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Lissoclibadin 1, a novel trimeric sulfur-bridged dopamine derivative, from the tropical ascidian *Lissoclinum* cf. *badium*

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Abstract—A novel tribenzotetrathiepin alkaloid, named lissoclibadin 1 (**1**), has been isolated from the ascidian *Lissoclinum* sp. (cf. *L. badium* Monniot and Monniot, 1996). The gross structure was assigned on the basis of the spectral data, and one of two possible isomers was selected by the computational modeling study. Lissoclibadin 1 inhibited the growth of the marine bacterium *Ruegeria atlantica* (15.2 mm at 20 μg/disk).
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Ascidians (tunicates) are a prolific source of diverse bioactive metabolites and also interesting organisms from the viewpoint of chemical ecology. Some ascidians live with photosynthetic prochlorons at the shallow water environments and some with bacteria of thioautotrophs. Aromatic alkaloids possessing polysulfide structures have been isolated from the later type of ascidians in the genera of *Lissoclinum*,[1–6] *Eudistoma*,[4] and *Polycitor*.[7] More than ten monomeric cyclic polysulfides[1–4,6,7] and four dimeric polysulfides[2,5,8] have been reported. 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 and Monniot, 1996)[9] collected at Manado, Indonesia, showed strong antimicrobial activity against the fungus *Mucor hiemalis* and the marine bacterium *Ruegeria atlantica*.

Bioassay-guided isolation yielded a novel trimeric alkaloid, named lissoclibadin 1 (**1**), together with two known monomeric polysulfides, *N*,*N*-dimethyl-5-(methylthio)-varacin (**2**) and 3,4-dimethoxy-6-(2′-*N*,*N*-dimethylaminoethyl)-5-(methylthio)benzotrithiane (**3**). Compounds **2** and **3** showed antimicrobial activity against *M. hiemalis* (17.4 and 19.6 mm at 20 μg/disk, respectively), and *R. atlantica* (14.2 and 15.8 mm at 5 μg/disk, respectively), and **1** against *R. atlantica* (15.2 mm at 20 μg/disk). We describe here the isolation and structure elucidation of lissoclibadin 1 (**1**).

The EtOH extract of the ascidian (200 g, wet) was dissolved in MeOH–H_2O (9:1) and extracted with hexane. Water was added to the aqueous MeOH layer to give 60% MeOH, and the solution was extracted with 1-butanol. The BuOH extract was subjected to ODS (MeOH–H_2O) and silica gel ($CHCl_3$–MeOH–NH_4OH, gradient elution) chromatographies followed by HPLC purification (ODS, 70% MeOH–H_2O containing 0.1% TFA) to yield lissoclibadin 1 (**1**, 5.4 mg). Two known compounds **2** (10 mg) and **3** (10 mg) were isolated from the hexane extract and assigned the structures on the basis of their spectral data and comparison with reported values.[4]

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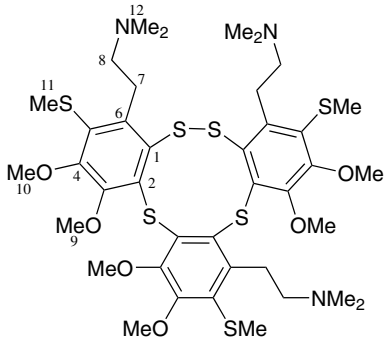
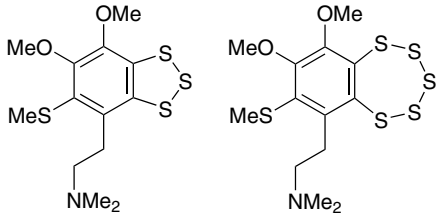


Lissoclibadin 1 (**1**)



2 **3**

Lissoclibadin 1 (**1**) was isolated as a Tris–TFA salt: $[\alpha]_D$ −3.6 (*c* 0.1, $CHCl_3$); UV λ_{max} 279 (ε 134,300), 318 nm (shoulder, ε 26,500); IR ν_{max} (KBr) 3440, 2928, 2851, 2816, 2775, 1635, 1446, 1381, 1268, 1059, 1024, 962 cm^{-1}. The molecular weight (887) and formula ($C_{39}H_{57}N_3O_6S_7$) were deduced from HRFABMS [*m*/*z* 888.2347 $[M + H]^+$ ($C_{39}H_{58}N_3O_6S_7$), requires 888.2371] 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. This SMe revealed an NOE with one of two methoxy methyl singlets due to 10-OMe in the NOESY spectrum. The ^1H signal of 10-OMe showed an HMBC correlation to an aromatic carbon signal (C-4) and an NOE with the 9-OMe singlet, which had an HMBC correlation to an aromatic carbon signal (C-3). HMBC correlations were observed from H_2-8 to a 6H broad singlet (NMe_2) 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) assigned as above were similar to those for **2** and **3**. Subtraction of the sum of three aromatic units from the molecular formula of **1** remained four sulfur atoms. Therefore, each aromatic unit was connected through one disulfide and two sulfide bonds. Thus, the

