A New Alkaloid from Marine-Derived Actinomycete

Actinoalloteichus cyanogriseus G631

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Abstract: A new alkaloid, isocyanogramide (1) together with six known compounds, including cyanogramide (2), marinacaroline F (3), marinacaroline H (4), caerulomycinamide (5), cerulomyconitrile (6), and 5′-deoxyuridine (7) were isolated from the agar culture of the marine-derived actinomycete Actinoalloteichus cyanogriseus G631. Compounds 1-7 were evaluated for antimicrobial activity against Gram (+) bacteria, Enterococcus faecalis, Staphylococcus aureus, and Bacillus cereus, Gram(−) bacteria, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, and the yeast, Candida albicans. All compounds significantly exhibited antibacterial effects on three tested Gram(−) bacteria and the yeast C. albicans with the MIC values ranging from 64 to 256 μg/mL. Compounds 4, 5, and 7 significantly inhibited B. cereus bacteria with MIC values of 16, 128, and 128 μg/mL, respectively.

Keywords: Actinoalloteichus cyanogriseus; marine actinomycetes; isocyanogramide; alkaloid; antibacterial activity; antifungal activity. © 2023 ACG Publications. All rights reserved.

1. Introduction

Actinoalloteichus genus belongs to the family Pseudonocardiaceae [1-3]. Actinoalloteichus species are also known as a producer of different types of secondary metabolites, including polycyclic tetramate macrolactams [4], polyene macrolactams [5], pyridine alkaloids [6, 7], and cyclopentenone

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derivatives [8]. As part of our project on investigating natural products that possess antimicrobial effects from marine-derived actinomycetes, the crude extract of Actinoalloteichus cyanogriseus G631 fermentation (isolated from the soft coral sample (Sarcophyton infundibuliforme Tixier-Durivault, 1958)) was found to exhibit antimicrobial effects against three Gram-(+) bacteria (E. faecalis, S. aureus, and B. cereus), one Gram-(–) bacteria (S. enterica), and one yeast (C. albicans) with MIC values of 64, 2, 16, 256, and 2 µg/mL respectively. Thus, chemical components of A. cyanogriseus G631 fermentation have been evaluated. Herein, we report the isolation, structural elucidation, and antimicrobial activity of one new and six known alkaloid compounds from the fermentation of the actinomycete strain A. cyanogriseus G631.

![Chemical structures of compounds 1-7](image)

**Figure 1.** Chemical structures of compounds 1-7

2. Materials and Methods

2.1. General Experimental Procedures and Fermentation

Detailed information of experimental procedure were given in supporting information file of the article.

2.2. Isolation and Taxonomy Identification

Actinoalloteichus cyanogriseus G631 strain was isolated from a soft coral (Sarcophyton infundibuliforme Tixier-Durivault, 1958), collected at a depth of 8 meters at Phu Quy, Binh Thuan, Vietnam in April 2021. The soft coral was identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources, VAST. A. cyanogriseus G631 were identified according to its morphological characteristics and 16S rRNA gene sequences (GenBank accession number OM190414).

2.3. Extraction and Isolation

The agar culture of A. cyanogriseus G631 (30 L) was minced and then extracted with ethyl acetate (EtOAc) (4 times, each 5 L) using a sonicator. The EtOAc layer was concentrated to give an EtOAc extract (EG631, 5.6 g). The EG631 extract was chromatographed on a silica gel chromatography column (CC) eluting with gradient solvents of n-hexane:CH₂Cl₂ (2:1, 1:1, 1:0, v/v) and then CH₂Cl₂:MeOH (40:1, 20:1, 10:1, 5:1, v/v) to afford seven fractions (F1-F7). F5 fraction (508 mg) was subjected to a Sephadex LH-20 column chromatography eluting with MeOH to give five fractions (F5.1-F5.7). F5.2 fraction was chromatographed on a silica gel CC using eluent solvent of n-
A new sesquiterpene from *Schisandra sphenanthera*

