, d, J = 13.8 Hz, H_b-3), 2.27 (1H, d, J = 12.6 Hz, H_b-1), 2.13 (3H, m, H-19), 2.73 (2H, m, H-7), 3.97 (1H, d, J = 11.4 Hz, H_a-14), 4.41 (1H, d, J = 11.4 Hz, H_b-14), 4.84 (1H, d, J = 15.6 Hz, H_a-15), 4.87 (1H, d, J = 10.8 Hz, H_a-11), 4.98 (1H, d, J = 10.8 Hz, H_b-11), 5.00 (1H, d, J = 15.6 Hz, H_b-15), 5.94 (1H, br d, J = 5.4 Hz, H-6), 7.49 (2H, t, J = 7.8 Hz, H-4′, 6′), 7.59 (1H, t, J = 7.2 Hz, H-5′), 8.01 (2H, d, J = 7.8 Hz, H-3′, 7′). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 17.9 (C-2), 20.6 (C-17), 21.0 (C-19), 27.8 (C-13), 29.1 (C-7), 31.9 (C-1), 37.2 (C-3), 37.8 (C-4), 40.6 (C-10), 54.3 (C-5), 66.0 (C-11), 66.5 (C-6), 67.0 (C-14), 71.3 (C-15), 122.9 (C-8), 128.8 (C-4′, 6′), 129.5 (C-2′), 129.7 (C-3′, 7′), 133.3 (C-5′), 165.6 (C-16), 165.6 (C-9), 166.1 (C-1′), 170.2 (C-18), 170.7 (C-16), 173.3 (C-12).

Cyclopiazonic acid (2): White solid, $[\alpha]_D^{25}$ = -89° (*c*. 0.2, $CHCl_3$), ESI-MS (*m/z*): 337 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 1.58 (3H,

s, H-22), 1.60 (3H, s, H-21), 2.39 (3H, s, H-20), 2.47 (1H, m, H-11), 3.00 (1H, dd, J = 5.4, 15.6 Hz, H_a-12), 3.06 (1H, dd, J = 3.6, 15.6 Hz, H_b-12), 3.54 (1H, dd, J = 5.4, 10.8 Hz, H-4), 3.87 (1H, d, J = 10.8 Hz, H-5), 6.80 (1H, d, J = 6.8 Hz, H-14), 7.03 (1H, dd, J = 7.2, 6.8 Hz, H-15), 7.14 (1H, s, H-2), 7.15 (1H, m, H-16). ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 25.8 (C-22), 26.3 (C-21), 27.6 (C-20), 27.9 (C-12), 38.1 (C-4), 55.3 (C-11), 63.4 (C-10), 71.7 (C-5), 106.5 (C-7), 109.5 (C-16), 111.7 (C-3), 116.6 (C-14), 122.1 (C-2), 123.3 (C-15), 127.5 (C-18), 130.2 (C-13), 135.2 (C-17), 173.8 (C-8), 184.7 (C-6), 195.2 (C-19).

Cyclopiamide F (3): Pale yellow solid, HR-ESI-MS *m/z* 253.0976 $[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 1.56 (6H, s, H-16, 17), 6.96 (1H, d, J = 6.6 Hz, H-13), 7.56 (1H, dd, J = 6.6, 9.0 Hz, H-12), 7.60 (1H, d, J = 9.0 Hz, H-11), 8.36 (1H, s, H-9), 8.96 (1H, br s, NH-6), 10.78 (1H, br s, NH-1). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 28.7 (C-16, C-17), 59.1 (C-7), 106.1 (C-13), 119.8 (C-11), 124.00 (C-3), 124.03 (C-9), 126.6 (C-15), 129.9 (C-4), 130.9 (C-12), 131.2 (C-10), 139.8 (C-14), 154.2 (C-8), 165.3 (C-5), 166.7 (C-2).

