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Exiguolide, a New Macrolide from the Marine Sponge *Geodia exigua*

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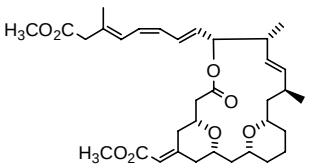

The structure of exiguolide was determined on the basis of spectroscopic data.

Exiguolide, a New Macrolide from the Marine Sponge *Geodia exigua*

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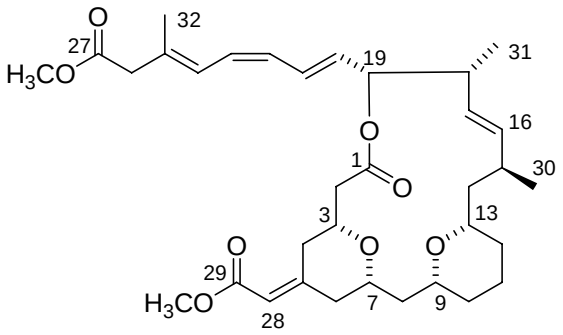
Abstract— A new 20-membered macrolide designated exiguolide has been isolated from the marine sponge *Geodia exigua*, and its structure determined by interpretation of spectroscopic data. Exiguolide specifically inhibited fertilization of sea urchin (*Hemicentrotus pulcherrimus*) gametes but not embryogenesis of the fertilized egg.

Marine sponges have provided a seemingly inexhaustible supply of bioactive metabolites.[1] In the course of our continuing search for inhibitors on echinoderm fertilization from marine sponges,[2–5] we isolated a new macrolide designated exiguolide (**1**) from the MeOH extract of the sponge *Geodia exigua* Thiele (order Astrophorida, family Geodiidae). This report describes the purification and the structure elucidation of **1**.



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Keywords: Marine sponge; *Geodia exigua*; Macrolide.

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The marine sponge *Geodia exigua* Thiele (order Astrophorida, family Geodiidae; 160 g, wet weight) collected off Amami-Oshima, Kagoshima Prefecture, Japan in July 2001, was cut into small pieces and steeped in MeOH.[6,7] The concentrated MeOH extracts were partitioned between water and hexane. The bioactive hexane-soluble portion (1.1 g) was separated on ODS column into several fractions by elution with 0–100% MeOH in H_2O. The fraction eluted with 88% MeOH–H_2O solvent system was further subjected twice to medium pressure column chromatography on ODS employing CH_3CN–H_2O gradient mixtures to afford **1** (3.5 mg, 0.002 % wet weight) as viscous oil, $[\alpha]_D^{25}$ –92.5˚ (*c* 0.069, $CHCl_3$).

The (+)-FABMS data of **1** exhibited a pseudomolecular ion peak at *m/z* 585 corresponding to $[M+H]^+$. The molecular formula of **1** was established to be $C_{34}H_{48}O_8$ on the basis of high-resolution FABMS data (*m/z* 585.3400 $[M+H]^+$, Δ–2.7 mmu). The IR spectrum displayed absorption bands at 1734, 1717, 1700, 1684, 1653, and 1636 cm^{-1} (C=O and C=C). The UV absorption at 276 nm (ε 42,200) suggested the presence of an α,β-unsaturated carbonyl group. The ^1H NMR spectrum measured in $CDCl_3$ displayed partially overlapped proton signals (Table 1). Therefore, extensive NMR measurements were also performed in C_6D_6 and in CD_3OD (Supplementary data). Inspection of the ^1H and ^{13}C NMR spectra together with DEPT and HMQC spectral data revealed the presence of two aliphatic methyls, an olefinic methyl, two oxygenated methyls, nine aliphatic methylenes, two aliphatic methines, five oxygenated methines, eight olefinic methines, two quaternary olefinic carbons, and three carbonyl carbons. The interpretation of ^1H-^1H COSY spectrum and 1D-TOCSY experiments revealed the presence of two partial structures (C-2 to C-4 and C-6 to C-24 containing C-30 and C-31). The connectivity between C-1 and C-2 was established on the HMBC correlations of H-2a and H-2b to C-1. The connectivity between C-4 and C-6 through C-5 and the presence of the methoxycarbonylmethylidene group attached at C-5 were established on the HMBC correlations (H-4_{ax}/C-5 and C-28; H-4_{eq}/C-5, C-6, and C-28; H-6_{ax}/C-5 and C-28; H-6_{eq}/C-5 and C-28; H-28/C-4, C-6 and C-29; 29-OCH_3/C-29). The absence of hydroxyl groups was suggested by the lack of an OH absorption band in the IR spectrum, which was supported by a deuterium-induced ^{13}C NMR isotope shift experiment of **1**. Typically, deuterium-induced upfield shifts (0.09–0.15 ppm) have been reported for carbons bearing a hydroxyl group.[8] The oxygen-bearing methine carbons (C-3, C-7, C-9, C-13, and C-19) of **1** showed shifts less than 0.04 ppm upon changing the NMR solvent from CD_3OH to CD_3OD. Although there were no HMBC correlation peaks which supported connectivity between C-3 and C-7 and between C-9 and C-13, NOE observations H-3/H-7 and H-9/H-13 indicated the presence of ether linkages between C-3 and C-7 and between C-9 and C-13 to form two tetrahydropyran ring systems. The large coupling constants ($J_{H\text{-}3,H\text{-}4ax}$ = 12.8 Hz, $J_{H\text{-}6ax,H\text{-}7}$ = 11.3 Hz, and $J_{H\text{-}9,H\text{-}10ax}$ = $J_{H\text{-}12ax,H\text{-}13}$ = 11.0 Hz) confirmed the chair conformations of the methoxycarbonylmethylidenetetrahydropyran ring (C-3 to C-7, C-28, and C-29) and the tetrahydropyran ring (C-9 to C-13). These structures of the tetrahydropyran ring systems were supported by the comparison of ^{13}C NMR chemical shift data with literature values.[9,10] The presence of the methoxycarbonylisopropylidene group at C-24 was established on the HMBC correlations (H-23/C-25; H-24/C-26 and C-32; H_2-26/C-24, C-25, C-27, and C-32; H_3-32/C-24, C-25, and C-26; 27-OCH_3/C-27). The HMBC correlation of H-19 to C-1 indicates the connectivity between C-1 and C-19 via an ester linkage, completing the macrocyclic ring in **1**. The geometry of disubstituted olefinic bonds was determined to be 16*E*,20*E*,22*Z* on the basis of the vicinal ^1H couplings ($J_{H\text{-}16,H\text{-}17}$ = $J_{H\text{-}20,H\text{-}21}$ = 15.3 Hz and $J_{H\text{-}22,H\text{-}23}$ = 11.0 Hz). The geometry of the C5/C28 olefinic bond was established as *Z* based on the NOESY correlation between H-6eq and H-28. The observations of NOEs between H-23 and H_3-32 and between H-24 and H_2-26 indicated the *E* geometry of Δ^{24}, leading to the gross structure depicted in structure **1**.

