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Stereochemical reassignment of heronamide A, a polyketide macrolactam from *Streptomyces* sp.

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ABSTRACT

The stereochemistry of heronamide A, a polyketide macrolactam from a marine-derived *Streptomyces* sp., was reassigned by detailed NMR analysis of the intact natural product and chemically modified derivatives.

Polyene macrolactam is a unique class of microbial metabolites. They exhibit a variety of biological activities, for example, antimicrobial and antitumor activity, modulation of Bcl-xL function, and inhibition of leukocyte adhesion,[1–6] and their modes of action are of interest. However, in many cases their stereochemistry remains to be determined partly due to their characteristic structural features.[4–10] For example, heronamide C (**2**)[11] has three chiral centers; two oxymethine carbons C-8 and C-9 next to each other and one isolated methine carbon C-19, which are connected through triene and tetraene chains (Fig. 1). Many polyene macrolactam compounds have similar structural features, making determination of the relative stereochemistry difficult.[3,4,6–9] Heronamide A (**1**) is a putative biosynthetic product *via* heronamide C (**2**), originally isolated from an Australian marine-derived *Streptomyces* sp.[11] Heronamide A has a hexahydropyrrolizin-3-one unit (ring A and B) and a cyclohexene ring (ring D), which are connected to form another 10-membered ring system (ring C). Although its relative stereochemistry was tentatively assigned by interpretation of the NMR spectra and the absolute stereochemistry of C-17 was determined by the modified Mosher analysis,[12] there was no direct evidence to conclude the absolute stereochemistry of ring D. During the course of our screening for novel secondary metabolites, we isolated heronamide A from a Japanese marine-derived *Streptomyces* sp. However, we found that the NMR data were not in agreement with the proposed stereochemistry. We herein report the reassignment of the absolute stereochemistry of heronamide A (**3**), elucidated by careful interpretation of the NOESY data and chemical modification. Our result indicates that the stereochemistry of other heronamide congeners should also be reinvestigated.

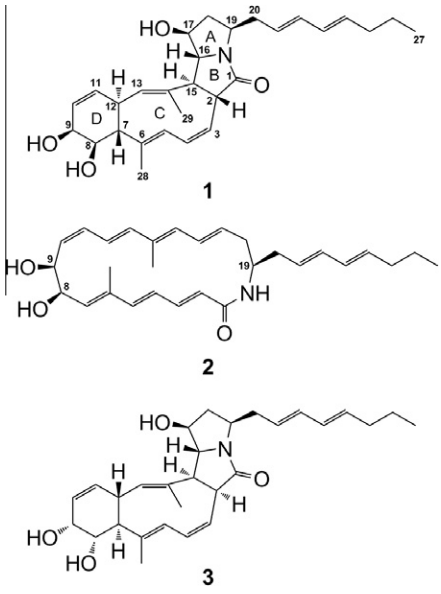

Figure 1. Chemical structures of heronamides. Heronamides A (**1**) and C (**2**) with proposed stereochemistry and heronamide A (**3**) with reassigned absolute stereochemistry are shown.

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Table 1
NMR data for heronamide A in pyridine-d_5

Position	δ_H (mult, J in Hz)[a]	NOESY
1		
2	3.81 (ddd, 9.1, 7.1, 2.3)	3, 5, 13, 15
3	5.81 (dd, 10.1, 7.1)	2, 4, 16, 29
4	6.75 (ddd, 10.1, 10.1, 2.3)	3, 28, 29
5	5.60 (d, 10.1)	2, 7, 13
6		
7	2.52 (dd, 11.3, 9.9)	5, 13
8	4.14 (m[b])	12, 28
9	4.61 (m[c])	8, 10
10	6.22 (ddd, 9.8, 5.1, 2.7)	9, 11
11	5.94 (dd, 9.8, 1.7)	10, 12, 13
12	2.92 (m[c])	8, 11, 28, 29
13	5.01 (d, 10.9)	2, 5, 7, 11, 15
14		
15	3.25 (dd, 9.1, 9.1)	2, 13, 17
16	4.30 (dd, 9.1, 6.9)	3, 18b, 29
17	4.16 (m[b])	15, 18a
18a	2.59 (ddd, 12.8, 7.4, 7.4)	17, 18b, 19,
18b	2.15 (ddd, 12.8, 8.0, 8.0)	16, 18a, 20b, 21
19	4.20 (m[b])	18a, 20a, 20b, 21
20a	2.80 (ddd, 13.6, 7.4, 4.5)	19, 20b, 21, 22
20b	2.66 (ddd, 13.6, 7.4, 7.4)	19, 20a, 21, 22
21	5.81 (ddd, 15.2, 7.4, 7.4)	16, 18b, 19, 20a, 20b, 23
22	6.27 (dd, 15.2, 10.4)	20a, 20b, 24
23	6.13 (dd, 15.2, 10.4)	21, 25
24	5.63 (dt, 15.2, 7.4)	22, 25
25	1.99 (dt, 7.4, 7.4)	23, 24
26	1.34 (tq, 7.4, 7.4)	24
27	0.84 (t, 7.4)	25
28	1.95 (s)	4, 8, 12, 29
29	1.58 (s)	3, 4, 12, 16, 28

