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# Anti-inflammatory cyclopeptides from the marine sponge *Theonella swinhoei*

Carmen Festa [a], Simona De Marino [a], Maria Valeria D'Auria [a], Maria Chiara Monti [b], Mariarosaria Bucci [c], Valentina Vellecco [c], Cécile Debitus [d], Angela Zampella [a,*]

[a] *Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy*
[b] *Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, via Ponte don Melillo, 84084 Fisciano (SA), Italy*
[c] *Dipartimento di Farmacologia Sperimentale, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy*
[d] *Institut de Recherche pour le Dèveloppement (IRD), Polynesian Research Center on Island Biodiversity, BP529, 98713 Papeete, Tahiti, French Polynesia*

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ABSTRACT

DCCC chromatography followed by HPLC purification on the polar extract of marine sponge *Theonella swinhoei* resulted in the isolation of five new cyclopeptides, perthamides G–K. The new structures, featuring unprecedented amino acid units, were determined by interpretation of extensive spectroscopic and spectrometric data (MS, $^{1}$H and $^{13}$C NMR, COSY, HSQC, HMBC, and ROESY). Pharmacological analysis demonstrated that these natural cyclopeptides are endowed with anti-inflammatory potential as assessed by their ability to reduce carrageenan-induced mouse paw oedema.



## 1. Introduction

Cyclic peptides and cyclodepsipeptides from sponges have been extensively studied for their significant biological activities and structurally unique features incorporating several modified amino acid residues.[1,2] Whereas many of these compounds were shown to be antifungal, antiviral and antiproliferative, few examples of anti-inflammatory peptides from sponges or, more generally, from marine habitats have so far been reported.[3–6]

As a part of our systematic study on secondary metabolites from marine organisms collected at Solomon Islands,[7] we found a single specimen of the sponge *Theonella swinhoei* as an extraordinary source of new metabolites,[8–12] among which were identified two classes of anti-inflammatory peptides, perthamides C–F (Fig. 1),[13–16] and solomonamides.[17] Perthamide C (**1**, Fig. 1), the most abundant component of the sponge's polar extracts, is an octapeptide with an unprecedented primary structure. Six of its eight residues are non conventional amino acids: γ-methylproline, $N^{\delta}$-carbamoyl-β-$OSO_3$asparagine ($N^{\delta}$-c-β-OHAsn), *o*-tyrosine, dAbu, *O*-methylthreonine, and the β-amino acid AHMHA (3-amino-2-hydroxy-6-methylheptanoic acid).

From a biological point of view, perthamide C showed promising anti-inflammatory activity measured as a dose-dependent reduction of mouse carrageenan-induced paw oedema and as a down-regulation of TNF-α and IL-8 release, two key biomarkers in the inflammatory response of primary human keratinocytes cells.[16]

In order to re-isolate further amounts of perthamide C for additional pharmacological studies, we re-examined the polar extracts of the sponge *T. swinhoei*, still available in our laboratories. In this paper we report the isolation of perthamide C and five new minor analogues, perthamides G–K (**2**–**6**), whose structures have been determined by NMR spectroscopy and mass spectrometry analysis (Fig. 2).

## 2. Results and discussion

The initial processing of the *T. swinhoei* (coll. No. R3170) was conducted according to procedures described previously.[13] The *n*-BuOH extract was purified by DCCC (*n*-BuOH/$Me_2CO$/$H_2O$, descending mode) followed by reverse-phase HPLC using a C-12 column (MeOH/$H_2O$ 59% with 0.1% TFA as eluent) to afford 69 mg of perthamide C (**1**), 10.8 mg of perthamide G (**2**), 17.8 mg of perthamide H (**3**), 16.6 mg of perthamide I (**4**), 12.2 mg of perthamide J (**5**), and 16.2 mg of perthamide K (**6**).

The HR-ESIMS spectrum of perthamide G (**2**) showed a major peak at *m*/*z* 1052.4046 $[M-Na]^-$ corresponding to a molecular formula $C_{43}H_{62}N_{11}NaO_{18}S$, 14 u.m.a. less than perthamide C.

Accordingly, comparison of the $^{1}$H NMR spectrum of perthamide G (**2**) with that of parent compound perthamide C (**1**)

* Corresponding author. Tel.: +39 (0)81 678525; fax: +39 (0)81 678552; e-mail addresses: angela.zampella@unina.it, azampell@unina.it (A. Zampella).

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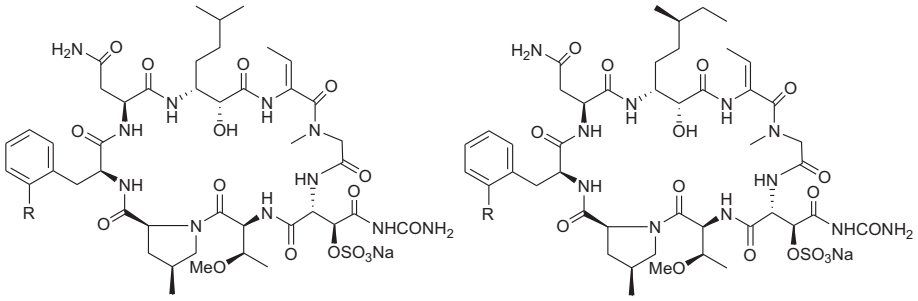
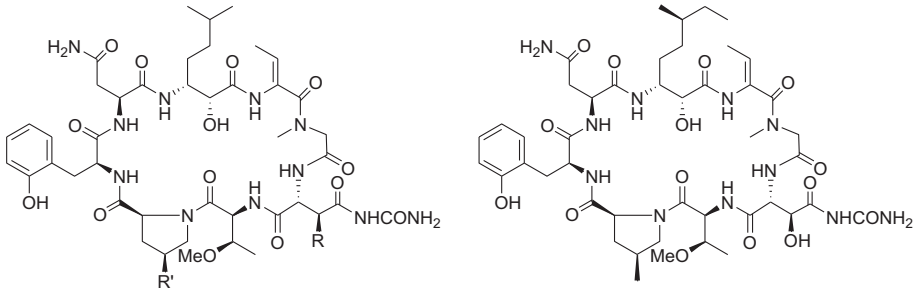


