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Isolation and Total Synthesis of Hoshinolactam, an Antitrypanosomal Lactam from a Marine Cyanobacterium

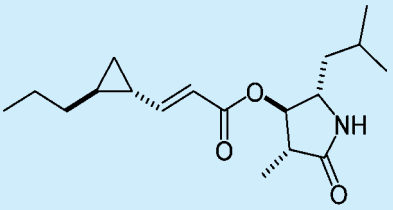
Hidetoshi Ogawa,[†] Arihiro Iwasaki,[†] Shimpei Sumimoto,[†] Masato Iwatsuki,[‡,§] Aki Ishiyama,[‡,§] Rei Hokari,[‡] Kazuhiko Otoguro,[‡] Satoshi Ōmura,[‡] and Kiyotake Suenaga*[,†]

[†]Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

[‡]Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, and [§]Graduate School of Infection Control Sciences, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan

Supporting Information

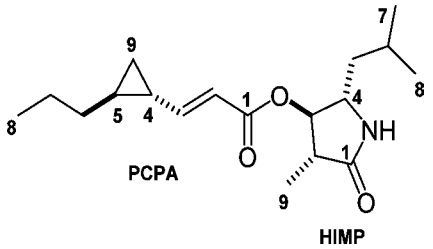
ABSTRACT: In the search for new antiprotozoal substances, hoshinolactam, an antitrypanosomal lactam, was isolated from a marine cyanobacterium. The gross structure was elucidated by spectroscopic analyses, and the absolute configuration was determined by the first total synthesis. Hoshinolactam showed potent antitrypanosomal activity with an IC_{50} value of 3.9 nM without cytotoxicity against human fetal lung fibroblast MRC-5 cells (IC_{50} > 25 μM).



Hoshinolactam

Tropical diseases caused by parasitic protozoa are a threat to human health, mainly in developing countries. Trypanosomiasis (Chagas disease and sleeping sickness) and leishmaniasis, inter alia, are classified as neglected tropical diseases, and over 400 million people are at risk of contracting these diseases.[1] In addition, a parasite of the *Trypanosoma* genus, *Trypanosoma brucei brucei*, is the causative agent of Nagana disease in wild and domestic animals, and this disease is a major obstacle to the economic development of affected rural areas.[2] Although some therapeutic agents for these diseases exist, they have limitations, such as serious side effects and the emergence of drug resistance.[1] Thus, new and more effective antiprotozoal medicines are needed.[1]

Marine natural products have recently been considered to be good sources for drug leads.[3] In particular, secondary metabolites produced by marine cyanobacteria have unique structures and versatile biological activities,[4] and some of these compounds show antiprotozoal activities. For example, coibacin A isolated from cf. *Oscillatoria* sp. exhibited potent antileishmanial activity,[5] and viridamide A isolated from *Oscillatoria nigro-viridis* showed antileishmanial and antitrypanosomal activities.[6] In our search for new antiprotozoal substances, we have focused on the constituents of marine cyanobacteria and reported an antitrypanosomal cyclodepsipeptide, janadolide.[7] Recently, we isolated a new antitrypanosomal lactam, hoshinolactam (**1**), from a marine cyanobacterium.[8] Structurally, **1** contains a cyclopropane ring and a γ-lactam ring. So far, some metabolites possessing either a cyclopropane ring or a γ-lactam ring have been discovered from marine cyanobacteria, such as majusculoic acid[9] and malyngamide A.[10] To the best of our knowledge, on the other hand, hoshinolactam (**1**) is the first compound discovered in marine cyanobacteria that possesses both of these ring systems. In addition, we clarified that **1** exhibited potent antitrypanosomal activity without cytotoxicity against human fetal lung fibroblast MRC-5 cells. Here, we report the isolation, structure elucidation, first total synthesis, and preliminary biological characterization of hoshinolactam (**1**).