[a] 500 MHz.
[b] Signals overlapped.
[c] Broad signals.

Heronamide A was obtained from the mycelium of *Streptomyces* sp. NSU893, isolated from a sea sediment sample collected in Uranouchi Bay, Kochi prefecture, Japan. Mycelium collected from a 5 L culture was extracted with MeOH. The MeOH extract (3.0 g) was concentrated and the residue was partitioned between 60% MeOH and $CHCl_3$. The $CHCl_3$ layer (1.2 g) was fractionated by SiO_2 column chromatography ($CHCl_3$/MeOH), followed by ODS flash column chromatography (H_2O/MeOH). Fractions containing heronamide A (134 mg) were subjected to RP-HPLC (Cosmosil 5C_8-MS) to give 11.6 mg of heronamide A. The physico-chemical properties including IR, optical rotation, UV, and MS data were comparable to those reported.[13] The ^1H and ^{13}C chemical shifts in methanol-d_4 also matched those reported.[14] However, the NOESY data (Table 1 and Fig. 2) did not agree with the stereochemistry proposed previously, for example, H-2, H-13, and H-15 mutually correlated, indicating the *cis* orientation of H-2 and H-15 although the *trans* orientation was proposed based on the large coupling constant ($^3J_{H\text{-}2/H\text{-}15}$ = 9.2 Hz). We also observed a large coupling constant, 9.1 Hz (Table 1). To assign the relative stereochemistry, we carefully analyzed the NOESY spectrum. H-2 and H-7 were both correlated to H-5 and H-13, whereas NOESY correlations between H-5 and H-13 were observed. In addition, H-2, H-13, and H-15 mutually correlated, suggesting that H-2, H-5, H-7, H-13, and H-15 are placed on the same face of the 10-membered ring C (Fig. 2). NOESY correlations from H_3-29 to H-4, H_3-28, H-12, and H-16, from H_3-28 to H-4, H-8, and H-12, and between H-8 and H-12 placed all these protons on the other face (Fig. 2). This interpretation was supported by two sets of large coupling constant values for $^3J_{H\text{-}7/H\text{-}8}$ and $^3J_{H\text{-}7/H\text{-}12}$ (11.3 and 9.9 Hz),[15] indicating the *anti*-orientation of H-7 and H-8, and H-7 and H-12. Thus, the relative stereochemistry of heronamide A was revealed to be 2S^*, 7S^*, 8S^*, 9R^*, 12R^*, 15S^*, 16R^*, 17S^*, 19R^*.[16]

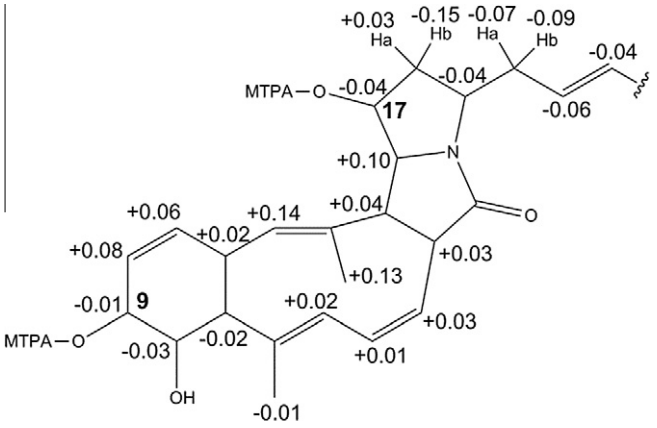

Figure 3. $\Delta\delta$ (=$\delta_S - \delta_R$, ppm) values for the bis-9,17-(*S*)- and bis-9,17-(*R*)-MTPA esters of heronamide A.

