Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3037–3039

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Exiguamide, a New Spirocyclic Sesquiterpene from the Marine Sponge *Geodia exigua* that Inhibits Cell Fate Specification During Sea Urchin Embryogenesis

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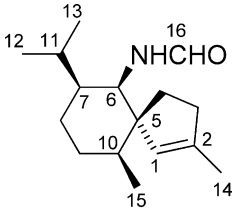
Received 24 June 2002; accepted 9 August 2002

Abstract—A new nitrogen-containing bicyclic spirosesquiterpene designated exiguamide which inhibited cell fate specification during sea urchin embryogenesis has been isolated from the marine sponge *Geodia exigua*. Its structure was determined by interpretation of spectral data and X-ray crystallographic analysis.

During the early development of sea urchins, the cell division synchrony in all blastomeres lasts only until the third cleavage. In the fourth cleavage, the four cells of the animal tier split meridionally into eight blastomeres, each having the same volume. The vegetal tier, however, undergoes an unequal equatorial cleavage to produce four large cells, the macromeres, and four smaller micromeres at the vegetal pole. Micromeres give rise to spiculogenetic primary mesenchyme cells at later developmental stages. The sea urchin embryo provides an excellent model system to study the molecular mechanisms of cell fate specification. This report describes the purification and the structure elucidation of a new nitrogen-containing bicyclic spirosesquiterpene designated exiguamide (**1**) which is capable of inhibiting the formation of micromeres without affecting cell division thereby producing spicule-deficient larvae.



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The marine sponge *Geodia exigua* Thiele (order Astrophorida, family Geodiidae; 160 g, wet weight) collected off Oshima, Kagoshima Prefecture, Japan in July 2001, was cut into small pieces and steeped in MeOH. The concentrated MeOH extracts were partitioned between water and hexane. The bioactive hexane-soluble fraction (1.1 g) was subjected to ODS column chromatography using 0–100% MeOH in H_2O as eluent. The bioactive fraction was purified by silica gel column chromatography using 0–100% ethyl acetate in hexane as eluent, followed by crystallization from aqueous MeOH to furnish **1** (8.0 mg; 0.005% wet weight) as colorless crystals, mp 139–140 °C, $[\alpha]_D^{25}$ +31.7° (*c* 0.08, $CHCl_3$).

The (+)-FABMS and (−)-FABMS data of **1** exhibited pseudomolecular ion peaks at *m*/*z* 250 and *m*/*z* 248 corresponding to $[M+H]^+$ and $[M-H]^-$, respectively. The molecular formula of **1** was established to be $C_{16}H_{27}NO$ on the basis of high-resolution FABMS data (*m*/*z* 250.2174 $[M+H]^+$, Δ +0.3 mmu). The IR spectrum displayed absorption bands at 3323 (NH) and 1657 cm^{-1} (amide and C=C). Inspection of the ^1H and ^{13}C NMR spectra (Table 1) together with DEPT and HMQC spectral data revealed the presence of three aliphatic methyls, an olefinic methyl, four aliphatic methylenes, four aliphatic methines, an olefinic methine, a formyl and two quaternary carbons. ^1H–^1H COSY correlations between the NH proton and both H-6 and H-16 provided evidence of the occurrence of the formylamino

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PII: S0960-894X(02)00662-5

Table 1. NMR spectral data of 1 in DMSO-d_6[a]

No.	δ_H multiplicity (*J* in Hz)	δ_C
1	5.58 br s	131.77 d
2		139.29 s
3a	2.34 ddd (15.8, 8.8, 7.5)	34.67 t
3b	1.99 br dd (15.8, 8.8)	
4a	1.80 ddd (12.6, 7.5, 2.1)	33.76 t
4b	1.42 ddd (12.6, 8.8, 8.8)	
5		56.16 s
6	3.82 dd (10.5, 3.5)	50.33 d
7	1.11 m	43.37 d
8ax	1.31 m	19.41 t
8eq	1.56 m	
9ax	1.74 tt (13.5, 5.4)	29.42 t
9eq	1.44 m	
10	1.47 m	36.34 d
11	1.30 m	28.99 d
12	0.86 d (6.5)	21.05 q
13	0.68 d (6.5)	20.36 q
14	1.68 br s	16.78 q
15	0.95 d (7.5)	15.87 q
16	8.08 br s	161.16 d
NH	7.62 br d (10.5)	

[a]The ^1H and ^{13}C NMR data were measured at 500 and 125 MHz, respectively.

group at C-6, which was supported by the observation of the ^1H–^{15}N long-range coupling between H-16 and the NH nitrogen in the ^1H–^{15}N HMBC spectrum. Analysis of the HMBC spectral data revealed **1** to be a spiro[4.5]decene having the formylamino, isopropyl, and two methyl groups, as shown in Figure 1.

