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Actinobenzoquinoline and Actinophenanthrolines A–C, Unprecedented Alkaloids from a Marine Actinobacterium

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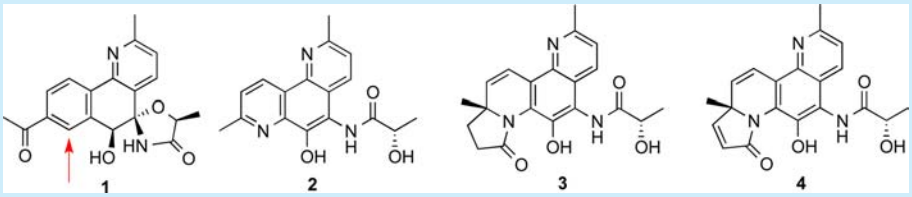
Supporting Information



ABSTRACT: Chemical investigation of a marine actinomycete within the family Streptomycetaceae (our strain CNQ-149) has led to the isolation of the unprecedented alkaloids, actinobenzoquinoline (**1**) and actinophenanthrolines A–C (**2**–**4**). The chemical structures of **1**–**4** were assigned by interpretation of NMR spectroscopic data, and their absolute configurations were assigned by X-ray analysis. Actinobenzoquinoline possesses a 5-methyloxazolidin-4-one moiety and a dihydrobenzo[*h*]quinoline core structure, while actinophenanthrolines A–C are composed of hydroxypropanamide-substituted 1,7-phenanthroline core skeletons.

Exploration of diverse, poorly examined environments will be important in the quest for new microbial strains producing novel natural products. Our program has focused on marine sediments as a source of actinomycete bacteria which differ from terrestrial strains.[1] In this regard, we have reported the identification of 13 marine actinomycete groups (MAR1–MAR13) based on phylogenetic analysis.[2] Chemical investigations of these groups have led to the discovery of unique secondary metabolites with a variety of bioactivities. These include a new class of macrocylic pyrroles, the marineosins A and B, from the MAR3 actinomycete CNQ-617,[3] several highly modified peptides, actinoramides A–C, from strain CNQ-027,[4] and more recently the isolation of a cytotoxic meroterpenoid, actinoranone,[5] as well as the known cyclic peptides, stendomycins.[6] Our strain CNQ-149 was isolated from a marine sediment sample collected off San Diego, CA. The strain requires seawater for growth and, based on all available sequence data, belongs to a subgroup of the streptomycetes that has only been cultured from marine samples. A comprehensive LC-MS-based investigation of the crude extract of this strain revealed several unknown compounds with molecular weights in the range of 300–400 amu possessing long wavelength UV chromophores. A large-scale fermentation followed by repeated purification of the organic extract resulted in the isolation of four new alkaloids, actinobenzoquinoline (**1**) and actinophenanthrolines A–C (**2**–**4**), representing two unprecedented natural alkaloid classes. Here we present the details of the isolation and characterization of these new alkaloids.

Actinobenzoquinoline (**1**)[7] was isolated as a pale yellow solid, which analyzed for the molecular formula $C_{19}H_{18}N_2O_4$ based on a pseudomolecular ion at *m*/*z* 361.1153 [M + Na]$^+$ in the HRESIMS data coupled with interpretation of ^{13}C NMR data. The ^{13}C NMR spectrum of **1** in combination with gHSQC data revealed three methyl, seven methine, and nine fully substituted carbons. The ^{1}H NMR spectrum of **1** further displayed five aromatic protons, two downfield protons, two methyl singlets, and one methyl doublet. Interpretation of 2D NMR spectroscopic data allowed three fragments to be constructed. The first fragment, a 1,2,4-trisubstituted benzene ring, was established based on interpretation of ^{1}H–^{1}H coupling constants and gCOSY NMR correlations [H-7 (δ 8.12, d, J = 1.0 Hz)/H-9 (δ 8.01, dd, J = 8.0, 1.0 Hz)/H-10 (δ 8.31, d, J = 8.0 Hz)]. gHMBC NMR correlations from the H-16 methyl singlet (δ 2.62) to the C-15 carbonyl carbon (δ 198.3) and to the C-8 aromatic carbon (δ 137.9) permitted the acetyl group to be positioned on C-8 of the benzene ring. Next, gCOSY NMR correlations between H-3 (δ 7.36, d, J = 8.5 Hz) and H-4 (δ 7.87, d, J = 8.5 Hz), and considering the ^{1}H–^{1}H coupling constants of these two protons, allowed the construction of a second fragment composed of a 2,3,6-trisubstituted pyridine ring. Positioning of a methyl group (H_3-14) at C-2 in the pyridine ring was facilitated by interpretation of gHMBC correlations from H_3-14 (δ 2.57) to carbons C-2 (δ 159.5) and C-3. The last fragment was assigned on the basis of gCOSY correlations between H_3-13 (δ 1.34, d, J = 6.8 Hz, 3H) and H-12 (δ 4.61, q, J = 6.8 Hz, 1H) and long-range