Hoshinolactam (**1**)

The marine cyanobacterium was collected at the coast near Hoshino, Okinawa. The collected cyanobacterium (2 kg, wet weight) was extracted with MeOH, and the extract was filtered and concentrated. The residue was partitioned between EtOAc and H_2O. The material obtained from the organic layer was partitioned between 90% aqueous MeOH and hexanes. The aqueous MeOH fraction was separated by reversed-phase column chromatography (ODS silica gel, MeOH−H_2O) and reversed-phase HPLC (Cosmosil 5C_{18}AR-II, MeOH-H_2O; Cosmosil 5C_{18}MS-II, MeOH-H_2O; Cosmosil Cholester, MeCN-H_2O) to give hoshinolactam (**1**) (6.5 mg). The molecular formula of hoshinolactam (**1**) was determined on the basis of positive HRESIMS data (m/z 308.2228, calcd for

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$C_{18}H_{30}NO_3$ $[M + H]^+$ 308.2225). Table 1 shows the NMR data for **1**. An analysis of the ^1H NMR spectrum indicated the

Table 1. ^1H and ^{13}C NMR Data for 1 in C_6D_6

unit	position	δ_C[a]	δ_H[b] (J in Hz)
HIMP	1	177.8, C	
	2	44.1, CH	2.51, dq (5.2, 7.6)
	3	80.8, CH	4.94, dd (4.6, 5.2)
	4	57.3, CH	3.49, ddd (4.6, 4.7, 9.4)
	5a	44.6, CH_2	1.21, m
	5b		1.36, m
	6	25.0, CH	1.61, m
	7	21.7, CH_3	0.74, d (6.2)
	8	23.2, CH_3	0.76, d (6.3)
	9	15.0, CH_3	1.33, d (7.6)
	NH		7.65, s
PCPA	1	166.0, C	
	2	117.4, CH	5.88, d (15.5)
	3	155.0, CH	6.59, dd (10.3, 15.5)
	4	22.4, CH	0.91, m
	5	23.3, CH	0.59, m
	6	35.7, CH_2	0.96, m
	7	22.5, CH_2	1.20, tq (7.1, 7.3)
	8	14.0, CH_3	0.78, t (7.3)
	9a	16.1, CH_2	0.35, ddd (4.5, 6.0, 8.2)
	9b		0.42, ddd (4.5, 4.5, 8.8)

[a]Measured at 100 MHz. [b]Measured at 400 MHz.

presence of four methyl groups (δ_H 0.74, 0.76, 0.78 and 1.33), four protons of the cyclopropane ring (δ_H 0.35, 0.42, 0.59 and 0.91), and two olefinic protons (δ_H 5.88 and 6.59). The ^{13}C NMR and HMQC spectra revealed the existence of two carbonyl groups (δ_C 166.0 and 177.8) and two sp^2 methines (δ_C 117.4 and 155.0). Examination of the COSY and HMBC spectra established the presence of two fragments derived from 4-hydroxy-5-isobutyl-3-methylpyrrolidin-2-one (HIMP) and 3-(2-propylcyclopropyl) acrylic acid (PCPA), respectively. The configuration of the C-2−C-3 olefinic bond in the PCPA was determined to be *trans* on the basis of the coupling constant ($^3J_{H2-H3}$ = 15.5 Hz). The connectivity of the two partial structures was determined from the HMBC correlation (H-3 of HIMP/C-1 of PCPA). As a result, the gross structure of **1** was established as shown in Figure 1.

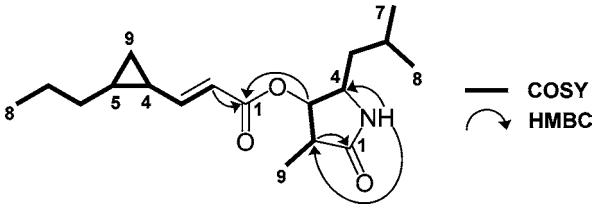
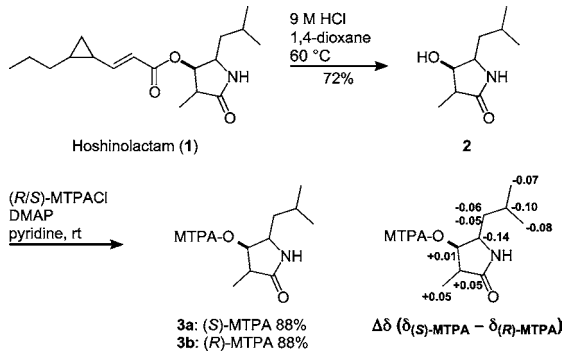


Figure 1. Gross structure of hoshinolactam (**1**) based on 2D NMR correlations.

