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Structure Revision of Poecillastrin C and the Absolute Configuration of the β-Hydroxyaspartic Acid Residue

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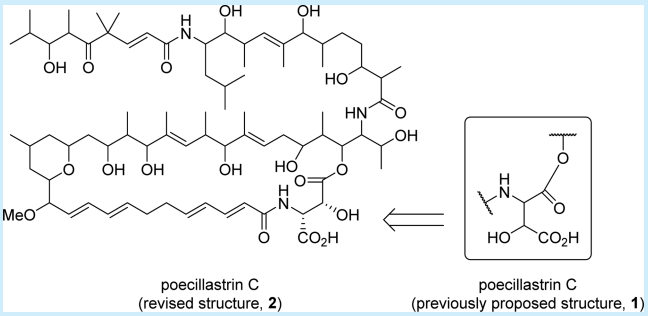
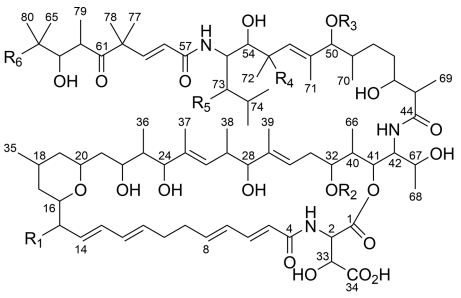
Supporting Information

ABSTRACT: The planar structure of poecillastrin C (**1**) was revised through selective reduction of the ester carbon. The absolute configuration of the β-hydroxyaspartic acid (OHAsp) residue was determined to be D-*threo* by Marfey's analysis. The acid hydrolysate of the reduction product of **1** liberated (2*R*,3*R*)-2-amino-3,4-dihydroxybutanoic acid, demonstrating that the β-carboxyl group in poecillastrin C was esterified. The structures of poecillastrins B–D and 73-deoxychondropsin A were also revised.



Chondropsins and poecillastrins are unique marine natural products. They were isolated only in small amounts from taxonomically diverse deep-sea sponges; chondropsins A, B, and D from *Chondropsis* sp.,[1,2] 73-deoxychondropsin A and chondropsin C from *Ircinia* sp.,[3] chondropsin A and 73-deoxychondropsin A from *Psammoclemma* sp.,[4] poecillastrins A–C from *Poecillastra* sp.,[5,6] poecillastrin C (**1**) and D from *Jaspis* sp.,[7] and mirabalin from *Siliquariaspongia* sp.[8] Chondropsins and poecillastrins were discovered as potent cytotoxic compounds with characteristic mean-graph profiles in the National Cancer Institute's 60-cell antitumor screen and later found to inhibit fungal and mammalian vacuolar H^+-ATPases (V-ATPases).[9] Due to their potent activity and unique structural features, this class of compounds is considered as promising leads for new therapeutic agents, especially as anticancer agents. However, further developmental studies have been hampered by restricted supply of the compounds from their natural sources. Total synthesis was unfeasible due to limited stereochemical information; only the relative stereochemistry of the THP ring has been reported, and none of the absolute configurations of 26 stereogenic centers in chondropsin A has been reported.[10]

The most difficult problem in the structure elucidation of chondropsin A was to assign which of the two carboxylic acid moieties in the OHAsp residue formed the ester linkage and which was free. The authors of ref 1 converted the free carboxylic acid group to a methyl ester and observed an NOE between the *O*-methyl signal and the oxymethine proton. From this analysis, they concluded that the carboxylic acid group



(previously proposed structures)

poecillastrin C (**1**): R_1 = OMe, R_2 = H, R_3 = H, R_4 = H, R_5 = H, R_6 = H
poecillastrin B: R_1 = OMe, R_2 = H, R_3 = H, R_4 = Me, R_5 = H, R_6 = H
poecillastrin D: R_1 = OMe, R_2 = H, R_3 = Me, R_4 = H, R_5 = H, R_6 = H
73-deoxychondropsin A: R_1 = H, R_2 = $COCH_2CH(OH)CO_2H$, R_3 = H, R_4 = H, R_5 = H, R_6 = CO_2Me

attached to the nitrogen-bearing methine formed the ester linkage. Although the distance between the carbomethoxy protons and the α-methine proton is shorter than that between the carbomethoxy protons and the β-methine proton, both distances fall within the range that can give observable NOEs. Because both carboxyl groups in the OHAsp residues are located within three bonds from either of the methine protons, it is not possible to assign the two carboxyl carbons by HMBC data, unless a HMBC cross-peak is observed from the amide proton (H-3) to one of the carboxyl carbons, which was not

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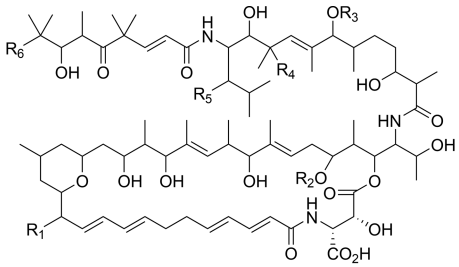
observed in chondropsin A. In the structure elucidation of other chondropsin congeners, the same mode of ester formation was proposed without firm evidence.

