# Generation of Heronamides A and B from Heronamide C Produced by a Marine-Derived *Streptomyces* sp. and Their Antifungal Activity

# 海洋放線菌由来のヘロナミドCからのヘロナミドAおよびBの生成と抗真菌活性

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## Abstract

Heronamide C is a 20-membered polyene macrolactam isolated from a marine-derived *Streptomyces* sp. and shows potent antifungal activity by targeting membrane phospholipids. Testing the stability of heronamide C under UV irradiation (365 nm) revealed that heronamide C is converted mainly to heronamide B and a minor product, heronamide A, as determined by LC-MS analysis. In addition, the antifungal activity of heronamides A and B as tested against fission yeast was much weaker than that of heronamide C. Taking these results together, further structure-activity relationship studies of the heronamides are helpful for developing a chemical probe and a promising lead compound for antifungal drugs.

**Keywords:** Heronamides, Polyene, Macrolactam, a Marine-derived *Streptomyces*, Antifungal activity, Liquid chromatography-mass spectrometry, Natural product chemistry, Chemical probe, Chemical biology

## 要旨

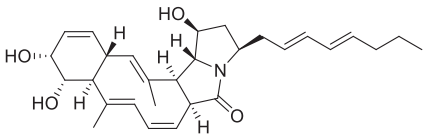
海洋放線菌由来の20員環ポリエンマクロラクタム化合物で抗真菌作用を持つヘロナミドCの紫外線照射（365 nm）による安定性試験を行った。また、ヘロナミドCと紫外線照射によって生成したヘロナミドA、Bの抗真菌作用を調べた。その結果、ヘロナミドCは紫外線照射によってヘロナミドA、B（主変換体）へと変換され、それらはヘロナミドCほどの抗真菌活性を示さないことが明らかになった。これらの結果から、ヘロナミドCが有する20員環ポリエンマクロラクタム骨格は抗真菌作用に重要であることが示唆され、さらなる構造活性相関研究はケミカルプローブ開発や新規抗真菌剤の開発に役立つことが期待される。

**重要語句：**ヘロナミド，ポリエン，マクロラクタム，海洋放線菌，抗真菌作用，LC-MS（Liquid Chromatography-Mass Spectrometry），天然物化学，ケミカルプローブ，ケミカルバイオロジー

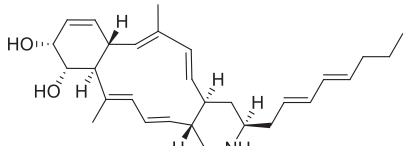
## Introduction

Natural products derived from medicinal plants, microbial metabolites, marine organisms, and other biological sources hold much promise for developing new medicine (1). For example, penicillin and amphotericin B antibiotics were produced by *Penicillium* sp. and *Streptomyces* sp., respectively. However, as severe problems such as drug-resistance have existed for some time, it is necessary to discover or synthesize new molecules or pharmacophores to overcome them.

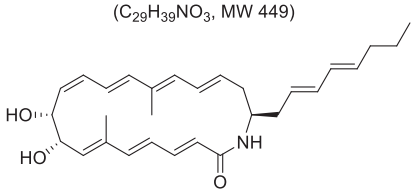
Heronamide C is a 20-membered polyketide macrolactam isolated



heronamide A
($C_{29}H_{39}NO_4$, MW 465)



heronamide B
($C_{29}H_{39}NO_3$, MW 449)



heronamide C
($C_{29}H_{39}NO_3$, MW 449)

Fig. 1. Chemical structures of heronamides A, B, and C.

The chemical structures of heronamides A, B, and C were originally reported in 2010 by Raju, R. *et al*. (2), and were recently revised by our extensive NMR analysis and synthetic methodology, as shown in Fig. 1 (3–5).

from a marine-derived *Streptomyces* sp (2,3). This macrolactam shows potent antifungal activity by targeting membrane phospholipids (3). The antifungal activity of heronamide C is as potent as that of amphotericin B, a well-known polyene macrolide that is used clinically. The chemical structures of heronamides A, B, and C were unambiguously revised very recently by our extensive NMR analysis and synthetic methodology, as shown in Fig. 1 (3–5).

