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# Nitrosporeusines A and B, Unprecedented Thioester-Bearing Alkaloids from the Arctic *Streptomyces nitrosporeus*

**Aigang Yang,[†] Longlong Si,[†] Zhenping Shi,[‡] Li Tian,*[,†] Dong Liu,[†] Demin Zhou,[†] Peter Proksch,[§] and Wenhan Lin*[,†]**

*State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, P. R. China, The First Institute of Oceannography SOA, Qingdao 266061, P. R. China, and Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, 40225 Duesseldorf, Germany*

*whlin@bjmu.edu.cn*

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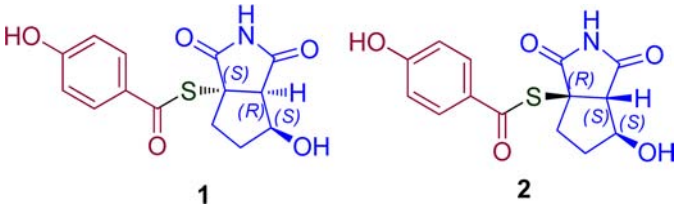
## ABSTRACT




**Chemical examination of an arctic actinomycete *Streptomyces nitrosporeus* resulted in the isolation of two new alkaloids named nitrosporeusines A (1) and B (2), an unprecedented skeleton containing benzenecarbothioc cyclopenta[*c*]pyrrole-1,3-dione. Their structures were determined through extensive spectroscopic analyses in association with X-ray single crystal diffraction. Both 1 and 2 exhibited inhibitory activities against the H1N1 virus in MDCK cells.**

Marine-derived microorganisms have proven to be rich sources for the generation of structurally unique and chemically diverse secondary metabolites with pronounced biological activities.[1,2] The genus *Streptomyces* represents the largest number of species and varieties in the genera of marine actinobacteria, which comprise about 10% of the bacteria colonising marine aggregates and can be isolated from various marine sources.[3] The strains of marine-derived *Streptomyces* have been proven to be efficient producers of bioactive metabolites with a wide range of activities such as antibacterial, antifungal, antitumor, and antivirus.[3] In comparison to the numerous diversity of bioactive metabolites discovered from territorial actinomycetes, the bioactive substances derived from marine *Streptomyces* are just beginning to be realized. The acetinomycete *S. nitrosporeus* from soil was reported to contain structurally unique and bioactive alkaloids, such as the noncytotoxic indole alkaloids madindolines possessing potent inhibition against the growth of interleukin-6 (IL-6) and IL-11 mediated cell lines,[4,5] while the indoline alkaloids benzastatins A−G protected neuronal cells and showed antivirus activities,[6−9] and foroxymithine is the angiotensin-converting enzyme inhibitor.[10] In the course of our investigation of the microorganisms from extreme environments for the discovery of bioactive and

[†] Peking University.
[‡] The First Institute of Oceannography.
[§] Heinrich-Heine University.

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**Figure 1.** Structures of **1** and **2**.

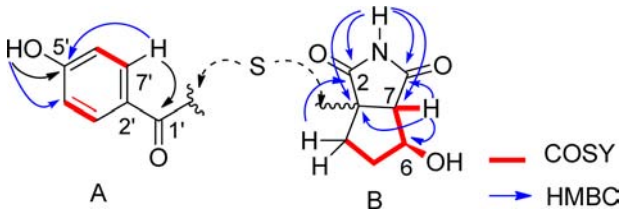


**Figure 2.** Key HMBC and COSY relationships of **1**.

structurally unique metabolites, the actinomycete *S. nitrosporeus* CQT14−24 was obtained in the sediments of the Arctic Chukchi Sea. Bioassay results indicated that the liquid fermentation broth exhibited strong insecticidal activity, while the HPLC fingerprint of the EtOAc extract displayed a major peak which was isolated and determined as a PKC inhibitor staurosporine.[11] Further examination of the minor components led to the isolation of two new compounds **1** and **2** (Figure 1).