5,6-Epoxyergosterol (4): White solid, ESI-MS (*m/z*): 413 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 0.66 (3H, s, H-18), 0.87 (3H, d, J = 7.2 Hz, H-27), 0.88 (3H, d, J = 6.6 Hz, H-26), 0.96 (3H, d, J = 6.6 Hz, H-21), 1.06 (3H, d, J = 6.6 Hz, H-28), 1.09 (3H, s, H-19), 3.56 (1H, d, J = 4.8 Hz, H-6), 4.01 (1H, dt, J = 11.4, 5.4 Hz, H-3), 5.24 (1H, dd, J = 15.3, 7.6 Hz, H-23), 5.25 (1H, dd, J = 15.3, 7.6 Hz, H-22), 5.28 (1H, d, J = 5.2 Hz, H-7). ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm) 11.8 (C-18), 17.2 (C-28), 18.2 (C-19), 19.0 (C-27), 19.4 (C-26), 20.2 (C-21), 21.9 (C-11), 22.9 (C-15), 28.1 (C-16), 30.8 (C-2), 32.9 (C-1), 33.4 (C-25), 36.9 (C-10), 39.5 (C-12), 39.7 (C-4), 40.8 (C-20), 42.9 (C-9, C-24), 43.4 (C-13), 54.9 (C-14), 56.4 (C-17), 67.4 (C-3), 73.2 (C-6), 75.9 (C-5), 118.1 (C-7), 132.3 (C-23), 135.9 (C-22), 142.8 (C-8).

3-Indolecarbadehyde (5): Pale yellow solid, ESI-MS (*m/z*): 146 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.31 (1H, m, H-5), 7.32 (1H, m, H-6), 7.44 (1H, m, H-7), 7.85 (1H, d, J = 2.8 Hz, H-2), 8.32 (1H, m, H-4), 10.08 (s, -CHO). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 111.5 (C-7), 119.8 (C-3a), 122.1 (C-4), 123.1 (C-5), 123.8 (C-3), 124.5 (C-6), 135.2 (C-2), 136.6 (C-7a), 185.6 (CHO).

2'-Deoxythymidine (6): Pale yellow solid, ESI-MS (*m/z*): 243 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 1.90 (3H, s), 2.25 (2H, m, H-2′), 3.74 (1H, dd, J = 3.6, 12.0 Hz, H-5′a), 3.82 (1H, dd, J = 3.6, 12.0 Hz, H-5′b), 3.93 (1H, q, J = 4.2 Hz, H-4), 4.41 (1H, m, H-3′), 6.29 (1H, t, J = 7.0 Hz, H-1′), 7.83 (1H, s, H-6). ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 12.4 (C-7), 41.2 (C-2′), 62.9 (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).

Uridine (7): Pale yellow solid, ESI-MS (*m/z*): 245 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 3.74 (1H, dd, J = 3.0, 12.0 Hz, H-5′a), 3.85 (1H, dd, J = 3.0, 12.6 Hz, H-5′b), 4.03 (1H, m, H-4), 4.17 (1H, t, J = 4.8 Hz, H-3′), 4.20 (1H, t, J = 5.4 Hz, H-2′), 5.72 (1H, d, J = 7.8 Hz, H-5), 5.92 (1H, d, J = 4.8 Hz, H-1′), 8.02 (1H, d, J = 7.8 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 62.3 (C-5′), 71.3 (C-3′), 75.7 (C-2′), 86.4 (C-4′), 90.7 (C-1′), 102.7 (C-5), 142.7 (C-6), 152.5 (C-2), 166.2 (C-4).

3. Results and discussion

Compound **1** was isolated as a white solid, $[\alpha]_D^{25}$ = -11.2° (c = 0.2, MeOH). Its ESI-MS showed a sodium adduct peak $[M+Na]^+$ at *m/z* 493. Analysis of the ^{1}H-NMR spectrum of **1** revealed signals of three methyl groups at δ_H 1.15 (13-CH_3), 1.89 (17-CH_3) and 2.13 (19-CH_3), a phenyl ring at δ_H 7.49 (2H, t, J = 7.8 Hz, H-4′, 6′), 7.59 (1H, t, J = 7.2 Hz, H-5′), 8.01 (2H, d, J = 7.8 Hz, H-3′, 7′), and the remaining protons in the aliphatic region at δ_H 1.21-5.94. The ^{13}C-NMR and DEPT spectra with the aid of HSQC spectrum of **1** showed the presence of twenty six carbons including four carbonyl groups (δ_C 166.1, 170.2, 170.7 and 173.3), 3 methyl groups (δ_C 20.6, 21.0 and 27.8), 7 methylene groups (δ_C 17.9, 29.1, 31.9, 37.2, 66.0, 67.0 and 71.3), five sp^2 methine groups (δ_C 128.8, 129.7 x 2 and 133.3 x 2), two sp^3 methine groups (δ_C 54.3, 66.5), and five non-protonated carbons (δ_C 37.8, 40.6, 122.9, 129.5 and 165.6). The chemical shifts of carbon signals at δ_C 66.5 (C-6), 67.0 (C-14), 71.3 (C-15) suggested their linkage to oxygen. These data were consistent with a drimane-type

