ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 17:6 (2023) 1024-1030

records of natural products

A New Alkaloid from Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* G631

Do Thi Quynh [1,2], Trinh Thi Thanh Van [1], Nguyen Thuy Linh [1], Le Thi Hong Minh [1], Vu Thi Quyen [1], Brian T. Murphy [3], Doan Thi Mai Huong [1,2,*] and Pham Van Cuong [1,2*]

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

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

[3]*Department of Pharmaceutical Sciences, Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States*

(Received May 7, 2023; Revised August 28, 2023; Accepted September 01, 2023)

Abstract: A new alkaloid, isocyanogramide (**1**) together with six known compounds, including cyanogramide (**2**), marinacarboline F (**3**), marinacarboline 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.

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

* Corresponding authors: E-Mail: huongdm@imbc.vast.vn (D.T.M Huang), phamvc@imbc.vast.vn (P.V. Cuong)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products November-December 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.412.2305.2776
Available online: September 18, 2023

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.

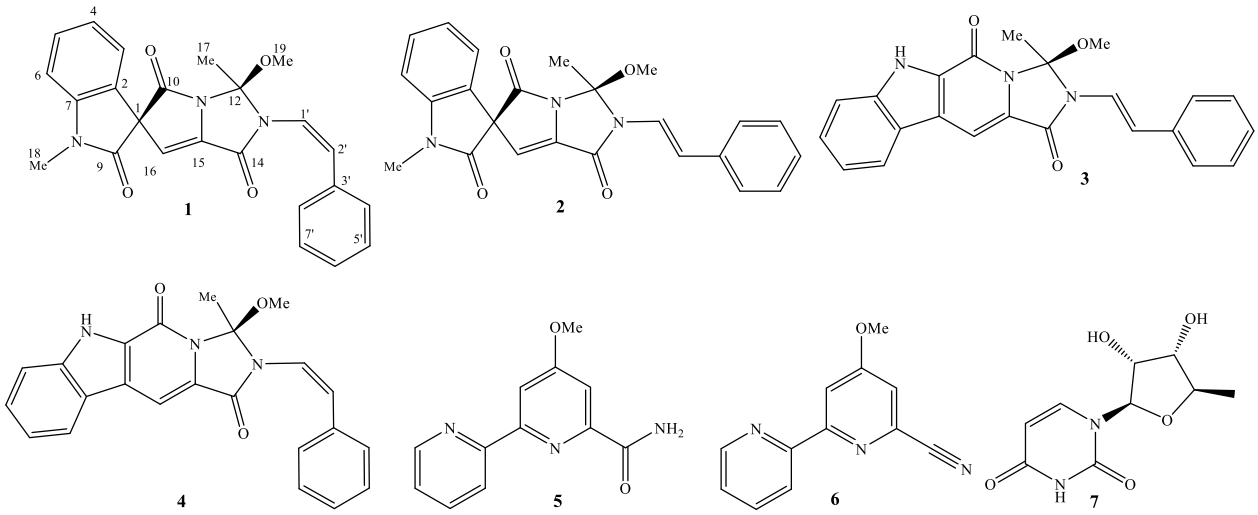

Figure 1. Chemical structures of compounds **1-7**

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:CH_2Cl_2 (2:1, 1:1, 1:0, v/v) and then CH_2Cl_2: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*-

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_R = 32 min) and **2** (15 mg, t_R = 27 min). The F5.5 fraction was separated on an HPLC eluting with 90 % MeOH in water to yield **3** (3.0 mg, t_R = 31 min) and **4** (2.5 mg, t_R = 36 min). F6 fraction was subjected to a Sephadex LH-20 CC eluting with MeOH and then followed by a silica gel CC (CH_2Cl_2: 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_2Cl_2:MeOH (5:1, v/v).

*Isocyanogramide (**1**)*: Yellow oil, $[\alpha]_D^{25}$ -59.3 (*c* 0.2, CH_2Cl_2); CD: λ_{max} ($\Delta\varepsilon$, 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 $[M+H]^+$ (calcd. for $[C_{24}H_{22}N_3O_4]^+$, 416.1605); ^{1}H-NMR and ^{13}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 $[M+H]^+$ at *m/z* 416.1610, confirming the molecular formula of $C_{24}H_{21}N_3O_4$. The ^{1}H-NMR spectrum of **1** showed proton signals of one mono-substituted benzene ring at δ_H 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_H 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_H 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_H 1.95, 3.27, and 3.31 (each 3H, s). The ^{13}C-NMR and HSQC spectra of **1** exhibited signals of 24 carbons, including three carbonyls at δ_C 154.3, 167.5, and 170.0, six-non- protonated carbons at δ_C 70.2, 98.6, 124.4, 135.0, 140.2, and 144.9, twelve olefinic methines at δ_C 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_C 23.8,

