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# Poecillastrin E, F, and G, cytotoxic chondropsin-type macrolides from a marine sponge *Poecillastra* sp.

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ABSTRACT

Poecillastrin E (**1**), F (**2**), and G (**3**) were isolated from a marine sponge *Poecillastra* sp. as the cytotoxic constituents. Their planar structures were determined by analyzing the MS and NMR spectra. They are closely related to the known poecillastrin C (**4**). The absolute configuration of the β-hydroxyaspartic acid (OHAsp) residue was determined to be D-*threo* by Marfey's analysis of the hydrolysate. The mode of lactone ring formation of OHAsp residue in **1**–**3** was determined by selective reduction of the ester linkage followed by acid hydrolysis.



## 1. Introduction

Chondropsin class of macrolides, chondropsins,[1–4] poecillastrins,[5–7] and mirabalin,[8,9] were isolated from taxonomically diverse marine sponges. These metabolites were reported to possess 35-membered macrolide ring encompassing a β-hydroxyaspartic acid residue (OHAsp) in common. Chondropsins contain either a methoxycarbonyl terminus of the side chain or a malate esterification at C33, or both, whereas poecillastrins contain a methoxy group at C16. Due to the highly functionalized and complex structures of chondropsins and poecillastrins, their structure elucidation was not straightforward. Recently, we revised the mode of the lactone ring formation of poecillastrin C by selective reduction of the ester linkage followed by acid hydrolysis and comparison with authentic samples.[10,11] Because this revision was applied to three other congeners, we consider that the structures of other members also need investigation in this context. On the other hand, absolute configuration of other numerous stereogenic centers are yet to be determined. This class of metabolites are considered as a new class of anticancer lead compounds, because they exhibit potent cytotoxicity by selectively inhibit vacuolar $H^+$-ATPase.[12]

During our continuing search for metabolites which cause characteristic morphological changes in rat embryonic fibroblast 3Y1 cells from marine organisms,[11] the extract of a marine sponge *Poecillastra* sp. collected at Miyako Sea Knoll exhibited activity, from which three new congeners of poecillastrin C have been isolated. We describe the isolation, structure elucidation, and cytotoxic activity of these new metabolites.

## 2. Results and discussion

### 2.1. Isolation of cytotoxic constituents

The combined EtOH and $CHCl_3$/MeOH (1:1) extract of the sponge (1.3 kg, wet weight) was partitioned between $CHCl_3$ and $H_2O$. The organic layer was further partitioned between *n*-hexane and 90% MeOH, and the $H_2O$ layer was extracted with *n*-BuOH. Two bioactive fractions, 90% MeOH and *n*-BuOH layers, were combined and fractionated by ODS flash chromatography. Finally, the major cytotoxic constituents were purified by reversed-phase (RP)-HPLC to give poecillastrin E (**1**, 2.5 mg), F (**2**, 3.7 mg), and G (**3**, 1.3 mg), and the known poecillastrin C (**4**, 8.4 mg).

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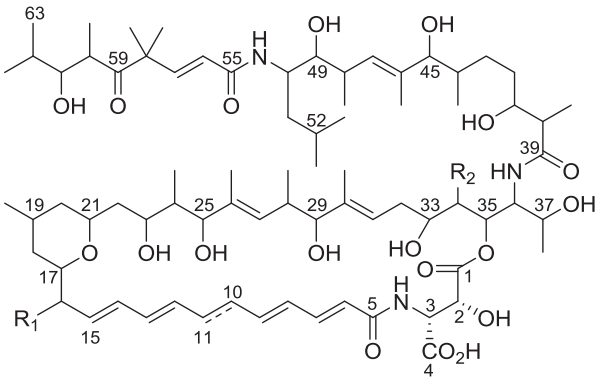

poecillastrin E (**1**): $\Delta^{10,11}$, $R_1$ = OMe, $R_2$ = Me
poecillastrin F (**2**): $R_1$ = H, $R_2$ = Me
poecillastrin G (**3**): $R_1$ = OMe, $R_2$ = H
poecillastrin C (**4**): $R_1$ = OMe, $R_2$ = Me

