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Lissoclibadins 1–3, three new polysulfur alkaloids, from the ascidian *Lissoclinum* cf. *badium*

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Dedicated to the memory of Dr. Kenneth L. Rinehart and Dr. Katsumi Kakinuma

Abstract—Three new polysulfur alkaloids, lissoclibadins 1 (**1**)–3 (**3**), were isolated from the ascidian *Lissoclinum* sp. (cf. *L. badium* Monniot, F. and Monniot, C., 1996). The structures of **1**–**3** were assigned on the basis of their spectral data, and the computational modeling study was utilized for **1**. Compound **1** had a trimeric structure of three identical aromatic anime moieties connected through two sulfide and a disulfide bonds. Compounds **2** and **3** were dimeric structures of the same unit as that of **1** connected through a sulfide and disulfide bonds (**2**) and two sulfide bonds (**3**). Compounds **1** and **2** inhibited the growth of the marine bacterium *Ruegeria atlantica* (15.2 mm at 20 μg/disk and 12.2 mm at 5 μg/disk, respectively) and **2** showed antifungal activity to *Mucor hiemalis* (13.8 mm at 50 μg/disk). Compounds **1**–**3** were cytotoxic against HL-60 (IC_{50}=0.37, 0.21, and 5.5 μM, respectively).

1. Introduction

Many interesting bioactive compounds have been obtained from ascidians (tunicates). Aromatic alkaloids possessing polysulfide structures have been isolated from ascidians of the genera *Lissoclinum*,[1–6] *Eudistoma*,[4] and *Polycitor*.[7] More than 10 monomeric cyclic polysulfides[1–4,6,7] and four dimeric polysulfides[2,5,8] have been reported. These compounds presented various biological activities, for example, antifungal activity,[1–3,7] antibacterial activity,[3,7] cytotoxicity,[1,3,8] antimalarial (*Plasmodium falciparum*) activity,[3] inhibition of protein kinase C,[4,5] and inhibition of IL-8 Rα and Rβ receptors.[5]

In the course of our study on the bioactive metabolites from marine organisms, we found that the ethanol extract of the ascidian *Lissoclinum* sp. (cf. *L. badium* Monniot, F. and Monniot, C., 1996)[9] collected at Manado, Indonesia, showed strong antimicrobial activity against the fungus *Mucor hiemalis* and the marine bacterium *Ruegeria atlantica*. Bioassay-guided separation gave three new polysulfide aromatic alkaloids, named lissoclibadins 1 (**1**),[10] 2 (**2**), and 3 (**3**), together with two known dimeric alkaloids, lissoclinotoxins E (**4**) and F (**5**), and two known monomeric compounds, 3,4-dimethoxy-6-(2′-*N*,*N*-dimethylaminoethyl)-5-(methylthio)benzotrithiane (**6**) and *N*,*N*-dimethyl-5-(methylthio)varacin (**7**). We have reported the structure of **1** in the previous communication[10] and describe here the isolation, structure elucidation, and biological activity of three new lissoclibadins 1–3 (**1**–**3**) and compounds **4**–**7**.

2. Results and discussion

2.1. Ascidian and isolation of alkaloids

Lissoclinum cf. *badium* was collected at Manado, Indonesia

Keywords: Tunicate; *Lissoclinum* sp.; Lissoclinotoxin; Polysulfur compound; Structure assignment.
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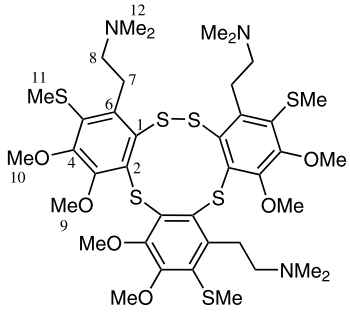
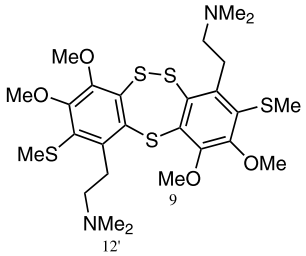
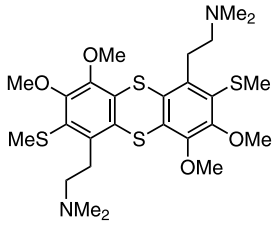