sesquiterpene, which was further supported by 2D-NMR data [5],[6],[7]. Analysis of the COSY spectrum of **1** indicated three spin-spin interaction systems of protons: H-1 (δ_H 1.35, 2.27)/H-2 (δ_H 1.65, 1.75)/H-3 (δ_H 1.21, 1.92), H-5 (δ_H 1.82)/H-6 (δ_H 5.94)/ H-7 (δ_H 2.73) and H-3′ (δ_H 8.01)/H-4′ (δ_H 7.59)/H-5′ (δ_H 7.49). Two acetyl groups were placed at C-14 and C-15 from the HMBC correlations of H-14 (δ_H 3.97, 4.41), H-17 (δ_H 1.89) to acetyl carbonyl carbon C-16 (δ_C 170.7) and H-19 (δ_H 2.13), H-15 (δ_H 4.84, 5.00) to C-18 (δ_C 170.2), respectively. Moreover, correlations between H-3′ (δ_H 8.01) and C-1′ (δ_C 166.1) suggested that the phenyl group was located at C-1′. The five-membered lactone ring of **1** was deduced from the cross-peaks of H-7 and H-11 with C-8, C-9, C-12. Thus, the planar structure of **1** were established as shown in Fig. 1. The coupling constant of H-6 to H-7 (J = 5.4 Hz) indicated that H-6 was equatorial and thus the benzoate group was in the axial position [8]. In addition, due to small coupling constant of H-5 to H-6 (br s), H-5 was assigned at α position [9],[10]. Careful analysis of the 2D-NMR spectra of **1** and comparision with reference data determined the structure of **1** as astellolide A [8].