hexane:acetone (5:1, v/v) to yield 6 (3.5 mg). The F5.3 fraction was purified on an HPLC (J’sphere H-80 column, 250 mm length × 20 mm ID, a flow rate of 3 mL/min eluting with 90 % MeOH in water) to yield 1 (2.0 mg, t<sub>R</sub> = 32 min) and 2 (15 mg, t<sub>R</sub> = 27 min). The F5.5 fraction was separated on an HPLC eluting with 90 % MeOH in water to yield 3 (3.0 mg, t<sub>R</sub> = 31 min) and 4 (2.5 mg, t<sub>R</sub> = 36 min). F6 fraction was subjected to a Sephadex LH-20 CC eluting with MeOH and then followed by a silica gel CC (CH<sub>2</sub>Cl<sub>2</sub>: acetone, 10:1, v/v) to yield compound 5 (4.0 mg). Compound 7 (4.5 mg) was obtained from the F7 fraction by purifying on a silica gel CC eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (5:1, v/v).

**Isocyanogramide (I):** Yellow oil, [α]<sub>D</sub><sup>25</sup> = -59.3 (c 0.2, CH<sub>2</sub>Cl<sub>2</sub>); CD: λ<sub>max</sub> (Δε, rel) 221 (-0.58), 238 (+0.55), 259 (-1.00), 278 (+0.21), and 309 (-0.27) nm; HR-ESI-MS m/z: 416.1610 [M+H]<sup>+</sup> (calcd. for [C<sub>23</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub>]<sup>+</sup>, 416.1605); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR, see Table 1.

2.4. Antimicrobial Assays

Antimicrobial activity studies were carried out in accordance with the method given in the literature. In order to avoid unnecessary repetition in the main text, the details are given in the supporting information file of this article in accordance with the relevant literature [9-11].

3. Results and Discussion

3.1. Structure Elucidation

Compound 1 was obtained as a yellow oil. The HR-ESI-MS of 1 showed a pseudo-molecular ion peak [M+H]<sup>+</sup> at m/z 416.1610, confirming the molecular formula of C<sub>23</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>. The <sup>1</sup>H-NMR spectrum of 1 showed proton signals of one mono-substituted benzene ring at δ<sub>H</sub> 7.28 (1H, d, J = 7.2 Hz, H-6'), 7.32 (2H, d, J = 7.2 Hz, H-4'/H-8'), and 7.37 (2H, t, J = 7.2 Hz, H-5'/H-7'), one ortho-disubstituted benzene ring at δ<sub>H</sub> 6.93 (1H, d, J = 7.8 Hz, H-6), 7.11 (1H, dd, J = 7.2, 7.8 Hz, H-4), 7.13 (1H, d, J = 7.8 Hz, H-3), and 7.40 (1H, dd, J = 7.2, 7.8 Hz, H-5), two olefinic protons at δ<sub>H</sub> 5.86 (1H, d, J = 9.6 Hz, H-1') and 6.65 (1H, d, J = 9.6 Hz, H-2'), suggested the presence of a double bond, three methyl groups at δ<sub>H</sub> 1.95, 3.27, and 3.31 (each 3H, s). The <sup>13</sup>C-NMR and HSQC spectra of 1 exhibited signals of 24 carbons, including three carbonyls at δ<sub>C</sub> 154.3, 167.5, and 170.0, six-non- protonated carbons at δ<sub>C</sub> 70.2, 98.6, 124.4, 135.0, 140.2, and 144.9, twelve olefinic methines at δ<sub>C</sub> 106.4, 109.2, 115.9, 123.5, 124.3, 128.3, 128.4 × 2, 128.5 × 2, 130.3, and 130.8, and three methyl carbons at δ<sub>C</sub> 23.8, 27.1, and 50.2 (Table 1). Analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR data of 1 indicated its structure was similar to that of cyanogramide (2), a compound that has been isolated from the same species, *Actinoalloteichus cyanogriseus* [12]. The HMBC correlations between H-16 (δ<sub>H</sub> 5.84) and C-1 (δ<sub>C</sub> 70.2)/C-2 (δ<sub>C</sub> 124.4)/C-10 (δ<sub>C</sub> 167.5)/C-14 (δ<sub>C</sub> 154.3)/C-15 (δ<sub>C</sub> 140.2) and between N-methyl protons (δ<sub>H</sub> 3.27) and C-7 (δ<sub>C</sub> 144.9)/C-9 (δ<sub>C</sub> 170.0) proved the presence of spiro rings at C-1 and carbonyl groups at C-9.