Herein we investigated not only the stability of heronamide C under UV irradiation (365 nm) but also the biological activities of the resulting converted products, heronamides A and B.

## Materials & Methods

Heronamide C Conversion to Heronamides A and B

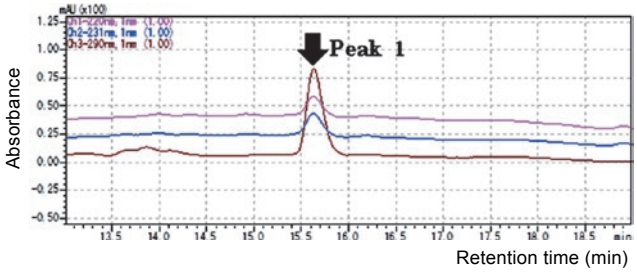
First, heronamides A, B, and C (1.0 µg) served as standard samples (3,4) for LC-MS analysis (ESI-IT-TOF; Shimadzu, Japan). Next, a solution of heronamide C (2.0 mM) in DMF was irradiated with UV (365 nm) for 6 min before, aliquots (1 µL) were removed for LC-MS analysis.

The LC conditions were as follows: Cosmosil 5$C_8$-MS (Nacalai Tesque, Japan), $\phi$3 × 150 mm, 5 µm; 0.2 mL/min; gradient elution from 60 to 100% aqueous MeOH over 10 min and 100% MeOH for 10 min). The three ion peaks (**1**, **2**, and **3**) observed on the chromatogram shown in Fig. 2 corresponded to heronamides C, A, and B, respectively. All MS spectra were taken in positive ion mode by ESI-IT-TOF.

Biological Activities of Heronamides A, B, and C against Fission Yeast Cells

Growth inhibition by heronamides was tested as previously described (6). Heronamides A, B, and C were added to fission yeast cells growing in liquid culture ($3.3 \times 10^{-3}$ $OD_{595}$, *Schizosaccharomyces pombe*, JY1 ($h^-$)) and incubated at 30 °C for 24 h. Changes in turbidity were determined by measuring the absorbance at 595 nm with a microplate reader (EnVision, Thermo Fisher Scientific, USA). The cell viability was calculated relative to that of DMSO-treated (control) cells.

a) Before UV irradiation




b) After UV irradiation for 6 min

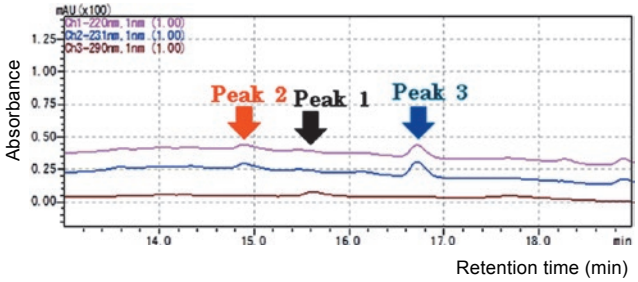



Fig. 2. Conversion of heronamide C to heronamides A and B under UV irradiation at 365 nm.

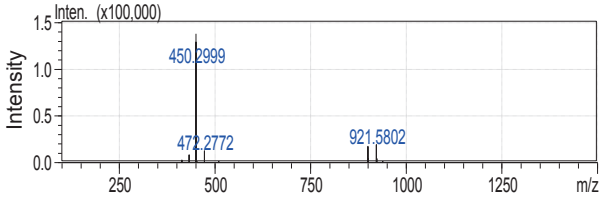
The LC profiles obtained by LC-MS analysis were recorded at 220 nm (pink), 231 nm (blue), and 290 nm (brown). Peak 1 shows heronamide C, while peaks 2 and 3 show heronamides A and B, respectively.

