ORGANIC LETTERS

2008
Vol. 10, No. 3
489−491

Aplaminal: A Novel Cytotoxic Aminal Isolated from the Sea Hare *Aplysia kurodai*

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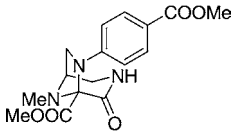
Received December 4, 2007

ABSTRACT

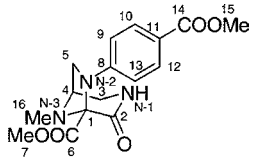


aplaminal (1)

Aplaminal (1), a novel triazabicyclo[3.2.1]octane framework metabolite, has been isolated from the sea hare *Aplysia kurodai*. The structure was determined by analysis of NMR data and confirmed by single-crystal X-ray diffraction analysis. Aplaminal (1) exhibits cytotoxicity against Hela S_3 cells.

The oceans are known as a good source of new biologically active materials. Over the past few decades, a considerable number of marine natural compounds have been reported. Many compounds used at the clinical trial level, such as halichondrin B,[1] bryostatin 1,[2] and dolastatin 10,[3] have been found in marine toxins. Therefore, marine natural products have been receiving increasing attention. Sea hares have been suggested to store some kinds of toxins to defend themselves from predators. The sea hare *Aplysia kurodai* contains various unique bioactive compounds,[4] such as, aplysin,[5] aplaminone,[6] aplyronine A,[7] and aplysiallene.[8]

During further exploration about biologically active compounds in sea hare extract, we found aplaminal (**1**), a novel triazabicyclo[3.2.1]octane framework metabolite. Herein we report the isolation and biological activity of **1**.



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Sea hare (18 kg) was extracted with methanol. The concentrated extract was partitioned between ethyl acetate and water, and the ethyl acetate layer was concentrated and partitioned between *n*-hexane and 90% aqueous methanol. The concentrated 90% aqueous methanol layer was partitioned between dichloromethane and 60% methanol. The 60% methanol layer was chromatographed on silica gel and aluminum oxide. Final purification was achieved by reversed-phase HPLC to give aplaminal (**1**) [2.0 mg; $[\alpha]^{20}_{D}$ −133 (*c* 0.02, methanol)] as colorless crystals (mp 235−237 °C). Aplaminal (**1**) exhibited cytotoxicity against HeLa S_3 (IC_{50} = 0.51 μg/mL).

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10.1021/ol7029307 CCC: $40.75
Published on Web 01/10/2008

The molecular formula of **1** was determined to be $C_{16}H_{19}N_3O_5$ by HRMS [$(M + H)^+$, *m*/*z* 334.1418, Δ 1.5 mmu]. A detailed analysis of the ^{1}H NMR, ^{13}C NMR, COSY, HMQC, and HMBC spectra in methanol-d_4 showed that **1** contained three methyl groups, two methylenes, one methine, a 1,4-disubstituted benzene moiety, three carbonyl groups, and one quaternary carbon. In addition, ^{1}H NMR spectra in acetone-d_6 showed that **1** possessed an amide proton (Table 1).

Table 1. NMR Data for Aplaminal (**1**) in CD_3OD

no.	^{13}C/ppm[a]	^{1}H/ppm[b] mult (*J* /Hz)	HMBC[c]
1	86.5 s		H-4, H-5b, H3-16
2	166.7 s[d]		H-3a, H-3b
3a	47.4 t	3.64 (1H, ddd, 1.4, 4.3, 11.9)	H-5a, H-5b
3b		3.16 (1H, d, 11.9)	
4	58.9 d	3.74 (1H, m)	H-3a, H-3b, H-5a, H-5b, H_3-16
5a	53.8 t	4.23 (1H, ddd, 1.4, 5.7, 9.4)	H-3a, H-3b
5b		3.34 (1H, d, 9.4)	
6	166.6 s[d]		H_3-7
7	53.3 q	3.84 (3H, s)	
8	150.5 s		H-5b, H-10, H-12
9,13	116.1 d	6.81 (2H, d, 8.6)	H-10, H-12
10,12	131.0 d	7.79 (2H, d, 8.6)	
11	120.1 s		H-9, H-13
14	168.7 s		H_3-15, H-10, H-12
15	52.1 q	3.83 (3H, s)	
16	38.0 q	2.49 (3H, s)	H-4

[a] Recorded at 151 MHz. Multiplicity was based on the HMQC spectrum. [b] Recorded at 270 MHz. [c] Based on the correlation with each carbon atom. $^{2,3}J_{CH}$ = 8 Hz. [d] Signals may be interchanged.