### 2.2. Structure elucidation of poecillastrins

Poecillastrin E (**1**) presented a molecular formula of $C_{78}H_{127}N_3O_{20}$ determined by HRESIMS. Analysis of the $^1$H NMR spectrum in conjunction with the HSQC data demonstrated the presence of 15 olefinic protons, 14 oxymethine protons, 18 methyl groups, and one methoxy group, which was reminiscent of chondropsin class of metabolites (Table 1). The UV absorption maximum observed at 356 nm suggested that the α,β,γ,δ-unsaturated carbonyl system observed in other poecillastrins was further extended as observed in mirabalin.[8,9] With this in mind, interpretation of the COSY and TOCSY spectra permitted the assignment of a pentaene system. Further analysis of the $^1$H–$^1$H spin system afforded ten partial structures (Fig. 1): partial structures **a** (C2–C3), **b** (C6–C23), **c** (C24 (Me)–C25), **d** (C26 (Me)–C29), **e** (C30 (Me)–C33), **f** (C34 (Me)–C38), **g** (C40 (Me)–C45), **h** (C46 (Me)–C54), **i** (C56–C57), and **j** (C60 (Me)–C64) as well as two singlet methyls. These partial structures are all observed in poecillastrin C (**4**) except of the pentaene, which possess an ethylene group in C10 and C11 in **4**. Interpretation of the HMBC data allowed us to connect these partial structures and showed that **1** is identical to poecillastrin C except for the additional double bond between C10 and C11. The geometries of all the olefins in the pentaene moiety were determined to be *E* by large vicinal coupling constants; $^3J_{H6\text{-}H7}$ (15.0 Hz), $^3J_{H8\text{-}H9}$ (14.5 Hz), $^3J_{H10\text{-}H11}$ (15.0 Hz), $^3J_{H12\text{-}H13}$ (14.7 Hz) and $^3J_{H14\text{-}H15}$ (14.7 Hz). The coupling constants of overlapped olefinic protons were determined by analysis of the HSQC spectrum. The configuration of $\Delta^{56,57}$-olefin was also determined to be *E* based on a large coupling constant of 15.4 Hz. The configurations of $\Delta^{26,27}$, $\Delta^{30,31}$, and $\Delta^{46,47}$-double bonds were all shown to be *E* on the basis of the $^{13}$C chemical shift values of methyls on C26 ($\delta_C$ 10.4), C30 ($\delta_C$ 10.6), and C46 ($\delta_C$ 11.8),[13,14] as well as NOE effects (26-Me/H28, 30-Me/H32, and 46-Me/H48). The absolute configuration and the direction of esterification through C1 or C4 in **1** were determined as previously reported.[11] Marfey's analysis of the acid hydrolysate of **1** revealed that the absolute configuration of the OHAsp residue was D-*threo* (Fig. S23), whereas reduction of **1** with $NaBH_4$ followed by acid hydrolysis and LC–MS analysis demonstrated the liberation of (2*R*,3*R*)-2-amino-3,4-dihydroxybutanoic acid (Fig. S24). Therefore, esterification of the β-carboxyl group of the OHAsp residue and (2*R*,3*R*)-configuration in **1** was demonstrated.

The molecular formula of poecillastrin F (**2**) was established to be $C_{77}H_{127}N_3O_{19}$ by HRESIMS, $CH_2O$ unit less than that of poecillastrin C (**4**). A *O*-methyl signal was absent in the spectrum of **2** when compared to that of **4**. Interpretation of the COSY and TOCSY data revealed that the C16-oxymethine in **4** was replaced by an allylic methylene ($\delta_H$ 2.09, 2.82 and $\delta_C$ 35.1, Table 2, Fig. S22). Further analysis of the 2D NMR data showed that the remaining structure was identical with that of **4**. The configurations of the eight olefins were all determined to be *E* based on the values of vicinal coupling constants, carbon chemical shifts of vinylic methyls, and NOE correlations. The absolute configuration and the mode of esterification of the OHAsp residue was determined by chemical degradation as described for **1**. Therefore, planar structure of poecillastrin F (**2**) was assigned as C16-desmethoxy poecillastrin C.

The molecular formula of poecillastrin G (**3**) was established by HRESIMS to be $C_{77}H_{127}N_3O_{20}$, which is $CH_2$ unit less than that of poecillastrin C (**4**). Comparison of the HSQC data between **3** and **4** showed that the C34 and C34-methyl signal in **4** were replaced by a methylene in **3**, otherwise, all the remaining structural units were present. As a result of this substitution, chemical shifts around C34 were perturbed. The C33 oxymethine proton ($\delta_H$ 3.29) in **3** was coupled to geminal methylene protons ($\delta_H$ 1.55 and 1.82), which was further correlated with the C35 oxymethine proton ($\delta_H$ 5.47) (Fig. S22). Although HMBC correlation from H35 to C1 and H3 to C4 were not observed due to the limited amount of the sample, downfield chemical shifts of the oxymethine proton at C35 ($\delta_H$ 5.47/$\delta_C$ 74.2) suggested that the oxygen atom at C35 was involved in forming the lactone ring. The NMR data indicated that **3** shared the same carbon backbone with that of **4**. Configurations of eight olefins were determined to be *E* by large vicinal coupling and NOE correlations. The absolute configuration and the mode of esterification of the OHAsp residue were determined by chemical degradation as described above. By comparing the $^1$H and $^{13}$C chemical shifts between **1** and **3**, we noticed significant perturbations of chemical shifts occur in signals of C40–C50 portion in **3**, but this phenomenon was not observed for **2** (Table 2). This suggests that C34-methyl group is specially close to the C40 to C50 portion, suggesting that the side chain folds over the macrocyclic portion. Poecillastrin E (**1**), F (**2**), G (**3**), and C (**4**) show potent cytotoxicity against rat embryonic fibroblast 3Y1 cells with the $IC_{50}$ values of 6.7 ng/mL, 1.2 ng/mL, 5.0 ng/mL, and 1.3 ng/mL, respectively.

Mirabalin, found in the sponge *Siliquariaspongia mirabilis*, has been the only example among the chondropsin class of metabolites with the conjugated pentaene moiety. Poecillastrin E (**1**) is the second example possessing this chromophore. Poecillastrin F (**2**) lacks the C16-methoxy group as observed in chondropsins; poecillastrin G (**3**) was devoid of the C34-methyl group as observed in mirabalin. We have added three congeners to the entry of chondropsin class of metabolites and determined their cytotoxic activity. Due to the inaccessibility of the samples, neither structure activity relationships nor stereochemistry of this class of metabolites has been established. Acquisition of such information is prerequisite for further utilization of this interesting class of metabolites as drug candidates.

## 3. Experimental section

### 3.1. General procedures

UV spectra were measured on a Shimadzu BioSpec-1600 spectrophotometer. Optical rotations were measured on a Jasco DIP-1000 polarimeter. NMR spectra were measured on a JEOL alpha 600 NMR spectrometer and referenced to solvent peaks: $\delta_H$ 3.30 and $\delta_C$ 49.0 for $CD_3OD$. ESI mass spectra were measured on a JEOL JMS-T100LC mass spectrometer. LC–MS experiments were