Only one set of signals observed in the ^1H NMR spectrum indicates that **1** predominantly exists in one conformation in solution. The magnetic non-equivalence of the geminal methylene protons at C-2, C-8, and C-14 suggested the rigidity of the macrocyclic ring in **1**. The relative stereochemistry of **1** was elucidated on the basis of NOESY correlations and vicinal ^1H couplings. The small $^3J_{H\text{-}18,H\text{-}19}$ (< 1 Hz) was indicative of a gauche orientation between H-18 and H-19. The NOE correlations were observed between H-17/H-19, H-18/H-20, and H-20/H_3-31, but not between H-17/H-20 or H-19/H_3-31. These observations indicate that the relative configuration between C-18 and C-19 is as shown in Figure 1.

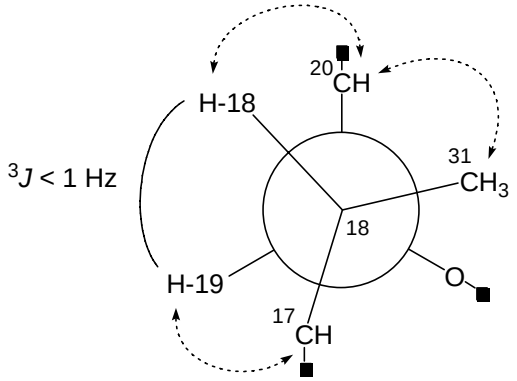

Figure 1. The relative configuration of the C-18–C-19 moiety of **1**. NOE correlations are shown by dotted arrows.

The relatively large vicinal ^1H couplings ($J_{H\text{-}2a,H\text{-}3}$ = 11.0 Hz, $J_{H\text{-}7,H\text{-}8a}$ = 9.2 Hz, $J_{H\text{-}8b,H\text{-}9}$ = 8.5 Hz, $J_{H\text{-}13,H\text{-}14a}$ = 11.0 Hz, $J_{H\text{-}14b,H\text{-}15}$ = 12.2 Hz, $J_{H\text{-}15,H\text{-}16}$ = 9.8 Hz, $J_{H\text{-}17,H\text{-}18}$ = 9.8 Hz) required anti orientations between H-2a/H-3, H-7/H-8a, H-8b/H-9, H-13/H-14a, H-14b/H-15, H-15/H-16, and H-17/H-18. The strong NOE correlations were observed between H-7/H-10eq, H-7/H-9, H-9/H-19, H-13/H-16, H-13/H-17, H-13/H-19, H-15/H-17, and H-16/H-18.

Conformational searches were performed for all the possible stereoisomers of **1** using CAChe CONFLEX program with the MM2 force field for energy minimization field (CAChe version 5.5; Fujitsu Co., Tokyo, Japan).[11,12] For only the stable conformational model with 3*R**,7*S**,9*R**,13*S**,15*S**,18*R**,19*R** configuration, the calculated proton distances and dihedral angles were in full agreement with the observed NOE correlations and vicinal ^{1}H couplings mentioned above, respectively. The calculated global minimum energy structure and the NOE correlations were shown in Figure 2. The relative stereochemistry of **1** was supported by the *J*-based configuration analysis method (Supplementary data).[13,14] At present, the absolute configuration of **1** remains to be determined.

