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Taumycins A and B, Two Bioactive Lipodepsipeptides from the Madagascar Sponge *Fascaplysinopsis* sp.

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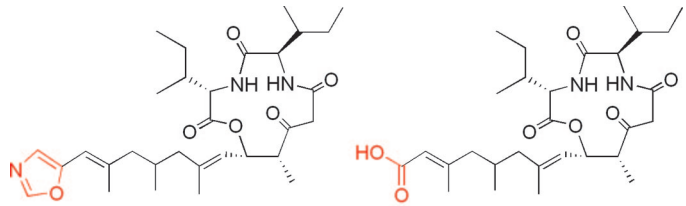
ABSTRACT



Taumycin A (1) **Taumycin B (2)**

Two closely related lipodepsipeptides, taumycins A and B (1 and 2) have been isolated from the Madagascar sponge *Fascaplysinopsis* sp. The two compounds have the same 12-membered oxodepsipeptide ring system in common. Both were toxic to brine shrimp larvae, and taumycin A (1 μM), but not taumycin B, inhibited growth of the human UT-7 leukemic cell line. The structure of the two compounds, likely to be derived from microorganisms, was established by MS and 1D and 2D NMR data.

In connection with our long-standing interest in bioactive compounds from marine invertebrates, we have found the extract of the Madagascar *Fascaplysinopsis* sp. sponge to be active in the brine shrimp test as well cytotoxic to cultured UT-7 cells.

Bioguided separation of the MeOH/$CHCl_3$ (1:1) extract derived from the marine sponge led to the identification of three groups of active compounds, namely the salarins (A−C), tulearins,[1,2] and the here-reported taumycins A and B (**1**, **2**; 7.6 mg, 0.38 wt % and 4.5 mg, 0.23 wt %, respectively) (Figure 1).

The HRCIMS of taumycin A (**1**) revealed a pseudo molecular ion $[M + H]^+$ at *m/z* 558.3559 corresponding to a molecular formula of $C_{31}H_{47}N_3O_6$ (Δ = 1.56 mmu) requiring 10 degrees of unsaturation.[3]

The ^1H, ^{13}C, COSY, HSQC, TOCSY, and HMBC spectra (Table 1 and Figure 2) revealed the presence of the following

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(3) **Taumycin A**: bright orange oil, becoming greenish with acid; $[\alpha]^{21}_D$ 39 (*c* 0.23, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2985, 1684, 1421, 1272 cm^{-1}; UV (MeOH) affords two absorptions at 214 and 247 nm. ^1H and ^{13}C NMR see Table 1; HRCIMS *m/z* 558.3559 $[M + H]^+$ (calcd for $C_{31}H_{48}N_3O_6$ 558.3543); FABMS *m/z* 558.4 $[M + H]^+$ (100).
(4) Martin, G. E.; Hadden, C. E. *J. Nat. Prod.* **2000**, *63*, 543–585.
(5) Hiemstra, H.; Houwing, H. A.; Possel, O.; Van Leusen, A. M. *Can. J. Chem.* **1979**, *57*, 3168–3170, The values for 4-subsituted oxazoles, on the other hand, are 210 and 230 Hz for CH(14) and CH(19), respectively.

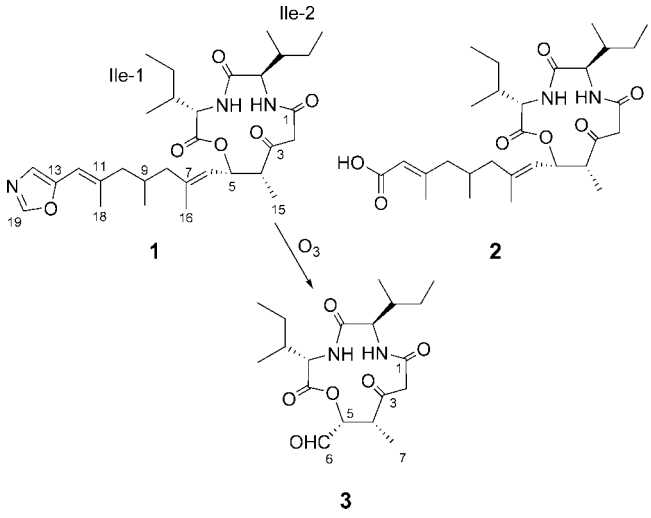

Figure 1. Taumycins A and B (**1** and **2**) and ozonolysis product **3**.