Perthamide C (**1**) R = OH
Perthamide D R = H

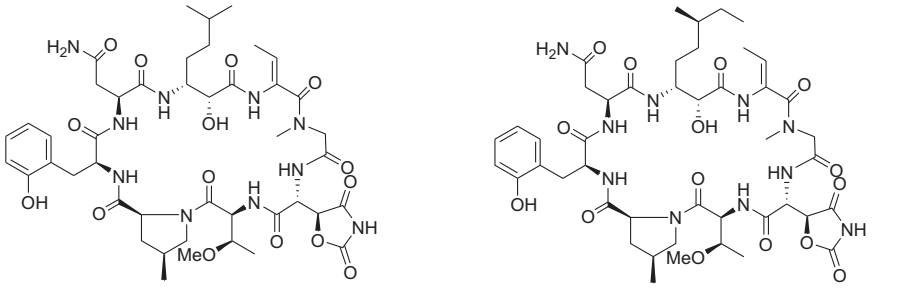
Perthamide E R = OH
Perthamide F R = H

**Fig. 1.** Perthamides C–F previously isolated from *Theonella swinhoei*.



Perthamide G (**2**) R = $OSO_3Na$ R' = H
Perthamide H (**3**) R = OH R' = $CH_3$

Perthamide I (**4**)



Perthamide J (**5**)

Perthamide K (**6**)

**Fig. 2.** New perthamide derivatives from *T. swinhoei*.

showed the absence of the methyl group signal at $\delta_H$ 1.04 assigned to 3-Me group in the γ-MePro residue. One spin system of the type X–CH–$CH_2$–$CH_2$–$CH_2$–X′ was easily defined using TOCSY, COSY, HSQC and HMBC data and indicated the presence of one proline unit. The remaining seven amino acid units were found to be the same of perthamide C, as suggested by 2D NMR spectroscopic data.

Marfey's method revealed L configuration for the Pro residue, whereas the relative and absolute configuration of L-Asn, L-*o*-Tyr, L-ThrOMe, *erythro*-D-$N^{\delta}$-c-β-OHAsn, (2*R*,3*R*)-AHMHA and *Z*-dAbu, were determined by comparison of NMR spectroscopic data and LC–MS analysis of the L/D-FDAA-derivatized hydrolysate of **2** with **1**.

The molecular formula of perthamide H (**3**), $C_{44}H_{65}N_{11}O_{15}$, was consistent with the lack of the sulfate group in the $N^{\delta}$-carbamoyl-β-$OSO_3$asparagine residue.

This hypothesis was confirmed by analysis of the NMR spectroscopic data. An additional exchangeable proton signal at $\delta_H$ 6.16 (br d, $J$=6.4) was observed in the $^1$H NMR spectrum of perthamide H (**3**), and was assigned to the β-OH group in the $N^{\delta}$-c-β-OHAsn residue on the basis of the COSY spin system NH/H-2/H-3/OH and the diagnostic HMBC correlations from the hydroxyl proton to C-2, C-3 and C-4 (Fig. 3). The downfield shift exhibited by the $NH^{\delta}$ proton (9.52 in **3** vs 8.90 in **1**) and C-2 (54.8 in **3** vs 53.0 in **1**) and C-4 (173.2 in **3** vs 170.5 in **1**) carbons and the upfield shift of H-2, H-3 and C-3 nuclei (Table 1) were all compatible with the presence of the $N^{\delta}$-c-β-OHAsn residue in **3**.

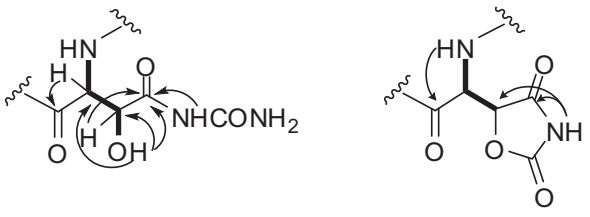


**Fig. 3.** $N^{\delta}$-c-β-OHAsn and ADAA residues in perthamides H and J, respectively, with COSY connectivities (bold bonds) and HMBC correlations (arrows).

The HR-ESIMS of perthamide I (**4**) showed an ion peak at $m/z$ 1000.4784 $[M-H]^-$ ($m/z$ 1000.4740, calcd for $C_{45}H_{66}N_{11}O_{15}$), 14 mass units higher than that of **3**. The NMR data for **4** (Table 2)