A detailed analysis of the ^{1}H−^{1}H COSY spectra of **1** allowed partial carbon−carbon connectivity, C3−C5 (Figure 1). Long-range coupling between H-3a/H-5a was observed,

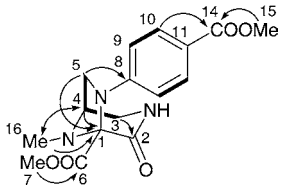

Figure 1. Gross structure of **1** determined by 2D-NMR spectroscopy (bold lines, ^{1}H−^{1}H COSY; arrows, HMBC correlations).

which suggested that the conformation of the C3−C5 chain was fixed tightly. Each carbon C-3 to C-5 could be connected to nitrogen atoms based on the ^{13}C NMR chemical shifts, establishing a partial structure of the 1,2,3-triaminopropane framework.

The HMBC correlations of H-5b/C-1 and H-4/C-1 suggested that **1** possessed a 5-membered ring system that included C-1, C-4, C-5, and two nitrogen atoms. The C-16 methyl group was established by the HMBC correlations of H-16/C-1, H-16/C-4, and H-4/C-16. The HMBC correlation of H-5/C-8 indicated that the 1,4-disubstituted benzene moiety was connected to C-5 via a nitrogen atom. Moreover, the methyl ester moiety, C-14 and C-15, was established by the HMBC correlations of H-10/C-14 and H-15/C-14. The HMBC correlations of H-3a/C-2 and H-3b/C-2, the ^{13}C NMR chemical shift of the C-3, and ^{1}H NMR in acetone-d_6 suggested that the C-2 carbonyl group was connected to the C-3 nitrogen to form a secondary amide group (see the Supporting Information). The HMBC correlation of H-7/C-6 confirmed that **1** possessed another methyl ester moiety. All elements of the molecular formula were accounted for, and one ring remained to be assigned. Therefore, quaternary carbon (C-1) should be linked to C-2. Although no HMBC correlations to the remaining methyl ester moiety (C-6 and C-7) were observed, the methyl ester moiety (C-6 and C-7) should be linked to C-1 considering the molecular formula. Thus, **1** was confirmed to be a 3,7,8-triazabicyclo[3.2.1]-octane ring compound as shown in Figure 1.

Crystals of **1** were obtained from NMR sample solution (CD_3OD): colorless plates, mp 235−237 °C. The stereostructure of **1** was confirmed by X-ray crystallographic analysis (Figure 2).[9] It corresponded completely with those

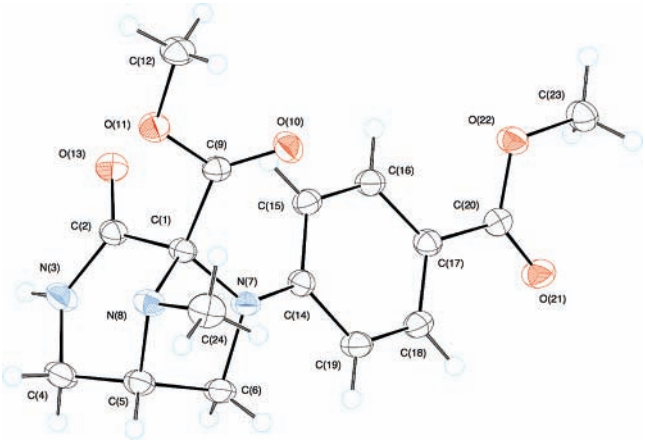

Figure 2. ORTEP drawing of **1**.

determined by the spectroscopic analysis described above. The absolute value of Flack's parameter is too small (−0.3) to determine the absolute stereochemistry of **1**.

Compound **1** has a 3,7,8-triazabicycle[3.2.1]octane skeleton, which has not been reported. It is interesting that the each bridge of this skeleton possesses a nitrogen atom.