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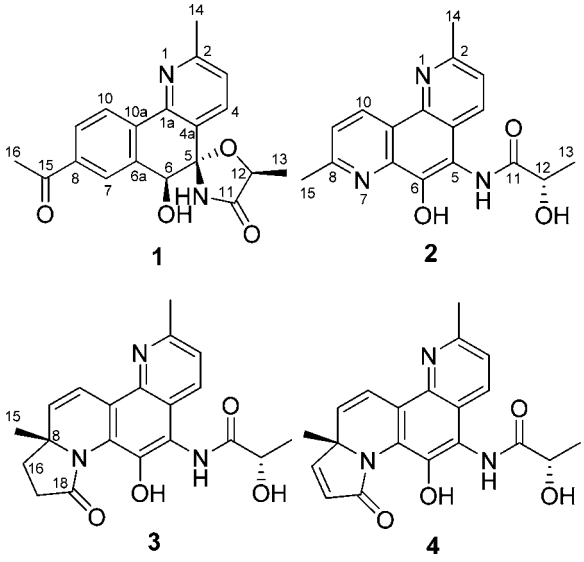
gHMBC correlations from protons H_3-13 to carbons C-11 (δ 174.7) and C-12 (δ 74.4).



The connection between the first and second fragments was achieved by interpretation of long-range gHMBC correlations (Figure 1a). The observation of three-bond HMBC correlations

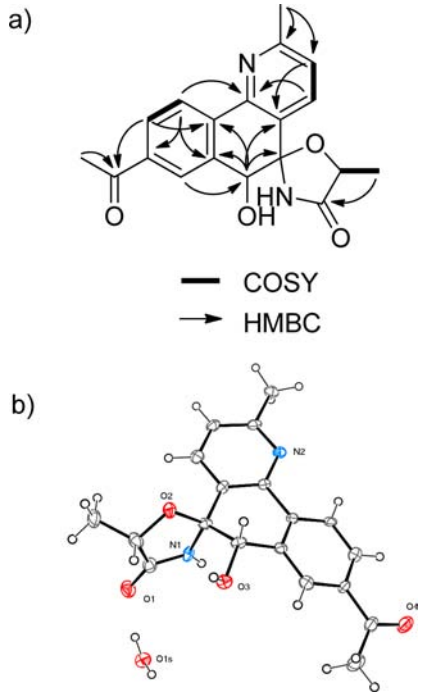


Figure 1. (a) gCOSY and key gHMBC correlations interpreted in the structure elucidation of actinobenzoquinoline (**1**). (b) Computer-generated X-ray crystal structure of actinobenzoquinoline **1**, showing a water of hydration and depicting the absolute configuration of 5*S*, 6*S*, and 12*S*.

from the H-4 and H-10 protons to carbon C-1a and from H-9 to C-10a permitted the connection of C-10a to C-1a. Three-bond gHMBC correlations from H-3 to C-4a and from H-4 to C-5 allowed the C-4a−C-5 bond to be constructed. In addition, long-range gHMBC correlations from the oxygenated methine proton H-6 to carbons C-4a, C-5, C-6a, and C-10a and from H-7 to carbon C-6 permitted the connection C-5−C-6−C-6a to be made. These data allowed construction of the benzo[*h*]quinoline core structure of **1** to be assigned. The ether linkage between C-5 and C-12 was established based on considering the carbon chemical shift of C-12 (δ 74.4). Lastly, the planar structure assignment of **1** was completed by connection of C-5 to C-11 by incorporation of NH, which was clearly indicated based upon the chemical shifts of C-5 (δ 92.7) and C-11 (δ 174.8).