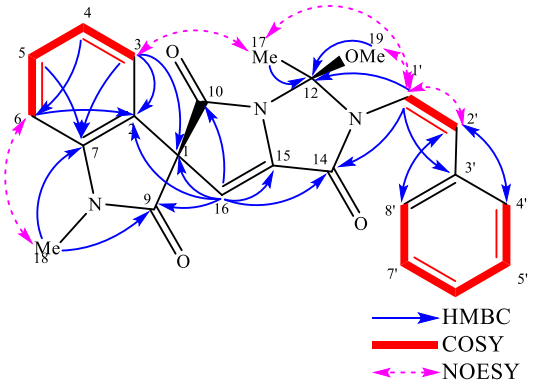

Figure 2. The key HMBC, COSY, and NOE correlations of **1**

27.1, and 50.2 (Table 1). Analysis of ^{1}H- and ^{13}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_H 5.84) and C-1 (δ_C 70.2)/C-2 (δ_C 124.4)/C-10 (δ_C 167.5)/C-14 (δ_C 154.3)/C-15 (δ_C 140.2) and between *N*-methyl protons (δ_H 3.27) and C-7 (δ_C 144.9)/C-9 (δ_C 170.0) proved the presence of spiro rings at C-1 and carbonyl groups at C-9

and C-10 (Figure 2). The *ortho*-disubstituted bezene ring 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 prence 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′ (8′) (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 tetrahydroimidazole 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

	1		***2***		
C	$\delta_C{}^a$	$\delta_H{}^a$ **mult. (*J* in Hz)**	$\delta_C{}^a$	$\delta_H{}^a$ **mult. (*J* in Hz)**	$\delta_C{}^b$
1	70.2	-	70.1	-	69.8
2	124.4	-	124.4	-	124.6
3	124.3	7.14 (1H, d, *J* = 7.8 Hz)	124.2	7.10 (1H, d, *J* = 7.5 Hz)	124.5
4	123.5	7.11 (1H, dd, *J* = 7.2, 7.8 Hz)	123.5	7.11(1H)*	123.1
5	130.3	7.40 (1H, dd, *J* = 7.2, 7.8 Hz)	130.3	7.40 (1H, dd, *J* = 7.5, 7.5 Hz)	130.1
6	109.2	6.93 (1H, d, *J* = 7.8 Hz)	109.2	6.93 (1H, d, *J* = 7.5 Hz)	109.6
7	144.9	-	144.9	-	144.8
9	170.0	-	169.8	-	169.5
10	167.5	-	167.4	-	167.4
12	98.6	-	99.1	-	98.7
14	154.3	-	154.6	-	154.6
15	140.2	-	139.3	-	138.6
16	106.4	5.84 (1H, s)	106.4	5.90 (1H, s)	107.2
17	23.8	1.95 (3H, s)	23.0	2.10 (3H, s)	21.8
18	27.1	3.27 (3H, s)	27.2	3.27 (3H, s)	26.9
19	50.2	3.31 (3H, s)	50.4	3.36 (3H, s)	49.4
1′	115.9	5.86 (1H, d *J* = 9.6 Hz)	118.5	7.22 (1H, d, *J* = 15.0 Hz)	118.9
2′	130.8	6.65 (1H, d *J* = 9.6 Hz)	118.7	6.96 (1H, d, *J* = 15.0 Hz)	118.0
3′	135.0	-	135.5	-	135.5
4′, 8′	128.4	7.32 (2H, d, *J* = 7.2 Hz)	126.1	7.42 (1H, d, *J* = 7.5 Hz)	126.1
5′, 7′	128.5	7.37 (2H, t, *J* = 7.2 Hz)	128.8	7.35 (1H, t, *J* = 7.5 Hz)	128.8
6′	128.3	7.28 (1H, t, *J* = 7.2 Hz)	127.8	7.27 (1H, t, *J* = 7.5 Hz)	127.6

[a)]recorded in CDCl3, [b)]δ_C of cyanogramide recorded in DMSO-d_6 [10], *overlappted signals.

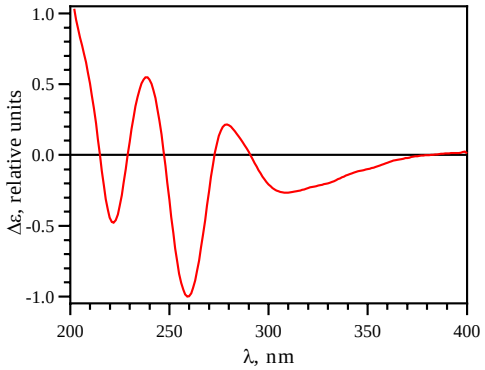

Figure 3. Experimental CD spectrum **1**

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

3.2 Antibacterial and Antifungal Activities

All compounds were evaluated for their antibacterial and antifungal activities against Gram-(+) and Gram-(–) bacteria 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. aurerus* 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 16, 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**

Compounds	**MIC (µg/mL)**						
	Gram-(+)			**Gram-(–)**			**Yeast**
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
1	128	64	-	-	-	-	128
2	128	128	-	32	64	-	32
3	64	128	-	256	-	-	64
4	64	128	16	-	64	-	128
5	128	64	128	-	-	-	32
6	128	128	-	-	-	-	128
7	64	256	128	-	-	-	64
Streptomycin	256	256	128	32	256	128	
Cyclohexamide							32

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

Acknowledgment

This research is funded by Vietnam Academy of Science and Technology (Grant No: TĐDLB0.03/20-22) and University of Illinois Campus Research Board grant (5D43 TW010530-05).