Nitrosporeusine A (**1**)[12] was isolated as a colorless needle crystal, and its molecular formula ($C_{14}H_{13}O_5NS$) was determined by HR-ESIMS (*m*/*z* 306.0442 [M − H]$^-$, calcd 306.0436) and NMR data, requiring nine degrees of unsaturation. The IR absorptions at 3229 and 1713 cm$^{-1}$ suggested the presence of hydroxyl and carbonyl groups. The $^1$H NMR spectrum exhibited an aromatic AABB spin system at $\delta_H$ 6.87 (2H, d, $J$ = 8.8 Hz, H-4′, H-6′) and 7.73 (2H, d, $J$ = 8.8 Hz, H-3′, H-7′) for a *para*-substituted phenyl ring, while the HMBC correlations from a phenol proton at $\delta_H$ 10.77 (s) to the aromatic carbons at $\delta_C$ 163.7 (s, C-5′) and 116.2 (d, C-4′,C-6′) and from H-3′/H-7′ to C-5′ and C-1′ ($\delta_C$ 190.1) assigned a *p*-hydroxyphenylcarbonyl unit. The $^{13}$C NMR and DEPT spectra of the remaining resonances were attributed to two carbonyl carbons at $\delta_C$ 179.3 (C-2) and 175.3 (C-8) and five alkyl carbons (Table 1). The COSY correlations (Figure 2) established a subunit from C-4 ($\delta_C$ 35.0) to C-7 ($\delta_C$ 60.2),

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(12) **1**: colorless needle crystal (MeOH/$H_2O$); mp 250−252 °C; $[\alpha]^{20}_D$ 46.0 (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ 3229, 2924, 1713, 1653, 1602, 1581, 1355,1281, 1218, 1167, 911 cm$^{-1}$, HRESIMS$^-$ *m*/*z* 306.0442 [M − H]$^-$ (calcd for $C_{14}H_{12}O_5NS^-$, 306.0436). **2**: colorless bulk crystal (MeOH/$CH_2Cl_2$); mp 235−237 °C; $[\alpha]^{20}_D$ −52.0 (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ 3229, 2924, 2854, 1713, 1602, 1581,1442,1355,1281, 1218, 1167, 911 cm$^{-1}$; HRESIMS$^-$ *m*/*z* 306.0442 [M − H]$^-$ (calcd for $C_{14}H_{12}O_5NS^-$, 306.0436).