## Results & Discussion

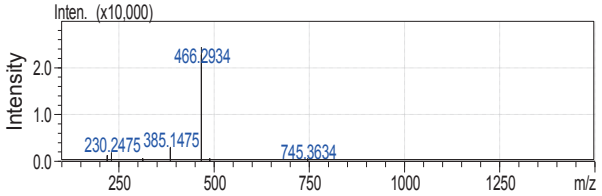
Heronamide C Conversion to Heronamides A and B

We investigated the stability of heronamide C under UV irradiation (365 nm). Peak **1**, corresponding to heronamide C in the LC-MS analysis, showed a retention time of 15.6 min before irradiation with an ion peak (*m/z*) of 450 (Figs. 2a & 3a). After UV irradiation for 6 min, peak **1** almost disappeared, whereas two new peaks appeared at 14.8 min and 16.8 min (Fig. 2b). The ion peak of the major product (peak **3**) was 432, which is identical to that of heronamide B; heronamide B showed a dehydration peak in this condition (Fig. 3c). In addition, the minor product (peak 2) was determined to correspond to heronamide A (Fig. 3b). These results indicate that heronamide C was converted mainly to heronamide B with a minor product heronamide A under UV irradiation at 365 nm.

a) MS analysis of peak 1




b) MS analysis of peak 2




c) MS analysis of peak 3

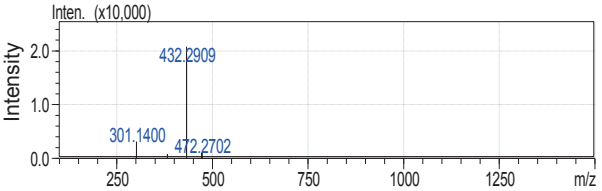



Fig. 3. MS profile of peaks **1**, **2**, and **3** obtained by LC-MS.

The ion peaks (*m/z*) observed for peaks **1** and **2** were established as 450 $(M+H)^+$ and 466 $(M+H)^+$, which are identical to those of heronamides C and A, respectively. The ion peak (*m/z*) of peak **3** was 432 $(M-H_2O+H)^+$, which matches that of heronamide B.

Biological Activities of Heronamides A, B, and C against Fission Yeast Cells

We investigated the biological activities of heronamides A, B, and C against fission yeast cells. The minimum inhibitory concentration (MIC) value calculated for heronamide C in this assay was 0.13 µM, whereas those for heronamides A and B were over 20 and 50 µM, respectively (Fig. 4). In contrast, the MIC value of amphotericin B was 0.27 µM (data not shown). These results suggest that the 20-membered polyene macrolactam ring in heronamide C plays a crucial role in its antifungal activity.

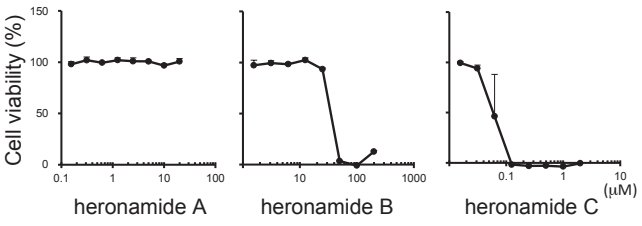



Fig. 4. Biological activities of heronamides A, B, and C against fission yeast cells.

Fission yeast cells were incubated in either the presence or absence of heronamides A, B, and C at 30 °C for 24h. Cell viability was determined by measuring the turbidity. The MIC values of heronamides A, B, and C were >20, 50, and 0.13 μM, respectively. Data represent the mean values of three independent experiments. Error bars indicate the SD.

In summary, we determined that heronamide C, the metabolite from a marine-derived *Streptomyces* sp., was mostly converted to heronamide B under UV irradiation (365 nm). In addition, we found that the antifungal activity of heronamides A and B was much weaker than that of heronamide C. Furthermore structure-activity relationship studies of heronamides will be helpful for developing a chemical probe as well as a promising lead compound for antifungal drugs.

## Acknowledgements

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