In the course of our search for bioactive marine metabolites, which cause morphological changes in rat embryonic fibroblast 3Y1 cells, we found activity in the extract of a marine sponge *Poecillastra* sp. collected at Oshima-shinsone. The combined EtOH and $CHCl_3$/MeOH (1:1) extract of the sponge (70 g, wet weight) was partitioned between $CHCl_3$ and H_2O, and the H_2O layer was further extracted with *n*-BuOH. The $CHCl_3$ and *n*-BuOH fractions were combined and fractionated by ODS flash chromatography and RP-HPLC to give 3.2 mg of poecillastrin C as the main active constituent (Supporting Information, SI). The structure of poecillastrin C had been assigned as **1**. We examined the NMR data of poecillastrin C and found that the spectroscopic data could not exclude the possibility of structure **2**. Therefore, we set out to obtain secure chemical evidence for the structure around the OHAsp moiety.

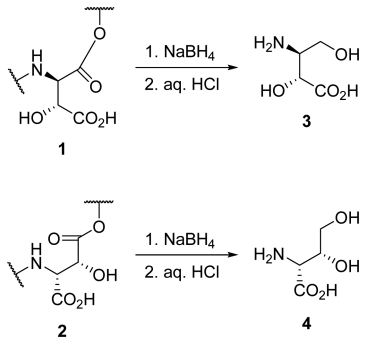


(revised structures)

poecillastrin C (**2**): R_1 = OMe, R_2 = H, R_3 = H, R_4 = H, R_5 = H, R_6 = H
poecillastrin B: R_1 = OMe, R_2 = H, R_3 = H, R_4 = Me, R_5 = H, R_6 = H
poecillastrin D: R_1 = OMe, R_2 = H, R_3 = Me, R_4 = H, R_5 = H, R_6 = H
73-deoxychondropsin A: R_1 = H, R_2 = $COCH_2CH(OH)CO_2H$, R_3 = H, R_4 =H, R_5 = H, R_6 = CO_2Me

We took advantage of the different reactivity of the ester and carboxylic acid moieties toward hydride reduction (Scheme 1).

Scheme 1. Differentiation of the Alternate Modes of Lactone Formation by Chemical Modification

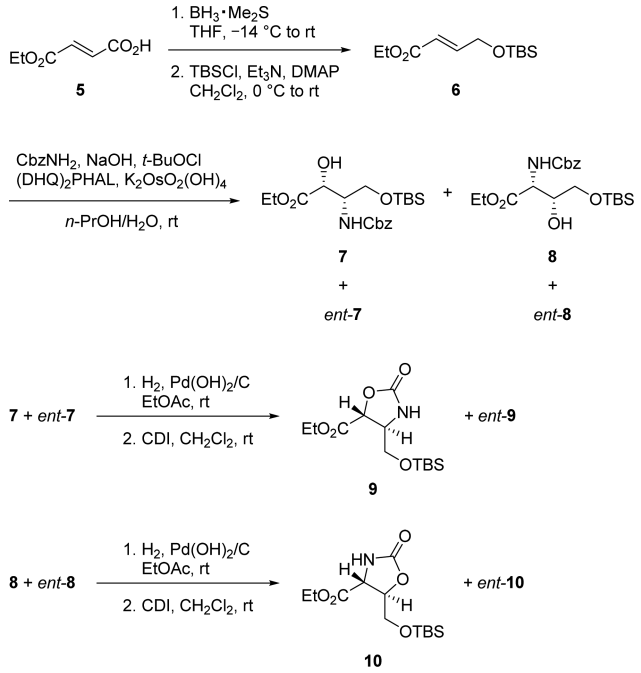


In structure **1**, reduction with $NaBH_4$ would convert the C-1 ester carbonyl carbon to a hydroxymethyl, while the C-34 carboxylic acid would stay intact, thereby affording 3-amino-2,4-dihydroxybutanoic acid following acid hydrolysis.[11] On the other hand, if a compound with structure **2** is reduced with $NaBH_4$ and then hydrolyzed, it would afford 2-amino-3,4-dihydroxybutanoic acid.