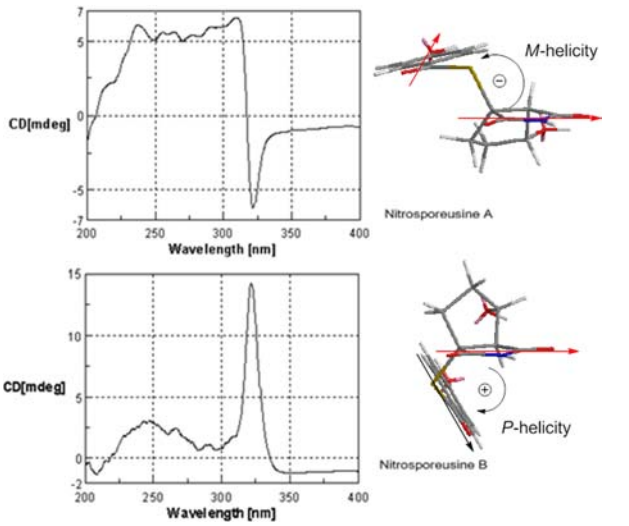


**Figure 3.** CD effects of **1** and **2**.

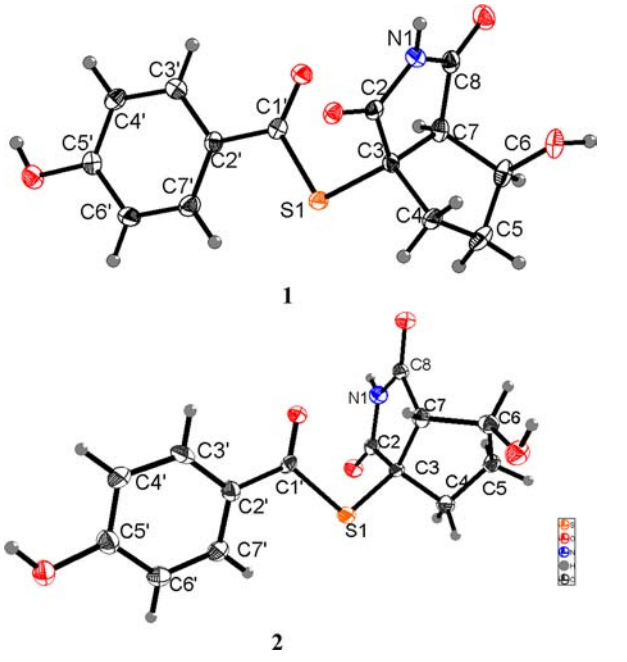


**Figure 4.** X-ray crystal structures of nitrosporeusines A and B.

whereas the HMBC interactions from H-7 ($\delta_H$ 3.25, *d*, $J$ = 7.3 Hz) to C-3 ($\delta_C$ 59.2) and C-4 indicated the existence of a cyclopentane ring. Additional HMBC interactions from an amide proton at $\delta_H$ 11.32 (s, NH-1) to C-2 ($\delta_C$ 179.3), C-3, C-8 ($\delta_C$ 175.3) and C-7 resulted in a unit of succinic imide, which was fused to the cyclopentane ring across C-3 and C-7. In addition, a $D_2O$ changeable proton at $\delta_H$ 5.25 (br) showed COSY correlation with H-6 ($\delta_H$ 4.46, dt, $J$ = 7.3, 6.7 Hz) confirmed the substitution of a hydroxyl group at C-6. Thus, the remaining sulfur atom in the molecule was assumed to connect between C-1′ and C-3

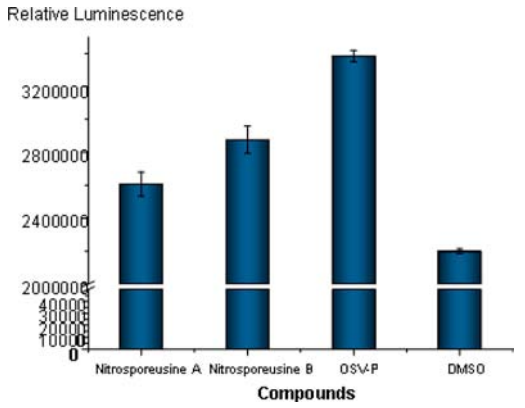


**Figure 5.** Inhibitory activities of **1** and **2** against influenza WSN virus.

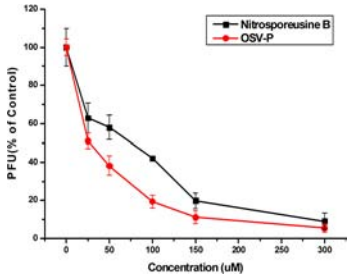


**Figure 6.** Inhibition of **2** against the progeny virus production.

to form a thioester. The NOE interactions between H-6/H-7 and OH-6/H-4b ($\delta_H$ 2.23) clarified the same orientation of H-6 and H-7. Based on the helicity rule of CD effects,[13,14] the negative CE at 322 nm for $^1L_b$ bond (Figure 3) reflected *M*-helicity, indicating 3*S* configuration. This assignment was confirmed by the X-ray diffraction of a single crystal using the Flack parameter,[15] which resulted in 3*S*, 6*S*, 7*R* configurations (Figure 4).