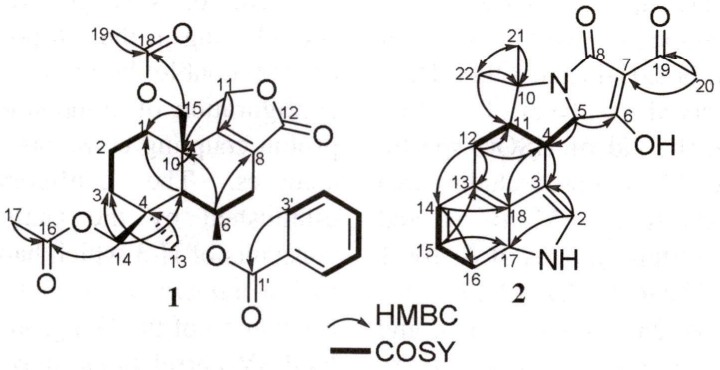

Fig. 3. Key COSY and HMBC interactions of compounds **1** and **2**

Compound **2** was isolated as a white solid, $[\alpha]_D^{25}$ = -89° (c = 0.2, $CHCl_3$). The ESI mass spectrum of **2** showed a pseudomolecular ion peak at m/z 337 $[M+H]^+$. In the ^{1}H-NMR spectrum of **2** proton signals of a 1,2,3-trisubstituted benzene ring at δ_H 6.80 (1H, d, J = 6.8 Hz, H-14), 7.03 (1H, dd, J = 7.2, 6.8 Hz, H-15), 7.15 (1H, m, H-16), two singlet methyl groups at δ_H 1.58 (3H, s, H-22), 1.60 (3H, s, H-21), one acetyl group at δ_H 2.39 (3H, s, H-20) were observed. The signals of 5 aliphatic protons at δ_H 2.47- 3.87 were also noted. The ^{13}C-NMR and DEPT spectra of **2** showed the presence of twenty carbons, including two carbonyl groups at δ_C 173.8 (C-8), 195.2 (C-19), four sp^2 methine groups at δ_C 109.5 (C-16), 116.6 (C-14), 122.1 (C-2), 123.3 (C-15), three sp^3 methine groups at δ_C 38.1 (C-4), 55.3 (C-11), 71.7 (C-5), one sp^3 methylene group at δ_C 27.9 (C-12), and seven non-protonated carbons. The chemical shifts of C-10 (δ_C 63.4) and C-5 (δ_C 71.7) suggested their linkage to nitrogen atom. In the COSY spectrum, two spin-spin interaction systems: H-14 (δ_H 6.80)/H-15 (δ_H 7.03)/H-16 (δ_H 7.15) and H-12 (δ_H 3.00, 3.06)/H-11 (δ_H 2.47)/H-4 (δ_H 3.54)/H-5 (δ_H 3.87) were observed. In the HMBC spectrum of **2** correlations between Me-21 (δ_H 1.60) and C-10 (δ_C 63.4), C-22 (δ_C 25.8) and C-11 (δ_C 55.3), between Me-22 (δ_H 1.58) and C-10 (δ_C 63.4), C-21 (δ_C 26.3) and C-11 (δ_C 55.3) confirmed the positions of Me-21 and Me-22 groups at C-10 as shown in Fig. 3. The HMBC correlations from the remaining methyl group at δ_H 2.39 (Me-20) to the carbonyl carbon C-19 (δ_C 195.2) and C-7 (δ_C 106.5) confirmed the position of this acetyl group at C-7. Thus, the planar structure of **2** was established as shown in Fig. 1. The large value of $^3J_{H\text{-}4,\ H\text{-}5}$ (10.8 Hz) prompted that H-4 and H-5 were in *trans*-configuration [11],[12]. Detailed analyses of the 2D-NMR spectra of **2** and comparison with the reference data determined the structure of **2** as cyclopiazonic acid [13],[14],[15]. Cyclopiazonic acid was an indole-tetramic acid described for the first time from

Aspergillus flavus in 1977 and this is the first report of the isolation of this compound from *Micromonospora aurantiaca* G657. Cyclopiazonic acid was reported to have moderate scavenging activity against DPPH with an IC_{50} value of 190.1 µg/mL [15].

Compound **3** was isolated as a pale yellow solid and its molecular formula of $C_{15}H_{13}N_2O_2$ was established by HR-ESI-MS at *m/z* 253.0976 $[M+H]^+$ (calcd. for $C_{15}H_{14}N_2O_2$ *m/z* 253.0977). The ^{1}H-NMR spectrum of **3** showed the signals of two methyl singlets at δ_H 1.56 (6H, s, H-16, H-17), four aromatic protons at δ_H 8.36 (1H, s, H-9), 7.60 (1H, d, J = 9.0 Hz, H-11), 7.56 (1H, dd, J = 6.6, 9.0 Hz, H-12), 6.96 (1H, d, J = 6.6 Hz, H-13). Additionally, the appearance of two broad singlet signals at δ_H 10.78 (1H, br s, H-1) and 8.96 (1H, br s, H-6), were also observed. Analyses of the ^{13}C-NMR and DEPT with the aid of HSQC spectra revealed the presence of 15 carbons, including two amide carbonyl carbons at δ_C 165.3 (C-5), and 166.7 (C-2), four sp^2 methine groups at δ_C 106.1 (C-13), 119.8 (C-11), 130.9 (C-12), 124.0 (C-9), two methyl groups at δ_C 28.7 (C-16, C-17), and seven non-protonated carbons at δ_C 124.0 (C-3), 126.6 (C-15), 129.9 (C-4), 131.2 (C-10), 139.8 (C-14), 154.2 (C-8). The chemical shift of C-7 (δ_C 59.1) of **3** suggested its linkage to nitrogen. The structure of **3** was then confirmed as cyclopiamide F by detailed analyses of the 2D-NMR spectra of **3**. Its NMR data were consistent with those reported in the literature [16].