As a prelude, we determined the absolute configuration of two chiral centers in the OHAsp residue. The acid hydrolysate of poecillastrin C was subjected to Marfey's analysis,[12] which showed that the OHAsp residue was D-*threo* (SI). Therefore, the acid hydrolysate of the reduction product of poecillastrin C would afford (2*R*,3*S*)-3-amino-2,4-dihydroxybutanoic acid (**3**) if the structure of poecillastrin C is **1**; the compound with structure **2** would give (2*R*,3*R*)-2-amino-3,4-dihydroxybutanoic acid (**4**).

The protected forms of compounds **3** and **4** were prepared as follows. The carboxyl group of monoethylfumarate (**5**) was reduced to the allyl alcohol (Scheme 2), which was protected

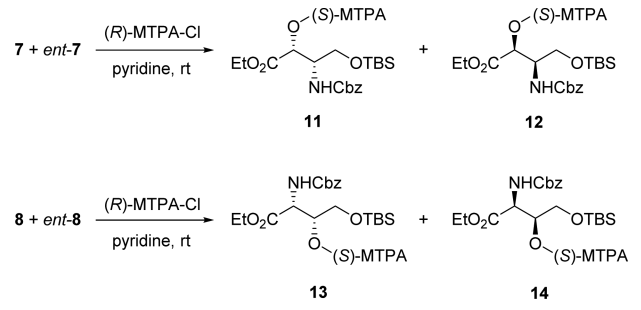
Scheme 2. Synthesis of 9, 10, and Their Enantiomers



by the TBS group. The resultant TBS ether (**6**) was subjected to the Sharpless asymmetric aminohydroxylation[13] to give a mixture of **7**, **8**, and their enantiomers. The enantiomeric mixtures were obtained after HPLC separation. The relative configurations of all the products were shown to be *syn* by conversion to the cyclic carbamates (**9**, **10**, and their enantiomers), in which a $^3J_{H2,H3}$ value of 4.8 Hz was observed for each.[14]

The optical resolution of the enantiomers and assignment of their absolute configurations were achieved by the modified Mosher's method (Scheme 3).[15] The mixture of **7** and its enantiomer was derivatized with (*R*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl) to give **11** and **12**, which were separated by HPLC. Their ^{1}H NMR data showed that the absolute configuration of **11** was (2*R*,3*S*). The mixture of **8** and

Scheme 3. Preparation of (*S*)-MTPA Esters 11−14



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its enantiomer was examined in the same way to give **13** and **14**, and the configuration of **13** was established as (2*R*,3*R*). Then, **11**, **13**, and the reduction product of poecillastrin C were hydrolyzed and subjected to Marfey's analysis, which demonstrated that the hydrolysate of **13** and the reduction product of poecillastrin C were identical (Figure 1, SI). Therefore, the structure of poecillastrin C was reassigned as **2**.

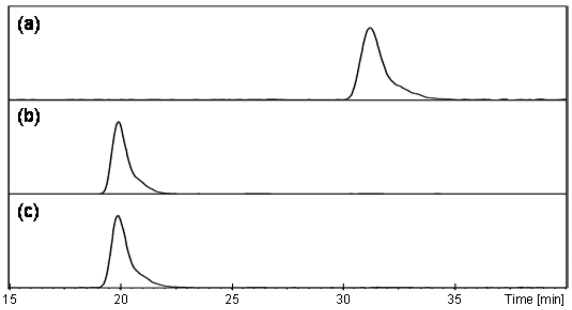

Figure 1. LC−MS chromatograms of Marfey's derivatives: (a) acid hydrolysate of **11**; (b) acid hydrolysate of **13**; (c) acid hydrolysate of the reduction product of poecillastrin C.

The same set of experiments run with authentic samples of poecillastrins B, D, and 73-deoxychondropsin A liberated **4**, demonstrating that the mode of ester formation in these compounds had to be revised (SI).

A combination of microscale reduction, hydrolysis, and derivatization reactions was performed on four different poecillastrin and chondropsin macrolides, and the resulting products were compared with appropriate synthetic standards. The natural products all provided derivatives of the OHAsp residue that are only consistent with macrolactonization occurring via the side chain carbonyl and not the α-amino acid carbonyl. In this work, we showed that the planar structures of the chondropsin/poecillastrin class of metabolites need to be revised, setting the stage for further stereochemical assignment of this class of bioactive compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02835.

Description of experimental procedure, LC−MS chromatograms, and NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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