Although no natural compounds with a 3,7,8-triazabicyclo-[3.2.1]octane skeleton have been reported, the 1,2,3-triami-

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(9) Crystal data for **1**: monoclinic $P2_1$, a = 7.7730(5) Å, b = 7.7870(5) Å, c = 12.9990(9) Å, α = 90°, β = 101.746(3)°, γ = 90°, V = 770.33(8) Å^3, Z = 2, D_{calcd} = 1.437 g/cm^3; T = −23 °C, R (R_w) = 0.0425 (0.1071) based on 1962 reflections {$I > 2\sigma(I)$} and 218 variable parameters.

nopropane framework of **1** can be found in tetrahydrofolic acid. The plausible biogenetic pathway for **1** is shown in Figure 3. Hydrolysis of the guanidine moiety of tetrahydrofolic acid and oxidative cyclization between C-1 and N-2

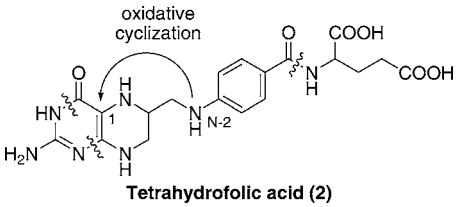


Figure 3. Plausible biogenetic pathway for the carbon framework of **1**.

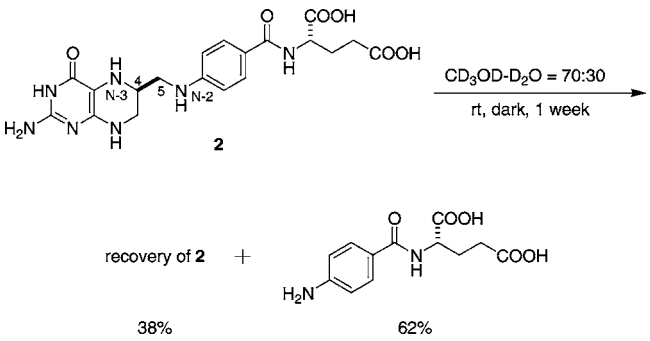
afford the framework of **1**.

This now raises the possibility that **1** could be an artifact of tetrahydrofolic acid in the extraction and isolation procedure. Tetrahydrofolic acid was exposed to CD_3OD-D_2O (7:3) at room temperature for 1 week, which corresponds to the extraction conditions of **1**, and the solution was analyzed by ^1H and ^{13}C NMR (Scheme 1). Tetrahydrofolic acid was recovered in 38% yield, and glutamyl *p*-aminobenzoate was obtained in 62% yield. Tetrahydrofolic acid is readily oxidized to C-4−N-3 imine by air, and then the C-5−N-2 bond was cleaved readily.[10] Because **1** was isolated as an optically active form, the pteridine moiety was hydrolyzed without the oxidation of the C-4−N-3 bond in the biosynthesis of **1**, in contrast with our experiment in which the nonenzymatic reaction resulted in the cleavage of the C-5−N-2 bond first. This result demonstrated that compound **1** is not likely produced under the protic and aerobic conditions.

About the stereochemistry of **1**, considering the biosynthesis of **1** from tetrahydrofolic acid, the configuration of

(10) The pteridine moiety that had connected with the glutamyl aminobenzoate was decomposed and could not be identified.

Scheme 1. Decomposition of Tetrahydrofolic Acid (Yields Determined by ^1H NMR)



C-4 of **1** could be the same as that of C-4 of tetrahydrofolic acid. Therefore, we deduced the absolute stereostructure of **1** as shown in Figure 1.

Folic acid plays important roles in the biosynthesis of several compounds. Therefore, folic acid derivatives could inhibit biosynthesis. Recently, several folic acid derivatives—methotrexate, azathioprine, and allopurinol—were reported to exhibit remarkable biological activities—antitumor, immunodepressive, and antigout, respectively. There is a possibility that, like those derivatives, aplaminal (**1**) exhibits new biological activity. Further biological studies on aplaminal (**1**) are in progress.

Acknowledgment. We would like to thank Professors Akira Sekiguchi and Masaaki Ichinohe (Univ. of Tsukuba) for the measurement of X-ray crystallographic analysis and helpful discussion.

Supporting Information Available: ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, HMQC, and HMBC spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL7029307