The relative stereochemistry of **1** was elucidated on the basis of difference NOE experiments. An enhancement of signals of three protons on C-15 and one of the protons on C-8 (H-8ax) upon irradiation of the NH proton indicated that they were all in *cis* 1,3-diaxial positions relative to one another around the six-membered ring, and defined a chair conformation for the cyclohexane ring, as shown in Figure 2. Irradiation of the olefinic proton (H-1) enhanced the signals of H-6, H-7, H-9ax, H-10 and H_3-14, placing these protons on the same face of the cyclohexane ring. Irradiation of H_3-15 enhanced signals of H-8ax, NH proton and one of the protons on C-4 (H-4a), supporting the relative stereochemistry of **1**. The relative stereochemistry of **1** was further confirmed by X-ray crystallographic analysis (Fig. 3).[1] At

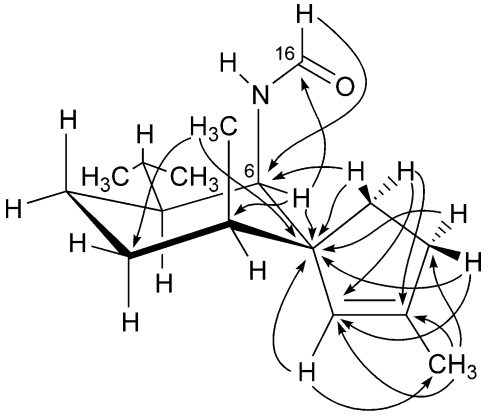

Figure 1. Key ^1H–^{13}C HMBC correlations observed for **1**.

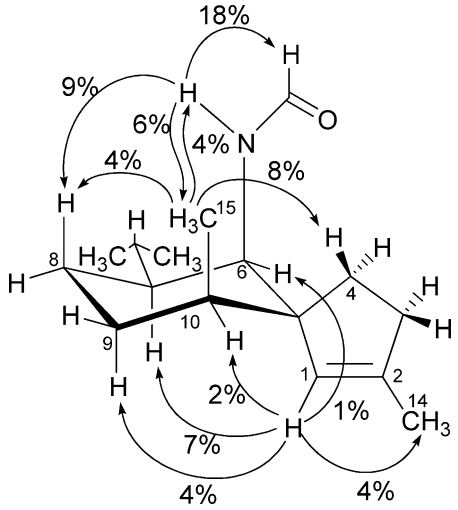

Figure 2. Key NOEs observed for **1**.

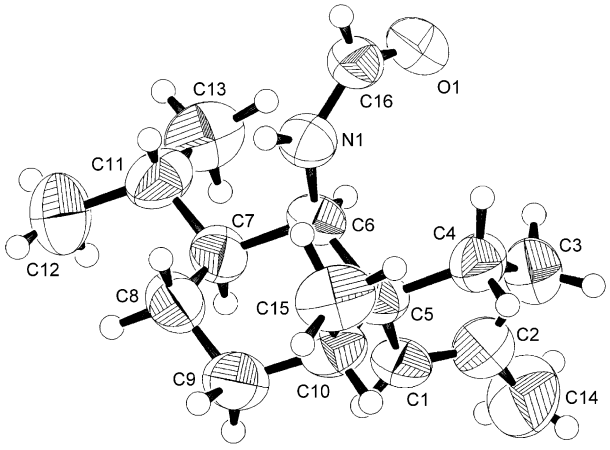

Figure 3. Perspective view of the crystal structure of **1**.

present, the absolute configuration of **1** remains to be determined.

When fertilized eggs of the sea urchin, *Hemicentrotus pulcherrimus*, were cultured in the presence of 0.4–12.0 μM **1**, they divided equally to form 16-cell embryos that were comprised of sixteen cells of the same size. After passing through the blastula and then gastrula stages, the **1**-treated embryos developed to form spicule-deficient plutei. There are very few substances having the biological activity.[4] An analogous sesquiterpene having the same spiro[4.5]decene skeleton with an isonitrile, a methoxycarbonylamino, or a 2-(methoxycarbonylmethyl-methyl-amino)-acetylamino group in place of the formylamino group of **1** did not exhibit such activity. Exiguamide (**1**) is considered to be a useful tool for elucidating the mechanism of cell fate specification during sea urchin embryogenesis.

Acknowledgements

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.

References and Notes

1. Crystal data for **1**: $C_{16}H_{27}NO$, $M = 249.40$, monoclinic, space group $C2$ (no. 5), $Z = 8$, $a = 24.4390$ (8), $b = 8.8390$ (4), $c = 16.8350$ (8) Å, $\beta = 115.786$ (2)°, $V = 3274.5$ (2) Å^3, $F(000) = 1104$, $\mu(\text{Mo-K}_\alpha) = 0.62\,\text{cm}^{-1}$, $D_c = 1.012\,\text{g cm}^{-3}$, $T = 295$ K. The reflection data were collected on a Mac Science DIP2030 imaging plate area detector with graphite monochromated Mo-K_α radiation ($\lambda = 0.71069$ Å). A total of 3076 independent reflections were collected of which 1568 were considered to be observed [$I > 2.90\sigma(I)$]. The structure was solved by direct methods[2] and expanded using Fourier techniques.[3] The non-hydrogen atoms were refined anisotropically by full-matrix least-squares refinement. Hydrogen atoms were refined isotropically. The structure was finally refined to $R = 0.056$ ($R_w = 0.076$). Crystallographic data for the structure in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 185123. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033; e-mail: deposit@ccdc.cam.ac.uk).

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