Since the planar structure of **1** was unprecedented in the alkaloid literature, and since the spectroscopic data did not define the configurations at C-5, C-6, and C-12, crystalline **1** was subjected to X-ray diffraction analysis. Slow evaporation of a MeOH/$CHCl_3$ mixture containing **1** provided X-ray quality crystals. The X-ray experiment confirmed the structure assigned for **1** and provided the absolute configuration as 5*S*, 6*S*, and 12*S*, respectively (Figure 1b).

Table 1. NMR Spectroscopic Data for Actinobenzoquinoline (1) in DMSO-d_6[a]

C#	δ_C, mult[b]	δ_H (mult, *J* (Hz))	HMBC
1			
1a	149.2, C		
2	159.5, C		
3	124.5, CH	7.36, d (8.5)	4a
4	135.2, CH	7.87, d (8.5)	1a, 2, 5
4a	130.4, C		
5	92.7, C		
6	73.2, CH	4.90, s	4a, 5, 6a, 7, 10a
6a	138.6, C		
7	126.8, CH	8.12, d (1.0)	6, 9, 10a, 15
8	137.9, C		
9	128.4, CH	8.01, dd (8.0, 1.0)	7, 10a, 15
10	125.4, CH	8.31, d (8.0)	1a, 6a, 8
10a	137.2, C		
11	174.8, C		
12	74.4, CH	4.61, q (6.8)	11
13	18.9, CH_3	1.34, d (6.8)	11, 12
14	24.8, CH_3	2.57, s	2, 3
15	198.3, C		
16	27.5, CH_3	2.62, s	15
5-NH		9.10, s	
6-OH		6.27, s	

[a]500 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. [b]Attached protons were determined by analysis of 2D spectroscopic data.

The molecular formula of actinophenanthroline A (**2**)[8] was assigned as $C_{17}H_{17}N_3O_3$, based on analysis of HRESIMS data. The ^{1}H NMR spectrum of **2** displayed four aromatic protons, one oxygenated proton, two methyl singlets, and one methyl doublet. The ^{13}C NMR spectrum of **2** showed 17 carbons, and the gHSQC data supported the assignment of three methyl, seven methine, and seven fully substituted carbons. Surprisingly, analysis of 2D NMR data revealed that **2** possessed a hydroxypropanamide-substituted 1,7-phenanthroline core structure instead of the benzo[*h*]quinoline core observed in **1**. An oxygenated methine proton H-12 (δ 4.32, d, *J* = 6.8 Hz) coupled to the H_3-13 methyl protons showed a long-range HMBC correlation to carbonyl carbon C-11 (δ 174.5), which permitted the assignment of the hydroxypropanamide side chain. gCOSY NMR correlations were observed between H-3 (δ 7.49, d, *J* = 8.5 Hz) and H-4 (δ 7.99, d, *J* = 8.5 Hz) and between H-9 (δ 7.66, d, *J* = 8.5 Hz) and H-10 (δ 9.30, d, *J* = 8.5 Hz). The ^{1}H−^{1}H coupling

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constants of these protons and the observation of long-range HMBC correlations from a methyl singlet H-14 to carbons C-2 and C-3 and from another methyl singlet H-15 to carbons C-8 and C-9 allowed two sets of 2,3-disubstituted 6-methylpyridine rings to be assigned.