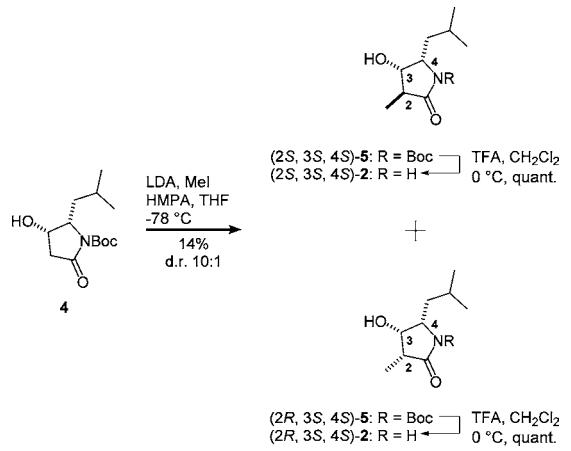
The absolute configuration of hoshinolactam (**1**) was established as follows. Regarding the HIMP moiety, there were three stereocenters: C-2, C-3, and C-4. This indicated that there were eight possible stereoisomers for the HIMP moiety. To determine the absolute configuration of C-3 of the HIMP moiety, the modified Mosher's method[11] was applied to **2**, which was prepared by the acid hydrolysis of **1**. Comparison of the ^1H NMR chemical shifts of (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) ester **3a** and (*R*)-MTPA ester **3b** revealed that the absolute configuration of C-3 of **2** was *R* (Scheme 1; see the Supporting Information).

Scheme 1. Preparation of MTPA Esters Derived from the HIMP of Hoshinolactam (1)



There were four possible combinations of the relative stereochemistries of C-2, C-3, and C-4: *syn* and *syn*, *syn* and *anti*, *anti* and *syn*, or *anti* and *anti*. To explore the possibility that the relative stereochemistry of C-3 and C-4 was *syn*, we synthesized two possible *syn* isomers, (2*S*,3*S*,4*S*)-**2** and (2*R*,3*S*,4*S*)-**2**, from the common intermediate **4**[12] (Scheme 2). As a result, the ^1H NMR spectra of the stereoisomers were

Scheme 2. Synthesis of Authentic Standards (2*S*,3*S*,4*S*)-2 and (2*R*,3*S*,4*S*)-2



not consistent with that of **2** from a natural source (see the Supporting Information). This indicated that the relative stereochemistry of C-3 and C-4 was *anti*. Therefore, the absolute configuration of C-4 was determined to be 4*S*.

To clarify the absolute configuration of C-2, we synthesized one of the two possible stereoisomers, (2*R*,3*R*,4*S*)-**2**, as follows (Scheme 3). Reduction of known Weinreb amide **6**[13] with lithium aluminum hydride followed by a non-Evans syn aldol reaction with the chiral auxiliary **7**[14] afforded the aldol adduct **8** as a single isomer (52% in two steps). Exposure of **8** to DMAP in MeOH gave the methyl ester **9** (78%). Deprotection of the methyl ester **9** followed by cyclization under basic conditions led to the desired lactam (2*R*,3*R*,4*S*)-**2** (87% in two steps). The absolute stereochemistry of C-2 of (2*R*,3*R*,4*S*)-**2** was confirmed to be 2*R* by application of the phenyl glycine methyl ester

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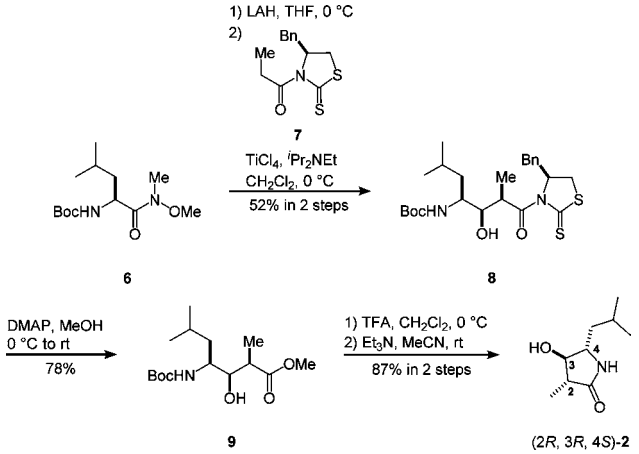
Scheme 3. Synthesis of Authentic Standard (2*R*,3*R*,4*S*)-2