Compound **4** was isolated as a white solid. ESI-MS of **4** showed a pseudomolecular ion peak at *m/z* 413 $[M+H]^+$. The ^{1}H-NMR spectrum of **4** showed signals of six methyl groups at δ_H 0.66 (3H, s, H-18), 0.87 (3H, d, J = 7.2 Hz, H-27), 0.88 (3H, d, J = 6.6 Hz, H-26), 0.96 (d, J = 6.6 Hz, H-21), 1.06 (3H, d, J = 6.6 Hz, H-28), 1.09 (3H, s, H-19), two *trans*-oriented olefinic protons at δ_H 5.24 (1H, dd, J = 15.3, 7.6 Hz, H-23), 5.25 (1H, dd, J = 15.3, 7.6 Hz, H-22), one olefinic proton at δ_H 5.28 (1H, J = 5.2 Hz, H-7), two oxygenated methine protons at δ_H 3.56 (1H, d, J = 4.8 Hz, H-6), 4.01 (1H, dt, J = 11.4, 5.4 Hz, H-3) and the remaining protons in the aliphatic region at δ_H 1.31-2.14. These ^{1}H-NMR data of **4** were suggestive of a steroid compound. In the ^{13}C-NMR and DEPT spectra of **4**, the presence of twenty eight carbon signals were noted, including six methyl groups (δ_C 11.8, 17.2, 18.2, 19.0, 19.4, 20.2), seven sp^3 methylene groups (δ_C 21.9, 22.9, 28.1, 30.8, 32.9, 39.5), three sp^2 methine groups (δ_C 118.1, 132.3, 135.9), eight sp^3 methine groups (δ_C 33.4, 36.9, 39.7, 40.8, 42.9 x 2, 54.9, 56.4, 67.4, 73.2), two sp^3 quaternary carbons (δ_C 36.9, 43.4), one oxygenated carbon (δ_C 75.9) and one sp^2 non-protonated carbon (δ_C 142.8). The chemical shifts of the carbon signals at δ_C 67.4 (C-3), 73.2 (C-6), 75.9 (C-5) suggested their linkage to oxygen. The HMBC correlations from H-7 (δ_H 5.28) to C-5, C-8, C-9, C-14 and from H-6 (δ_H 3.56) to C-5, C-7, C-8, along with the ESI-MS data of **4** suggested the formation of an epoxide ring in the 5,6-position and the position of the double bond at C-7/C-8. The relative configuration of compound **4** was suggested by proton coupling constants and NOESY spectrum analyses. The configuration of C-3 was established by the analysis of the coupling constants of H-3 which had an *anti* (J = 11.4 Hz) and a *gauche* (J = 5.4 Hz), indicating a β-orientation of the OH group at C-3 [17],[18]. The NOESY correlations of H-3 with H-6 suggested the α configuration of the 5,6-epoxide ring. The NOESY correlations between Me-18, Me-19 and H-20 indicated that they were oriented on the same side of the molecule. The configuration at C-24 of compound **4** was determined by comparison of the ^{13}C-NMR chemical shifts with those of the related structures [19],[20]. Accordingly, due to the chemical shift difference of 0.4 ppm between C-26 and C-27 and the chemical shift of C-28 at δ_C 17.2 ppm, *R*-configuration at C-24 was suggested for compound **4**. Furthermore, a large coupling constant between H-22/H-23 (J = 15.3 Hz) assigned *E*-configuration for the C-22/C-23 double bond. Thus, detailed analysis of the 2D-NMR, MS data of **4** and comparison with the reported values identified the structure of **4** as 5,6-epoxyergosterol [21],[22]. Compound **4** was reported to exhibit significant cytotoxic effect against HL-60 cell line with IC_{50} value of 7.3 µg/mL [23].

Compound **5** was obtained as a pale-yellow solid. The ESI-MS of **5** indicated a pseudo-molecular ion peak at *m/z* 146 $[M+H]^+$. The ^{13}C-

NMR and DEPT spectra of **5** exhibited signals of nine carbons including a carbonyl group (δ_C 185.6), five sp^2 methine groups (δ_C 111.5, 122.1, 123.1, 124.5 and 135.2), and three sp^2 non-protonated carbons (δ_C 119.8, 123.8 and 136.6). Analysis of the ^{1}H-NMR spectrum of **5** showed the characteristic signals of an indole skeleton including a doublet proton at δ_H 7.85 (1H, d, J = 3.0 Hz, H-2), four aromatic protons (δ_H 7.31, 7.32, 7.44 and 8.32) and one proton at the down-field NMR region at δ_H 10.08 (s, -CHO) which was characteristic for an aldehyde group. Thus, analyses of the MS and 1D-NMR spectra of **5** and comparison with reported data identified compound **5** as 3-indolecarbadehyde [24].