Assignment of a phenanthroline core structure was achieved from gHMBC spectroscopic data analysis. The observation of gHMBC correlations from H-4 and H-10 to C-1a and from H- 9 to C-10a provided the connectivity of C-10a and C-1a. The connection from C-4a to C-5 was also determined by a three-bond gHMBC correlation from H-4 to C-5. Because of the lack of protons neighboring carbons C-5 and C-6, it was very difficult to assign the connectivity of the remaining carbons. However, considering the biosynthetic pathway of **1** and chemical shift of C-5 (δ 114.2) and C-6 (δ 145.8), the most plausible structure of **2** was drawn. However, to assign this structure meant placing a N atom at position 7, in stark contrast to the C atom at that position in compound **1**. Within the same mixture, this transposition seemed unlikely.

To unequivocally establish the structure of **2**, the compound was crystallized from a MeOH/CH_3Cl solution to obtain X-ray quality crystals. Confirmation of structure and assignment of the *S* configuration of C-12 was obtained in the X-ray experiment (Figure 2a).

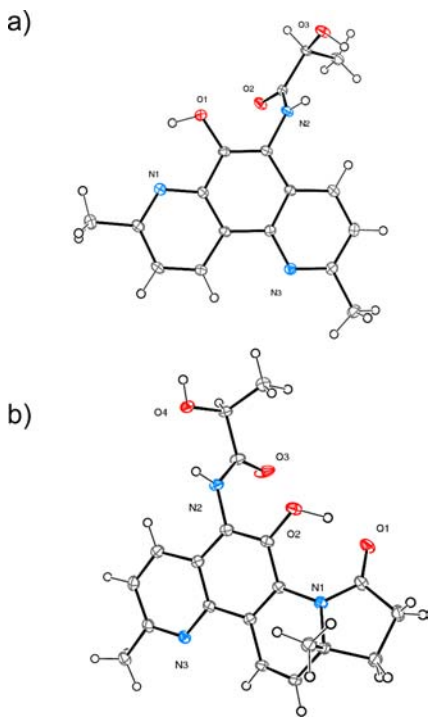

Figure 2. X-ray crystal structures for (a) actinophenanthroline A (**2**) and (b) actinophenanthroline B (**3**), depicting their absolute configurations of 12*S*.

A related compound, actinophenanthroline B (**3**), was also isolated as a pale yellow solid and was assigned the molecular formula $C_{20}H_{21}N_3O_4$ based upon a pseudomolecular ion at *m*/*z* 390.1427 $[M + Na]^+$ in the HRESI mass spectrum. By the difference in the molecular formula of **2**, alkaloid **3** was recognized to have an added three-carbon fragment. The ^1H NMR spectrum of **3** was almost identical to that of **2** except for the presence of two additional sets of methylene protons [H-16 (δ 2.35, 2.26) and H-17 (δ 2.46, 2.82)]. The ^{13}C NMR spectrum of **3** also illustrated three additional carbons [C-16 (δ 32.4), C-17 (δ 29.5), and C-18 (δ 177.1)], which corresponded to two methylenes and a carbonyl carbon. A gCOSY NMR correlation between H-16 and H-17 and the observation of long-range gHMBC NMR correlations from a methyl singlet (H_3-15) to carbons C-8, C-9, and C-16 and from H-17 to carbons C-8, C-16, and C-18 indicated that **3** possessed an α,β-saturated lactam ring. Interpretation of gCOSY, gHSQC, and gHMBC NMR data allowed the planar structure of **3** to be assigned by comparison with data from **2**. However, as in **1**, the full structure assignment as a 1,7-phenanthroline and the absolute configurations were determined by an X-ray diffraction experiment. X-ray crystal data confirmed the structure as assigned but also provided the absolute configurations of C-8 and C-12 as *R* and *S*, respectively (Figure 2b).