Nitrosporeusine B (**2**) had the same molecular formula as that of **1** as determined by the HRESIMS and NMR data. Analyses of 1D and 2D NMR data revealed the gross structure of **2** to be the same as that of **1**, indicating a stereoisomer. The distinction was attributed to the significantly downfield-shifted C-6 ($\delta_C$ 74.5) and C-7 ($\delta_C$ 64.0) in addition to the small coupling constant of $J_{H\text{-}6/H\text{-}7}$. The NOE interaction between OH-6 ($\delta_H$ 5.25) and H-7 ($\delta_H$ 3.10) indicated H-6 and H-7 to be in opposite orientation. The positive CE at 322 nm for $^1L_b$ bond (Figure 3) indicated a *P*-helicity. Thus, the configuration of C-3 was assigned to 3*R*. The X-ray diffraction of single crystal using

**Table 1.** $^1$H and $^{13}$C NMR Data of Nitrosporeusines A (**1**) and B (**2**) in DMSO-$d_6$

| | **1** | | **2** | |
|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 11.32, s | | 11.55, s | |
| 2 | | 179.3, qC | | 179.4, qC |
| 3 | | 59.2, qC | | 58.2, qC |
| 4 | 2.23, m | 35.0, $CH_2$ | 2.21, m | 33.8, $CH_2$ |
| | 1.89, m | | 2.17, m | |
| 5 | 1.89, m | 32.8, $CH_2$ | 1.79, m | 32.4, $CH_2$ |
| | 1.64, m | | 1.56, m | |
| 6 | 4.46, dt (7.3,6.7) | 72.3, CH | 4.38, brt (6.5) | 74.5, CH |
| 7 | 3.25, d (7.3) | 60.2, CH | 3.10, brs | 64.0, CH |
| 8 | | 175.3, qC | | 176.9, qC |
| 1′ | | 190.1, qC | | 190.5, qC |
| 2′ | | 127.0, qC | | 126.8, qC |
| 3′,7′ | 7.73, d (8.8) | 130.0, CH | 7.76, d (7.6) | 130.1, CH |
| 4′,6′ | 6.87, d (8.8) | 116.2, CH | 6.88, d (7.6) | 116.2, CH |
| 5′ | | 163.7, qC | | 163.8, qC |
| 6-OH | 5.25, br | | 5.25, brs | |
| 5′−OH | 10.77, brs | | 10.68, s | |

the Flack parameter revealed the absolute configurations of **2** to be 3*R*,6*S*,7*S*.

Compounds **1** and **2** were tested against the influenza virus strains A/WSN/33(H1N1) that were propagated in MDCK cells.[16,17] The cytotoxic assay indicated both **1** and **2** were no inhibitory activity against the uninfected MDCK cells at 300 $\mu$M. However, compounds **1** and **2** showed inhibitory activity against the influenza WSN virus (H1N1) in MDCK cells with the inhibitory rates of 18.6% and 30.9%, respectively, with the dose of 50 $\mu$M, whereas the same dose of the positive control oseltamivir phosphate (Osv-P)[18] inhibited 54% virus (Figure 5). In an in vitro plaque reduction assay, nitrosporeusine B (**2**) exhibited dose-dependent reduction of the production of the viral progeny which was produced by the infected MDCK cells with influenza A/WSN/33 virus (Figure 6). The $EC_{50}$ value of nitrosporeusine B for the inhibition of viral plaque formation was 112.7 ± 4.4 $\mu$M, whereas the $EC_{50}$ value of Osv-P was 67.0 ± 1.6 $\mu$M. As influenza viruses have developed resistance toward current drugs, discovery of new inhibitors that prevent viral replication through different inhibitory mechanisms become urgently challenge. Such far, a few natural compounds are found to be potent inhibition against influenza viruses. The bioassay results implied compounds **1** and **2** may provide the structural models for further modification under molecular modeling.

The present work reported two unprecedented benzenecarbothioc alkaloids with a unique skeleton from the Arctic actinomycete. However, the biogenetic pathway is unknown. In comparison with the structural patterns

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derived from the same species isolated from soil, the microorganism origins from the extreme environments really produced dramatically different metabolites. This finding suggested the extreme factors may activate "silent genes" to stimulate a new biogenetic pathway.

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**Supporting Information Available.** Experimental procedures, 1D and 2D NMR, IR, HRESIMS, CD spectra, and X-ray crystallographic data of **1** and **2** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.