substructures: (a) two isoleucine amino acids in, according to the unequal resonances, different surroundings (Table 1); (b) an oxazole ring (δc 151.3s, 123.9d, 150.3d and a characteristic nitrogen resonance of 262 ppm);[4] and (c) a 5-oxygenated-4,7,9,11-tetramethyl-12-substituted-3-oxododecan-6,11-dienoic acyl moiety [δ_H 2.90d and 3.15d (isolated AB-system), four methyl groups (δ_H 1.00d, 1.79s, 0.81d and 1.73s; Me 15−18), δ_H 5.58dd (H-5), δ_H 5.54d (H-6) and δ_H 6.00s (H-12)].

Assembling the above substructures was deduced from CH-correlations (Figure 2); that is, the oxazole was connected to the terminus of the dodecanoic unit to C-12, and the Ile-Ile dipeptide, on its carboxylic side to C(5)H−O, to form a lactone, and on the amino edge to the carboxylic functionality C-1 of the dodecanoic acid to form an amide. The above connectivities created a 12-membered lipodepsipeptide, satisfying the ten degrees of unsaturation, thus completing the gross structure of taumycin A (**1**) except for the place of substitution of the oxazole ring. The attachment of the chain to the oxazole ring i.e. to C-13 or C-14 was evidenced from $^1J_{CH}$ values of CH(14) and CH(19), namely, 197 and 230 Hz, respectively.[5] The latter values, measured by a Gated experiment, were compared to published values for 4- and 5-substituted oxazoles;[5] hence, it could be concluded that the chain is attached to C-13 (the 5 position of the oxazole ring).

The relative chirality of the four chiral centers of the depsipeptide was determined by Marfey's method,[6] establishing one L- and one D-Ile, and analysis of NOESY data (Table 1 and Figure 3). The key NOE in both **1** and its ozonolysis carboxaldehyde product **3**, vide infra, was the transannular correlation between NH(2) and H-4. The latter correlation suggests a quite rigid conformation of the ring

(6) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(7) So far we obtained only amorphic material.
(8) Interestingly, short ozonolysis cleaved first the 11(12) bond of **1**. Ozonolysis producted **3** analyzed for molecular formula $C_{19}H_{30}N_2O_6$ by FABMS data *m/z* 405.6 $[M + Na]^+$.

Table 1. NMR Spectroscopic Data for Taumycin A (**1**)