![Figure 2. The key HMBC, COSY, and NOE correlations of 1](image-url)
and C-10 (Figure 2). The ortho-disubstituted benzene at C-2/C-7 was confirmed by HMBC correlations between H-3 (δ_H 7.14) and C-1 (δ_C 70.2)/C-2 (δ_C 124.4)/C-7 (δ_C 144.9), between H-6 (δ_H 6.93) and C-2 (δ_C 124.4)/C-7 (δ_C 144.9) as well as the COSY correlations of H-3 (δ_H 7.14)/H-4 (δ_H 7.11)/H-5 (δ_H 7.40)/H-6 (δ_H 6.93). The presence of phenyl ethenyl was confirmed by HMBC correlations between H-2' (δ_H 6.65) and C-1' (δ_C 115.9)/C-3' (δ_C 135.0)/C-4' (δ_C 128.4) and COSY correlations of H-4' (δ_H 7.32)/H-5' (δ_H 7.37)/H-6' (δ_H 7.28)/H-7' (δ_H 7.37)/H-8' (δ_H 7.32) and H-1' (δ_H 5.86)/H-2' (δ_H 6.65). In addition, the coupling constant between H-1' and H-2', J = 9.6 Hz and also NOESY correlations between H-1' (δ_H 5.86) and H-2' (δ_H 6.65) suggested the geometry of the double bond of C-1' and C-2' is Z [11]. The position of this group at N-13 of tetrahydroimidazolone was confirmed by HMBC correlations from H-1' (δ_H 5.86) and C-12 (δ_C 98.6)/C-14 (δ_C 154.3). Both methyl and methoxy groups at C-12 was proved by HMBC correlations from H-17 (δ_H 1.95) and methoxy proton (δ_C 3.31) to C-12 (δ_C 98.6). Compound 1 has two stereogenic centers, C-1 and C-12. Thus, the experimental ECD has been recorded and shown the negative Cotton effects (CEs) 221, 259 and 309 nm and positive CEs at 238 and 278 nm (Figure 3), similar to that of cyanogramide (2) [13], suggesting the absolute configurations of two stereogenic centers at C-1 and C-12 to be R and S, respectively. Consequently, new structure of compound 1 was elucidated as spirocyclic pirrolo[1,2-c]imidazole, named isocyanogramide.

Table 1. NMR Data for Compounds 1 and 2, and reference compound

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*a*recorded in CDCl₃, *b*δ_C of cyanogramide recorded in DMSO-d₆ [10], *overlapped signals.
A new sesquiterpene from *Schisandra sphenanthera*

The known compounds were determined to be cyanogramide (2) [12], marinacarbinol F (3) [14], marinacarbinol H (4) [14], caerulomycinamide (5) [6], cerulomyconitrile (6) [6], and 5'-deoxyuridine (7) [14]. Their chemical structures were elucidated by their spectroscopic data and comparing with those reported NMR data in the literature data.

3.2 Antibacterial and Antifungal Activities

All compounds were evaluated for their antibacterial and antifungal activities against Gram-(+), Gram-(−), and yeast, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus*, and *Candida albicans* (Table 2). Streptomycin and cycloheximide were used as positive controls. All compounds exhibited significant antibacterial effects on Gram-(+) bacteria, *E. coli, S. aureus* and the yeast *C. albicans* with MIC values ranging from 64 to 256 µg/mL. Compounds 4, 5, and 7 significantly inhibited *B. cereus* bacteria with MIC values of 64, 128, and 128 µg/mL, respectively. In addition, compounds 2 and 4 also showed potent inhibitory activity against the Gram-(−) bacteria *P. aeruginosa* with a MIC value of 64 µg/mL. This is the first report of the antimicrobial activity of isolated compounds. These compounds may be potential antibacterial sources against Gram-(+) bacteria.

**Table 2. Antibacterial and antifungal activities of compounds 1-7**

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(-) compound did not exhibit antimicrobial and antifungal activities (MIC >256 µg/mL)

**Acknowledgment**

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

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