Compound **6** was obtained as a pale yellow solid. Its ESI-MS showed the protonated molecular ion peak at *m/z* 243 $[M+H]^+$. The ^{1}H-NMR spectrum of **6** showed the singlet signals of one methyl group at δ_H 1.90 (3H, s) and one aromatic proton at δ_H 7.83 (1H, s, H-6). Additionally, the appearance of proton signals at δ_H 2.25-6.29 were also observed. The ^{13}C-NMR and DEPT spectra of **6** exhibited signals of 10 carbons, including two carbonyl groups (δ_C 152.4 and 166.4), two methylene groups (δ_C 41.2 and 62.9), one sp^2 methine groups (δ_C 138.2, C-6), three sp^3 methine groups (δ_C 72.2, 86.3 and 88.8), and one sp^2 non-protonated carbon (δ_C 111.5). The chemical shifts of four carbon signals at δ_C 62.9, 72.2, 86.3 and 88.8 of **6** suggested their linkages to oxygen and nitrogen. Complete analyses of the 1D-NMR spectra and MS data of **6** and comparison of the NMR data with the reference data indicated that compound **6** was 2'-deoxythymidine [25].

Compound **7** was obtained as a pale yellow solid. Its ESI-MS showed the protonated molecular ion peak at *m/z* 245 $[M+H]^+$. The ^{1}H- and ^{13}C-NMR spectra of **7** were similar to those of **6**, except for the replacement of one methyl group and one methylene group in **6** with two methine groups. Detailed analysis of the 1D-NMR spectra, along with MS data and comparison with reported values in the literature determined the structure of **7** as uridine [26].

4. Conclusion

Analysis of an antimicrobial extract prepared from culture broth of the marine-derived actinomycete *Micromonospora aurantiaca* G657 led to the isolation of seven compounds. They were identified as astellolide A (**1**), cyclopiazonic acid (**2**), cyclopiamide F (**3**), 5,6-epoxyergosterol (**4**), 3-indolecarbadehyde (**5**), 2'-deoxythymidine (**6**) and uridine (**7**). To the best of our knowledge, this is the first report of the isolation of astellolide A (**1**), cyclopiazonic acid (**2**), cyclopiamide F (**3**) from *Micromonospora aurantiaca.* These compounds are commonly found in fungi (*Penicillum, Aspergillus* sp.).

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CEMBRANOID CONSTITUENTS FROM THE SOFT CORAL *SARCOPHYTON INFUNDIBULIFORME*

Nguyen Thi Huong, Ninh Thi Ngoc, Tran Thi Hong Hanh, Tran Hong Quang
Nguyen Van Thanh, Nguyen Phuong Thao, Nguyen Xuan Cuong*, Nguyen Hoai Nam
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)
*Corresponding author: cuongnx@imbc.vast.vn
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Summary

Cembranoid Constituents from the Soft Coral *Sarcophyton infundibuliforme*

Five cembranoids were isolated from the methanolic extract of the Vietnamese soft coral *Sarcophyton infundibuliforme* using combined chromatographic experiments. Their chemical structures were elucidated as sarcrassin D (**1**), emblide (**2**), 7α,8β-dihydroxydeepoxysarcophine (**3**), lobocrasol B (**4**), and sarcophine (**5**) on the basis of detailed analysis of the 1D and 2D NMR spectroscopic data in comparison with the literature values. This is the first report of these compounds from the soft coral *Sarcophyton infundibuliforme*.

Keywords: *Sarcophyton infundibuliforme, Soft coral, Cembranoid.*

1. Introduction

Soft corals are well-known as excellent sources of marine-derived natural products. Among them, members of the genera *Sarcophyton*, *Sinularia*, and *Lobophytum* are the most prolific and especially attractive targets for marine natural product research. These marine invertebrates constitute an important group of marine invertebrates widely distributed in the coral reefs of the world oceans and have proven to be a biochemical warehouse for terpenes, particularly cembranoid diterpenes [1]. *Sarcophyton* species contain diterpenes up to 10% of their dry weight, and this large quantity