P	δ_C (mult)[a]	δ_H, mult (J in Hz)[b]	NOESY	HMBC (H−C)
1	165.2 s			
2	53.6 t	3.15 d (10.5)	2b, 15	1, 3
		2.90 d (10.5)	2a, NH″, 15	1, 3
3	202.6 s			
4	47.9 d	2.91 m[c]	5, 15, NH″	3, 5
5	74.2 d	5.58 dd (10.0, 3.7)[d]	4, 6, 15, 16	3, 4, 6, 1′
6	121.3 d	5.54 d (10.0)[d]	5, 8a, 8b, 15, 17	7, 8
7	140.8 s			
8	47.5 t	1.90 dd (12.4, 6.1)	6, 8b, 16	6, 7, 9, 17
		1.78 m[c]	6, 8a	6, 7, 9, 17
9	29.1 d	1.78 m[c]	9, 17	8, 10, 17
10	47.7 t	2.04 dd (13.4, 5.7)	10a, 12	8, 9, 11, 12,
		1.59 m[c]	10b, 12	17
				9, 11, 12, 17
11	140.5 s			
12	112.6 d	6.00 s	10a, 10b, 9, 17	10, 11, 13, 14
13	151.3 s			
14	123.9 d	6.90 s	18	12, 19
15	13.8 q	1.00 d (7.0)	2a, 2b, 4, 5, 6, 16	3, 4, 5
16	16.4 q	1.79 s	8a, 5,, 15	6, 7, 8
17	15.3 q	0.81 d (7.6)	6, 8a, 9, 12, 18	8, 9, 10
18	18.2 q	1.73 s	14, 17	10, 11, 12
19	150.3 d	7.24 s		14
Ile-1				
1′	169.2 s			
2′	57.7 d	4.56 dd (9.7, 4.8)	3′, 5′, 6′,NH′	1′, 3′
3′	36.7 d	1.69 m[c]	2′, 6′, NH′	2′, 6′
4′	25.3 t	1.39 m[c]	4b′, 5′	3′, 5′, 6′
		1.08 m[c]	4a′, 5′, NH′	3′, 5′, 6′
5′	10.9 q	0.69 t (7.4)	4a′, 4b′	4′
6′	19.2 q	0.82 d (5.7)	2′, 3′, NH′	2′, 3′, 4′
NH′		7.04 d (9.7)	2″, 3′, 4b′, 6′	1″
Ile-2				
1″	171.7 s			
2″	58.3 d	4.30 t (9.7)	3″, 4b″, 5″, NH′, NH″	1″, 3″
3″	32.8 d	2.12 m[c]	2″, 6″, 5″, NH″	2″, 6″
4″	26.4 t	1.58 m[c]	4b″, 5″	3″, 5″, 6″
		1.09 m[c]	4a″, 5″	3″, 5″, 6″
5″	11.3 q	0.88 t (7.6)	4a″, 4b″	4″
6″	14.5 q	0.89 d (5.7)	2″, 3″	2″, 3″, 4″
NH″		6.53 d (9.7)	2b, 4, 2″, 3″, 6″	1

[a] Data recorded in C_6D_6 on Bruker Avance 500 and 400 MHz instruments (100 MHz for ^{13}C). [b] The CH correlations were assigned by an HSQC experiment. [c] Multiplicities were not determined because of overlapping with other signals. [d] Protons 5 and 6 were well separated in $CDCl_3$; H-5 δ_H 5.47 dd (9.8, 4.3) and H-6 δ_H 5.20 d (9.8).

as well as established, assuming 2″*R** configuration, see below, the 4*S** chirality, i.e., H-4 pointing toward NH(2) and CH_3-15 outward. Next, a correlation between H-4 and H-5 suggested the 5*S** configuration, namely, the chain or aldehyde group of **3** being pseudoequatorial. The measured 3.7 Hz coupling constant between H-4 and H-5 agrees well with a ca. 30° between these two protons, whereas, an opposite configuration would give a H-4,H-5 dihedral angle of ca. 85° resulting in a ca. 8 Hz coupling constant. The chirality of the fourth stereogenic center of the ring has, according to one D- and one L-Ile, to be 2′*S**. The impos-

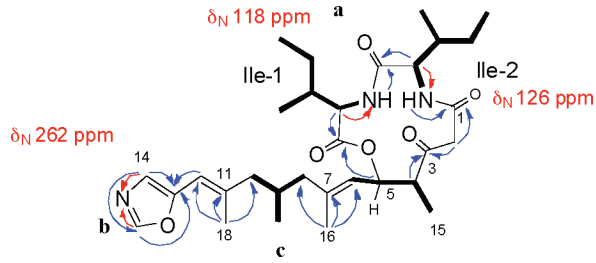

Figure 2. COSY (—), key HMBC correlations (blue arrows), and ^{15}NH-HMBC correlations (red arrows) of **1**.