To determine the absolute stereochemistry of heronamide A, we applied the modified Mosher analysis.[12] The previous report also applied this method to heronamide A.[11] However, the isopropylidene derivative was converted to the MTPA esters and only 17-OH was esterified. We reacted intact heronamide A with (*R*)- or (*S*)-MTPACl to obtain 9,17-bis-(*S*)- or 9,17-bis-(*R*)-MTPA derivatives, respectively (Fig. 3).[17] Analysis of the differences between

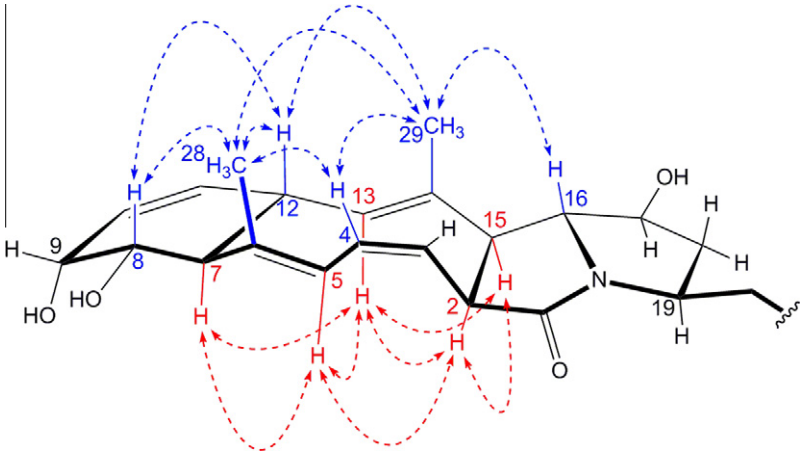

Figure 2. Key NOESY correlations of heronamide A.

the ^{1}H NMR chemical shifts of these two MTPA derivatives confirmed the 17*S* stereochemistry while it revealed the 9*R* configuration (Fig. 3), supporting our model led by the NMR analysis of the intact compound (Fig. 2). We now conclude that the absolute stereochemistry of heronamide A (**3**) was 2*S*, 7*S*, 8*S*, 9*R*, 12*R*, 15*S*, 16*R*, 17*S*, 19*R* (Fig. 1).

In summary, we isolated heronamide A from a Japanese marine-derived *Streptomyces* sp. NSU893 and reassigned the stereochemistry. Our assignment differed from the previously proposed structure in two points; H-2 and H-15 were placed in a *cis* configuration and ring D had opposite stereochemistry. We assigned the relative stereochemistry by precise interpretation of the NOESY data, while the absolute stereochemistry was unambiguously determined by the modified Mosher analysis. Since heronamides B and C possess the same diol function, we suggest that their stereochemistry should also be reinvestigated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01.012.

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13. Yellow oil; IR (neat) λ_{max} 3360 (br), 2926 (m), 2360 (m), 1666 (brs) cm^{-1}; $[\alpha]_D^{20}$ −42 (*c* 0.10 in MeOH); UV (MeOH) λ_{max} (log ε) 224 nm (4.44); HR MS (ESI) *m/z* 466.2948 [M+H]$^+$ calcd for $C_{29}H_{40}NO_4$, 466.2952.
14. The ^{1}H NMR signals of heronamide A in pyridine-d_5 dispersed well while many signals overlapped in methanol-d_4. Hence we analyzed the NOESY data in pyridine-d_5.
15. Two coupling constant values were deduced by interpretation of the peak shape of H-7. However, we could not determine which value is for $^3J_{H\text{-}7/H\text{-}8}$ (or $^3J_{H\text{-}7/H\text{-}12}$) since the ^{1}H NMR peaks corresponding to H-8 and H-12 were broad. In methanol-d_4, a large coupling constant value for $^3J_{H\text{-}7/H\text{-}8}$ (11.9 Hz) was observed, confirming their *anti*-orientation.
16. Two hydroxyl groups at C-8 and C-9 were placed in a *cis* relationship by analyzing the NOESY data of an isopropylidene derivative as reported previously. In addition, NOESY correlations around rings A and B were comparable to those reported. Our data also assigned the relative configurations as 15*S**, 16*R**, 17*S**, 19*R**.
17. A solution of heronamide A (1.0 mg, 2.1 μmol), (*S*)-MTPACl (5.0 mg, 20 μmol), and DMAP (1.6 mg, 13 μmol) in pyridine/CH_2Cl_2 (100/400 μL) was stirred at room temperature for 12 h. The reaction was quenched with satd aq $NaHCO_3$ and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and fractionated by ODS flash column chromatography, followed by HPLC (Cosmosil 5C$_8$-MS) to yield the 9,17-bis-(*R*)-MTPA ester (0.1 mg, 0.11 μmol) as a white powder. The 9,17-bis-(*S*)-MTPA ester was prepared in the same way.