Supporting Information

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

ORCID
Do Thi Quynh: 0009-0004-1539-5460
Trinh Thi Thanh Van: 0009-0003-2766-2215
Nguyen Thuy Linh: 0000-0002-1817-0687
Le Thi Hong Minh: 0000-0002-1710-942X
Vu Thi Quyen: 0009-0000-3606-2375
Brian T. Murphy: 0000-0002-1372-3887
Doan Thi Mai Huong: 0000-0003-3252-870X
Pham Van Cuong: 0000-0003-1317-6382

References

[1] M. Girão, I. Ribeiro and M. d. F. Carvalho.(2022). Actinobacteria from marine environments: A unique source of natural products, In: Natural products from *Actinomycetes*, ed: Rai, R. V., Bai, J. A. Springer Singapore, Singapore, pp. 1-45.

[2] T. Tamura, L. Zhiheng, Z. Yamei and K. Hatano (2000). *Actinoalloteichus cyanogriseus* gen. nov., sp. nov, *Int. J. Syst. Evol. Microbiol*. **50**, 1435-1440.

[3] D. Cao Duc, T. Cao Duc, V. T. Quyen, A. Nguyen Mai, H. Doan Thi Mai, C. Pham Van, M. Chau Van and L. T. H. Minh (2018). Identification and antimicrobial activity of *Actinomycetes* strains isolated from samples in the coastal area of Thanh Hoa – Quang Binh – Quang Tri, *Vietnam J. Sci. Tech*. **56**, 424-433.

[4] M. Xiangui, W. Liping, W. Dongyang, F. Jie and Z. Weiming (2017). Polycyclic tetramate macrolactams from the marine-derived *Actinoalloteichus cyanogriseus* WH1-2216-6, *Chinese J. Org. Chem.* **37**, 2352-2360.

[5] K. Fujita, R. Sugiyama, S. Nishimura, N. Ishikawa, M. A. Arai, M. Ishibashi and H. Kakeya (2016). Stereochemical assignment and biological evaluation of BE-14106 unveils the importance of one acetate unit for the antifungal activity of polyene macrolactams, *J. Nat. Prod.* **79**, 1877-1880.

[6] P. Fu, S. Wang, K. Hong, X. Li, P. Liu, Y. Wang and W. Zhu (2011). Cytotoxic bipyridines from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6, *J. Nat. Prod.* **74**, 1751-1756.

[7] X. Mei, M. Lan, G. Cui, H. Zhang and W. Zhu (2019). Caerulomycins from *Actinoalloteichus cyanogriseus* WH1-2216-6: isolation, identification and cytotoxicity, *Org. Chem. Front.* **6**, 3566-3574.

[8] X. J. Wang, J. Zhang, P. T. Qian, J. D. Wang, C. X. Liu and W. S. Xiang (2014). Three new cyclopentenone derivatives from *Actinoalloteichus nanshanensis* NEAU 119, *J. Asian Nat. Prod. Res.* **16**, 587-592.

[9] J. M. Andrews (2001). Determination of minimum inhibitory concentrations, *J. Antimicrob. Chemother*. **48**, 5-16.

[10] L.Yanping, S. Sheng J.Feng, Y. Wang, J. Guo, Y. Jiang and W. Wang (2022). New cyclic Peptides from the endophytic *Aspergillus versicolor* 0312 with their antimicrobial activity, *Rec. Nat. Prod*. **16**(**6**), 585-591.

[11] N. Peerakam, P. Phoowiang, S. Chansakaow, C. Thongpoon and S. Hadpech (2022). Chemical profiling revealed a dominant compound trans-anethole and biological evaluation of an edible plant clausena harmandiana containing essential oil, *Rec. Nat. Prod*. **16**(**2**), 118-127.

[12] P. Fu, F. Kong, X. Li, Y. Wang and W. Zhu (2014). Cyanogramide with a new spiro [indolinone-pyrroloimidazole] skeleton from *Actinoalloteichus cyanogriseus*, *Org. Lett.* **16**, 3708-3711.

[13] L. Qin, W. Yi, X. Y. Lian and Z. Zhang (2020). Bioactive alkaloids from the actinomycete *Actinoalloteichus* sp. ZZ1866, *J. Nat. Prod.* **83**, 2686-2695.

[14] T. Bavaro, S. Rocchietti, D. Ubiali, M. Filice, M. Terreni and M. Pregnolato (2009). A versatile synthesis of 5′-functionalized nucleosides through regioselective enzymatic hydrolysis of their peracetylated precursors, *Eur. J. Org. Chem.* **2009**, 1967-1975.