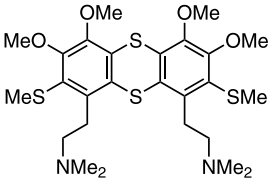
Lissoclibadin 1 (**1**)



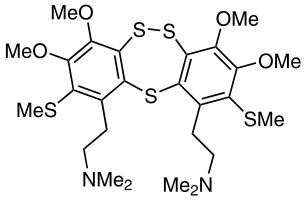
Lissoclibadin 2 (**2**)



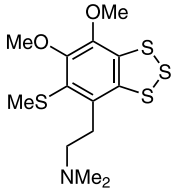
Lissoclibadin 3 (**3**)



Lissoclinotoxin E (**4**)



Lissoclinotoxin F (**5**)



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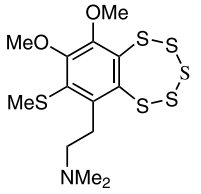

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in 2003 and 2004 and extracted with ethanol. The ethanol extract was redissolved in MeOH–H_2O (9/1) and extracted with *n*-hexane. The aqueous MeOH layer was diluted with water and extracted with *n*-BuOH. The BuOH extract showed moderate antimicrobial activity and gave **1**, **2**, **3**, and **5** by ODS and SiO_2 column chromatographies followed by HPLC. The hexane extract revealed strong antifungal and antibacterial activities and was separated by SiO_2 column chromatography followed by HPLC to yield **6** and **7**. Compound **4** was isolated from the ascidian collected in 2004 together with **1**–**3** and **5**–**7**.

Structures of three known compounds **5**–**7** were assigned on the basis of their spectral data and comparison with those for the reported values.[4,8]

2.2. Structure of lissoclibadin 1

Lissoclibadin 1 (**1**) was isolated as a Tris–TFA salt. The molecular weight (887) and formula ($C_{39}H_{57}N_3O_6S_7$) were deduced from HRFABMS and NMR data (Table 1). Three sets of ^1H and ^{13}C NMR signals were observed in the NMR spectra of **1** and assigned to three identical aromatic amine moieties by the analysis of ^1H–^1H COSY, HMQC, HMBC, ROESY, and NOESY spectra.

The geminal couplings and connectivity of two methylene groups at the 7 and 8 positions were revealed by ^1H–^1H COSY spectrum. HMBC correlations were detected from H_2-7 to three aromatic carbon signals (C-1, 5, and 6) and C-8. The ^{13}C signal assigned as C-5 showed an HMBC correlation from a methyl singlet of SMe (H_3-11). This SMe revealed an NOE with one of two methoxy methyl singlets due to H_3-10 in the NOESY spectrum. The ^1H signal of H_3-10 showed an HMBC correlation to an aromatic carbon signal (C-4) and an NOE with the H_3-9 (OMe) singlet, which had an HMBC correlation to an aromatic carbon signal (C-3). HMBC correlations were observed from H_2-8 to NMe_2 (C-12) and vice versa. Therefore, three sets of ^1H and ^{13}C NMR signals were assigned except each one aromatic carbon signal, which had no cross peak in any 2D NMR spectra and was deduced as C-2.

^1H and ^{13}C NMR data for three identical aromatic units

Table 1. ^{13}C (150 MHz) and ^1H NMR (600 MHz) data for the three units (TFA salts) in **1** (CD_3OD)

C#	Unit 1		Unit 2		Unit 3		HMBC
	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	
1	142.2[a]		140.3[b]		140.3[c]		
2	142.3[d]		141.2[d]		137.9[d]		
3	156.9		158.8		159.0		
4	153.6		153.5		158.2		
5	135.1		134.4		133.6		
6	136.9[a]		134.9[b]		135.0[c]		
7	31.3	3.80, m	30.7	3.71, m	30.6	3.68, m	1, 5, 6, 8
		4.01, m		3.92, m		3.85, m	1, 5, 6, 8
8	58.3	3.00, m	58.3	3.25, m	58.2	3.23, m	7, 12
		3.27, m		3.36, m		3.32, m	7, 12
9	61.1	3.99, s	60.5	3.70, s	60.8	3.67, s	3
10	60.8	3.34, s	60.4	3.15, s	62.3	3.88, s	4
11	19.3	2.53, s	19.1	2.46, s	19.1	2.44, s	5
12	43.4	3.06, br s	43.4	3.06, br s	43.4	2.96, br s	8, 12

[a,b,c,d] Signals are interchangeable within the same letters.