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		
	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	HMBC
1	142.2[a]		140.3[b]		140.3[c]		
2	137.9		141.2		142.3		
3	158.2		153.5		153.6		
4	156.9		158.8		159.0		
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	62.3	3.88, s	60.4	3.15, s	60.8	3.34, s	3
10	61.1	3.99, s	60.5	3.70, s	60.8	3.67, s	4
11	19.3	2.53, s	19.1	2.46, s	19.1	2.44, s	5
12	43.4	2.96, br s	43.4	3.06, br s	43.4	3.06, br s	8, 12

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

Table 2. Antimicrobial activity of compounds **1**–**3**

Compd	*M. hiemalis*[a]		*R. atlantica*			*S. cerevisiae*		*S. aureus*	*E. coli*	
	50[b]	20	50	20	5	50	20	50	50	20
1	—[c]	—	23.4[d]	15.2	—	—	—	—	—	—
2	23.0	17.4	32.4	23.3	14.2	11.8	—	10.3	17.8	14.4
3	26.2	19.6	30.0	24.5	15.8	15.2	10.5	14.2	17.1	13.1

[a] Test microorganisms: *M. hiemalis* IAM 6088, *R. atlantica* TUF-D, *S. cerevisiae* IAM 1438T, *S. aureus* IAM 12544T, *E. coli* IAM 12119T.
[b] Amount (μg/disk).
[c] Not active.
[d] Inhibition zone (mm).

gross structure of **1** was assigned to have a tetracyclic ring composed of trimeric aromatic amines and a 10-membered polysulfur ring.

Four geometric isomers were possible for lissoclibadin 1 with the orientation of three aromatic units, *cyclo*(-1-S-S-1-2-S-1-2-S-2-) (clockwise, structure **1** shown in scheme), *cyclo*(-1-S-S-2-1-S-1-2-S-2-) (**1a**), *cyclo*(-2-S-S-1-2-S-1-2-S-1-) (**1b**), and *cyclo*(-2-S-S-2-1-S-1-2-S-1-) (**1c**). An NOE was detected between two 9-OMe signals (δ_H 3.88 and 3.34) in the different aromatic units. Therefore, **1b** and **1c** was eliminated from the isomer of lissoclibadin 1.

Although the geometry of two dimeric compounds, lissoclinotoxin D[2] and lissoclin disulfoxide,[5] was not discussed in the original papers, these compounds were subjected to the computational energy minima calculations together with two new dimeric compounds, lissoclinotoxins E and F by Ireland and co-workers.[8] We have, therefore, employed the same calculation method to two possible isomers of lissoclibadin 1.

Monte Carlo conformational analysis in vacuo was performed on noncharged isomers (**1**, **1a**, **1b**, and **1c**) with MM2 force field utilizing MacroModel® software.[10] The global energy minima calculations revealed that the isomer shown as **1** had the lowest global energy minimum value, and the isomer **1a** showed 0.8 kcal/mol higher value than **1** (isomers **1b** and **1c** had, respectively, 3.7 and 4.6 kcal/mol higher values than **1**). Consequently, we have tentatively assigned the isomer **1** as the structure of lissoclibadin 1.

However, the other isomer (**1a**) cannot be excluded since the difference of energy minimum value was small and since biosynthetic enzymes sometimes construct the natural products that are thermodynamically more unfavorable structures considered from computational modeling studies. The isomers **1** and **1a** may be distinguished by the reduction and methylation[4] of lissoclibadin 1 to a linear dithioether compound to identify the positions of newly generated two SMe groups by HMBC and NOESY spectra. Unfortunately, the reaction was not successful because of the small sample sizes obtained. Collection of the ascidian sample will be carried out to isolate enough amounts of **1** for the chemical transformation.

Lissoclibadin 1 (**1**) inhibited the growth of the marine bacterium *R. atlantica* strain TUF-D[11] (15.2 mm at 20 μg/disk) but was not active against *Escherichia coli* IAM 12119T, *Staphylococcus aureus* IAM 12544T, *Saccharomyces cerevisiae* IAM 1438T, *M. hiemalis* IAM 6088 at 50 μg/disk (Table 2).

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