(PGME) method[15] to the hydrolysate of **9** (see the Supporting Information). Moreover, the ^{1}H NMR spectrum of this compound did not match that of (2*R*,3*S*,4*S*)-**2**. This indicated that the absolute stereochemistry of C-3 was 3*R*. The ^{1}H NMR spectrum of (2*R*,3*R*,4*S*)-**2** was in agreement with that of the natural lactam **2** (see the Supporting Information). Thus, the absolute configuration of C-2 of **2** was determined to be 2*R*, and the stereochemistry of the HIMP moiety was clarified to be (2*R*,3*R*,4*S*).

Meanwhile, the relative stereochemistry of the two stereogenic centers of the PCPA moiety was determined on the basis of analyses of the coupling constants and NOESY correlations, as described below (Figure 2). Based on detailed decoupling

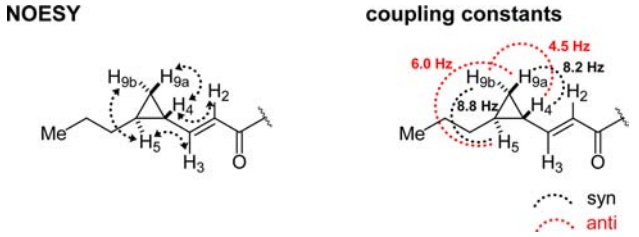
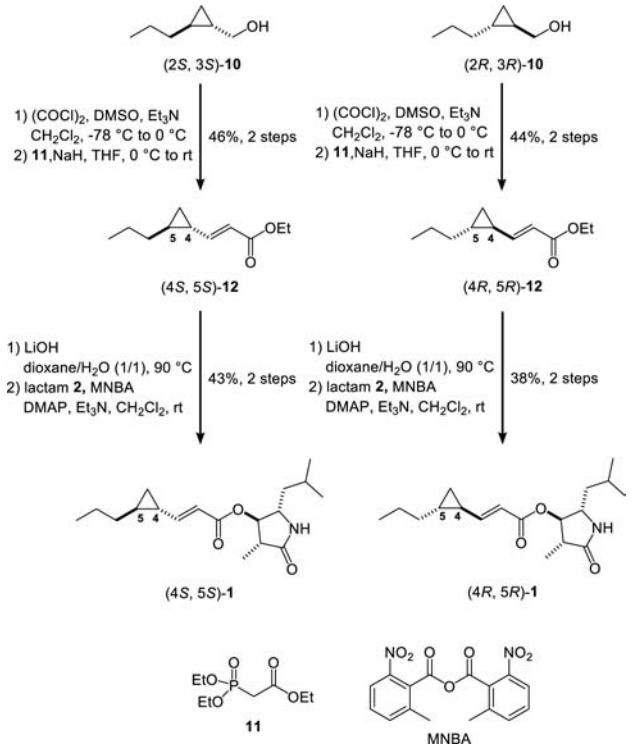


Figure 2. Relative stereochemistry of the PCPA moiety based on NOESY correlations and coupling constants.

experiments, the following coupling constants were observed: $^{3}J_{\text{H-4/H-9a}}$ = 8.2 Hz, $^{3}J_{\text{H-4/H-9b}}$ = 4.5 Hz, $^{3}J_{\text{H-5/H-9a}}$ = 6.0 Hz, and $^{3}J_{\text{H-5/H9-b}}$ = 8.8 Hz. The two large coupling constants indicated that H-4/H-9a and H-5/H-9b were in a *cis* relationship, respectively. On the other hand, the small values indicated that H-4/H-9b and H-5/H-9a were in a *trans* relationship, respectively. In addition, this conclusion was supported by the four NOESY correlations, H-2/H-4, H-4/H-9a, H-3/H-5, and H-5/H-9b. Thus, the relative stereochemistry of C-4 and C-5 of PCPA was determined to be *trans*. However, the absolute stereochemistry remained to be clarified. Therefore, we sought to determine the stereochemistry through the total synthesis of hoshinolactam (**1**) as follows (Scheme 4).

