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<td>山前, 結</td>
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<td>ELCAS Journal (2016), 1: 63-65</td>
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<td>Issue Date</td>
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<td>2016-03</td>
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<td><a href="http://hdl.handle.net/2433/216480">http://hdl.handle.net/2433/216480</a></td>
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<td>Kyoto University</td>
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Generation of Heronamides A and B from Heronamide C Produced by a Marine-Derived Streptomyces sp. and Their Antifungal Activity

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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

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 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

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

Heronamide A (C_{20}H_{29}NO_5, MW 465)
Heronamide B (C_{20}H_{29}NO_5, MW 449)
Heronamide C (C_{20}H_{29}NO_5, MW 449)
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 5C8-MS (Nacalai Tesque, Japan), φ3 × 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 × 10^{-3} OD$_{595}$, Schizosaccharomyces pombe, JY1 (h$^-$)) and incubated at 30 ºC 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.

**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.

**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.
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**

We thank the ELCAS Program for the opportunity to perform this study at Kyoto University. We are grateful to Prof. Onaka, H. (The University of Tokyo) and Mr. Sugiyama, R. (Kyoto University) for the support in the heronamides-producing *Streptomyces* sp. strain and valuable discussions, respectively.

**References**