Actinophenanthroline C (**4**) was isolated as a pale yellow solid. The molecular formula of $C_{20}H_{19}N_3O_4$, also incorporating an additional three-carbon fragment, was deduced from the pseudomolecular ion peak at *m*/*z* 388.1270 $[M + Na]^+$ in the HRESI mass spectrum. The ^1H NMR spectral data of **4** were almost identical to those of **3** except for the presence of two new olefinic protons replacing the two methylene sets in **3**. A gCOSY NMR correlation between H-16 (δ 7.73) and H-17 (δ 6.35) and long-range gHMBC correlations from a methyl singlet H-15 to carbons C-8, C-9, and C-16 suggested that the three-carbon unit was in the form of an α,β-unsaturated γ-lactam ring in the molecule. Thus, the planar structure of **4** was assigned by analogy to **3**. Based on the analogy to **3**, we suggest that the absolute configurations for C-8 and C-12 can be assigned as *S* and *S*, based on its obviously related biosynthesis and upon the consistent negative optical rotations of **3** and **4**.

The core structures of actinobenzoquinoline and actinophenanthrolines A–C are unprecedented in the alkaloid literature.[9] In addition, the oxazolidin-4-one ring in **1** is also unusual among natural products. Four representative microbial natural product classes, the ergot alkaloids,[10] IT-62-B,[11] indolmycins,[12] and lipoxazolidinones,[13] contain the oxazolidin-4-one functionality. The benzo[*h*]quinoline core of **1** has, to the best of our knowledge, not been observed in natural products.

Actinophenanthrolines A–C (**2**–**4**) are the first examples of natural products containing the 1,7-phenanthroline alkaloid core. Synthetic 1,7-phenanthrolines are well-known and have been demonstrated to bind metals and to induce the biosynthesis of drug-metabolizing enzymes.[14] 1,10-Phenanthrolines are known as inhibitors of zinc metallopeptidases,[15] and several studies also reported that 1,10-phenanthrolines displayed antimalaria activities.[16] From a biosynthetic viewpoint, it seems highly unusual to observe two distinct alkaloid skeletons presumably derived from the same biosynthetic pathway. That one class, actinobenzoquinoline (**1**), contains a single nitrogen atom and that the actinophenanthrolines A–C (**2**–**4**) contain two nitrogen atoms provides for a potentially intriguing biosynthesis. We are currently pursuing the biosynthesis of **1**–**4** by analysis of genome sequence data.

Actinobenzoquinoline and actinophenanthrolines A–C were tested in our standard cytotoxicity-based assays. We did not observe significant in vitro activity against HCT-116 colon carcinoma up to 128 μg/mL nor activity against methicillin-resistant *Staphylococcus aureus* up to 256 μg/mL.

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■ ASSOCIATED CONTENT

Supporting Information

Details of the isolation, taxonomy, cultivation, and extraction of strain CNQ-149, as well as complete NMR spectral data for **1**−**4** and X-ray data for **1**−**3**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01387.

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Notes

The authors declare no competing financial interest.

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(7) Actinobenzoquinoline (**1**): $[\alpha]_D^{21}$ −53 (*c* 0.2, methanol); IR (KBr) ν_{max} 3051, 2337, 1677, 1602, 1375, 1293, 1165, 757 cm^{-1}; UV (methanol) λ_{max} (log ε) 242 (4.3), 281 (3.1), 295 (3.1), 310 (3.1) nm; ^1H and ^{13}C NMR data, see Table 1; ESI-TOF *m*/*z* 361 $[M + Na]^+$; HR ESI-TOF *m*/*z* 361.1153 (calcd for $C_{19}H_{18}N_2O_4Na$, 361.1159). Details of the identification and cultivation of strain CNQ-149 and the isolation of **1**−**4** are found in the Supporting Information.

(8) Actinophenanthroline A (**2**): $[\alpha]_D^{21}$ +2 (*c* 0.5, methanol); IR (KBr) ν_{max} 3189, 1712, 1595, 1444, 1148, 758 cm^{-1}; UV (methanol) λ_{max} (log ε) 238 (4.5), 290 (4.4), 305 (3.5) nm; ^1H and ^{13}C NMR data, see Table S1; ESI-TOF *m*/*z* 312 $[M + H]^+$; HR ESI-TOF *m*/*z* 312.1344 (calcd for $C_{17}H_{18}N_3O_3$, 312.1343). Details of the isolation of actinophenanthroline A, as well as additional details of the structure assignments for actinophenanthrolines B and C (**3** and **4**), are provided in the Supporting Information.

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