Regarding the PCPA moiety, two possibilities for the stereochemistry remained, (4*S*,5*S*) or (4*R*,5*R*). To clarify the absolute configuration of the PCPA moiety, we synthesized two ethyl esters, (4*S*,5*S*)-**12** and (4*R*,5*R*)-**12**, from known alcohols (2*S*,3*S*)-**10**[16] and (2*R*,3*R*)-**10**[17] by Swern oxidation followed by the Horner−Wadsworth−Emmons reaction with phosphonate

Scheme 4. Synthesis of (4*S*,5*S*)-1 and (4*R*,5*R*)-1



11, respectively. Next, hydrolysis of (4*S*,5*S*)-**12** and (4*R*,5*R*)-**12** and condensation with HIMP (**2**) using the Shiina reagent afforded (4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**, respectively. Surprisingly, their ^{1}H NMR spectra were very similar to each other, and only proton signals corresponding to the cyclopropane ring were slightly different. In addition, their ^{13}C NMR spectra were completely consistent. On the other hand, their specific optical rotations were completely different [(4*S*,5*S*)-**1**: $[\alpha]^{26.6}_{D}$ +59 (*c* 0.50, $CHCl_3$), (4*R*,5*R*)-**1**: $[\alpha]^{22.7}_{D}$ −54 (*c* 0.80, $CHCl_3$)]. As a result, the spectroscopic data (^{1}H and ^{13}C NMR spectra, and specific optical rotation) of (4*S*,5*S*)-**1** were fully consistent with those of natural hoshinolactam (**1**) (see the Supporting Information), and thus, the absolute configuration of hoshinolactam (**1**) was established as shown in **1**.

Hoshinolactam (**1**) showed potent antitrypanosomal activity against *Trypanosoma brucei brucei* GUTat 3.1 strain with an IC_{50} value of 3.9 nM, which was equivalent to the commonly used therapeutic drug pentamidine (IC_{50} 4.7 nM) (Table 2). On the

Table 2. Antitrypanosomal Activity of Hoshinolactam (1)

	IC_{50} (nM)	
compd	antitrypanosomal activity	cytotoxicity
hoshinolactam (**1**)	6.1	>25000
synthetic **1**	3.9	>25000
pentamidine[a]	4.7	16800

[a]Drug used to treat trypanosomal disease.

other hand, **1** did not show any cytotoxicity against human fetal lung fibroblast MRC-5 cells (IC_{50} > 25 μM). This indicated that hoshinolactam (**1**) recognized specific biomolecules for *Trypanosoma* protozoa.

In conclusion, hoshinolactam (**1**), an antitrypanosomal lactam, was isolated from a marine cyanobacterium. The gross structure was elucidated by spectroscopic analyses, and

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the absolute configuration was determined by the first total synthesis. This synthetic process should provide routes for obtaining synthetic analogues. In addition, hoshinolactam (**1**) showed potent antitrypanosomal activity without cytotoxicity against human fetal lung fibroblast MRC-5 cells. Studies on the structure−activity relationships of synthetic analogues should contribute to the development of new antitrypanosomal drugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00047.

^{1}H, ^{13}C, COSY, HMQC, HMBC, and NOESY NMR spectra in C_6D_6 and ^{1}H and ^{13}C NMR spectra in CD_3OD for hoshinolactam (**1**); ^{1}H NMR spectra in CD_3OD for lactam **2** from **1**; ^{1}H NMR spectra in C_6D_6 for MTPA esters and PGME amides; spectroscopic data and ^{1}H and ^{13}NMR spectra for synthetic products; detailed experimental procedures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: suenaga@chem.keio.ac.jp.

ORCID

Kiyotake Suenaga: 0000-0001-5343-5890

Notes

The authors declare no competing financial interest.

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