Table 1. NMR spectral data of **1** in $CDCl_3$[a]

No	δ_H multiplicity (*J* in Hz)	δ_C	No	δ_H multiplicity (*J* in Hz)	δ_C
1		170.7 s	15	2.53 m	33.1 d
2a	2.57 dd (14.0, 11.0)	41.6 t	16	5.08 dd (15.3, 9.8)	135.5 d
b	2.55 dd (14.0, 2.4)		17	5.52 dd (15.3, 9.8)	132.5 d
3	3.79 dddd (12.8, 11.0, 4.3, 2.4)	74.2 d	18	2.34 br dq (9.8, 7.3)	42.1 d
4ax	1.97 dd (13.4, 12.8)	34.8 t	19	5.26 br d (7.3)	78.6 d
eq	3.87 br d (13.4)		20	5.66 dd (15.3, 7.3)	131.4 d
5		156.7 s	21	6.67 dd (15.3, 11.6)	127.6 d
6ax	2.23 br dd (12.8, 11.3)	42.5 t	22	5.97 dd (11.6, 11.0)	128.0 d
eq	2.12 br d (12.8)		23	6.19 dd (11.6, 11.0)	125.5 d
7	3.04 br dd (11.3, 9.2)	74.9 d	24	6.38 br d (11.6)	124.1 d
8a	1.78 br dd (13.4, 9.2)	44.1 t	25		132.5 s
b	1.56 br dd (13.4, 8.5)		26a	3.13 s	45.4 t
9	3.30 br dd (11.0, 8.5)	75.4 d	b	3.13 s	
10ax	1.12 br ddd (12.8, 11.0, 11.0)	32.5 t	27		171.8 s
eq	1.62 br d (12.8)		28	5.70 br s	115.0 d
11ax	1.51 m	23.9 t	29		166.8 s
eq	1.77 br d (12.8)		30	0.94 d (6.7)	21.8 q
12ax	1.21 br dddd (12.8, 11.0, 11.0, 3.7)	31.6 t	31	1.05 d (7.3)	14.4 q
eq	1.41 br d (12.8)		32	1.85 br s	16.8 q
13	3.21 br t (11.0)	76.0 d	27-OCH_3	3.70 s	51.9 q
14a	1.47 ddd (14.0, 11.0, 3.1)	43.1 t	29-OCH_3	3.69 s	51.0 q
b	1.09 br dd (14.0, 12.2)				

[a] The ^{1}H and ^{13}C NMR data were measured at 500 and 125 MHz, respectively. Chemical shifts were referenced to residual $CHCl_3$ (δ_H 7.26) and $CDCl_3$ (δ_C 77.0).

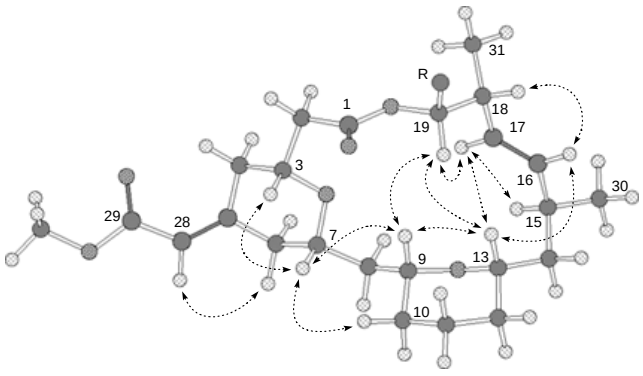

Figure 2. Key NOE correlations and the relative stereochemistry of **1**. NOE correlations are shown by dotted arrows. To clarify the backbone structure, the side chain at C-19 was omitted.

Compound **1** was also detected (by MS) in the crude ethanol extract, clearly indicating that the substance is a genuine natural product and not an artifact.

Although many biologically active macrolides have been obtained from marine organisms,[1] there are very few compounds having the methoxycarbonylmethylidenetetrahydropyran ring among them. Antineoplastic compounds, bryostatins, are known to have the unique chemical structure containing one or more methoxycarbonylmethylidenetetrahydropyran rings.[15,16]

We have treated sea urchin (*Hemicentrotus pulcherrimus*) gametes with **1** to investigate the effects of **1** on fertilization and egg activation. Concentrations at or higher than 21 μM of **1** prevented fertilization as revealed by the egg's incapability of forming the fertilization envelope by

insemination. However, **1** at 100 μM did not affect the development of fertilized eggs up to the gastrula stage.

Acknowledgments

We thank Captain A. Goh and the crew of R/V Toyoshio-Maru of Hiroshima University for the help in the collection of the sponge sample, Professor Patricia R. Bergquist, The University of Auckland, New Zealand, for the identification of the sponge specimen, and Mr. Hitoshi Fujitaka, Hiroshima University, for the NMR measurements. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supplementary data

Supplementary data (NMR spectral data in C_6D_6 and in CD_3OD and $^3J_{HH}$, $^2J_{CH}$, and $^3J_{CH}$ data of **1**) associated with this article can be found, in the online version, at ...

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