sibility of distinguishing between the location of the two D- and L-isoleucine amino acids was the reason for only determining the relative configuration of **1** (2′*S**, 2″*R**, 4*S**, 5*S**). Additionally, NOE's between H-6 and -8 and between H-10 and -12 established the *E*-configuration of both side-chain double bonds. Additional NOEs (Table 1) between Me-15 and Me-16, H-5 and Me-16, H-12 and Me-17, and H-14 and Me-18 as well as a coupling constant of 10 Hz between H-5 and -6 confirmed the structure of the side chain and pointed to a preferred conformation. Attempts to crystallize taumycin A (**1**) failed, the likely cause being the flexible appendix on the depsipeptide ring;[7] therefore, we decided to remove the oxazolyl side chain. Reductive ozonolysis of **1** (Me_2S) afforded an aldehyde **3** [δ_C 47.2d (C-4), δ_H 3.20qd, J = 7.4, 3.7 Hz (H-4); δ_C 79.3d (C-5), δ_H 5.00d, J = 3.7 Hz (H-5); δ_C 195.2d (C-6), δ_H 9.52s (H-6); δ_C 12.4q (Me-7), δ_H 1.39d, J = 7.4 Hz (H-7)].[8] A NOESY experiment of compound **3** (Figure 3) confirmed, once again, the stereochemistry of the depsipeptide part of **1**.[9]

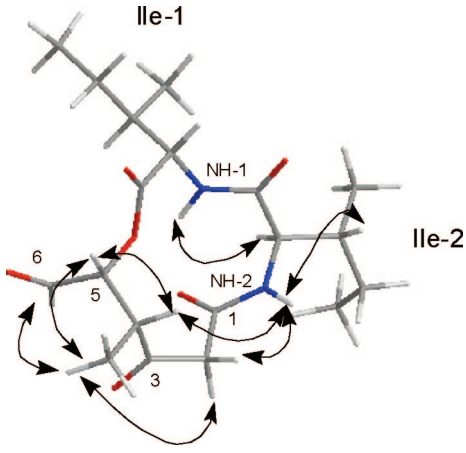

Figure 3. Key NOESY correlations (arrows) observed for compound **3** (and **1**).

A 14-aminotetradecanoic moiety is known in nature, for example, in erythromycin; however, as the latter originates from seven molecules of propionate there are, alternately, seven methyl groups in the molecule. In the taumycins, on the other hand, only three out of the four methyl groups are alternating.

The second closely related compound devoid of the oxazole ring, taumycin B (**2**), was isolated and analyzed for molecular formula $C_{29}H_{46}N_2O_7$ (Δ = 3.3 mmu) by HRESIMS data *m*/*z* 557.3197 $[M + Na]^+$ requiring eight double-bond equivalents (DBE), and this formula was fully supported by NMR data.[10] Compound **2** was found to be identical in structure to **1** except for the side chain end. NMR data evidenced a α,β-unsaturated carboxylic group at C-11 [δ_C 161.2s (C-11); δ_C 116.9d (C-12); δ_H 5.77 s; δ_C 169.8s (C-13); δ_C 18.4q (C-18), δ_H 2.15s (Me-18)].

Twelve-membered cyclic depsipeptides as in the taumycins are rare. Hapalosin, the first reported one, as well as the recent reported acremolides A−D[11] and stereocalpin A[12] are all derived from microorganisms, the first from a cyanobacteria, the second from a marine-derived fungus, and the third from an Antarctic lichen. It is, thus, very likely that the taumycins also derive from microorganism(s) living within the sponge.

Taumycin A and B are toxic to brine shrimp larvae with IC_{50} values of 10 μg/mL.

Taumycin A, at 1 μM, inhibited growth of the erythropoietin-dependent human leukemic UT-7 cell line.[13] Notably, taumycin B as well as several derivatives of taumycin A did not possess an antiproliferative effect on these cells.

Acknowledgment. We thank Dr. A. Sacher (of the Maiman Institute for Proteome Research, Tel Aviv University) for performing the electrospray mass measurements.

Supporting Information Available: NMR data (^{1}H NMR and ^{13}C NMR) for taumycins A and B and ozonolysis product including COSY, HSQC, HMBC, NOESY, and ^{15}NH-HMBC for taumycin A. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) For complete absolute configuration of **1**, taumycin A or a derivative including C-9 will have to be analyzed by X-ray.

(10) **Taumycin B**: yellow oil; $[\alpha]^{26}_D$ 68 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2986, 1678, 1421, 1271 cm^{-1}; HR-ESIMS (QqTOF) *m*/*z* 557.3230 $[M + Na]^+$ (calcd for $C_{29}H_{46}N_2O_7Na$ 557.3197).

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