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Tausalarin C: A New Bioactive Marine Sponge-Derived Nitrogenous Bismacrolide

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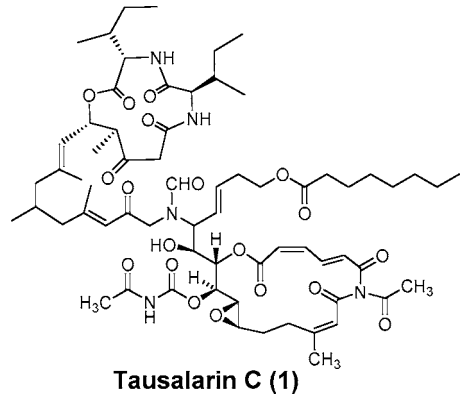
ABSTRACT



Tausalarin C (1)

A novel nitrogenous bismacrolide, designated tausalarin C (1), was isolated from the Madagascar sponge *Fascaplysinopsis* sp. The structure of the compound was elucidated by interpretation of MS and 1D and 2D NMR spectra. It is suggested that tausalarin C is assembled from salarin A (2) and pretaumycin A. The relative configuration of the chiral centers of salarin A was determined by X-ray diffraction. Tausalarin C was found to inhibit proliferation of K562 leukemia cells. A possible biogenesis is discussed.

Marine sponges have proven to be a rich source of secondary metabolites possessing novel structures and biological activities.[1] We recently reported the isolation and structure elucidation of three unprecedented groups of compounds: the salarins, tulearins, and taumycins, from the Madagascan sponge *Fascaplysinopsis* sp.[2−5] Changes in chemical composition and relative amounts of compounds, from the three groups, in over two dozen collections of the sponge and mainly structural similarities to microorganism and fungal metabolites (e.g., the cyanobacteria *Lyngbia bouillonii* metabolites madangolide and laingolide A)[6,7] suggest that these compounds may originate from guest microorganisms rather than from the host sponge.[8−10]

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(1) Munro, M. H. G.; Blunt, J. W. *Marine Literature Database*; Department of Chemistry, University of Canterbury: New Zealand, 2007.
(2) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 153–456.
(3) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Tetrahedron Lett.* **2008**, *49*, 4355–4358.
(4) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 4307–4309.
(5) Bishara, A.; Rudi, A.; Aknin, M.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **2009**, *50*, 3820–3822.

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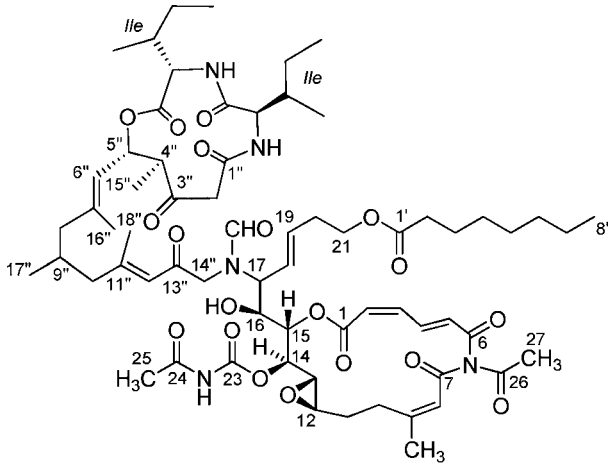
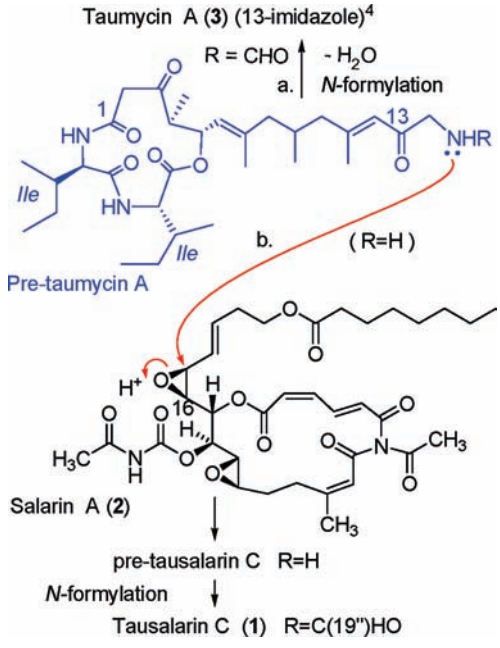


Structure of tausalarin C (**1**)[12]

All collections of the sponge were carried out in Salary Bay ca. 100 km north of Tulear, Madagascar, in a depth of 25−35 m. From three of the collected samples, we have isolated another metabolite designated tausalarin C (**1**) (13 mg, 0.012% dry wt). Spectral similarities of the NMR data of compound **1** to those of salarin A (**2**) and taumycin A (**3**)[11] (Scheme 1) caused us to initially think that we were

Scheme 1. Suggested Biogenesis of Tausalarin C (**1**) and Taumycin A (**3**)



* Position numbers according to salarin A and taumycin A.

dealing with a 1:1 mixture of two compounds; however, unsuccessful HPLC separation efforts and mainly NMR correlations from one-half of the molecule to the other, vide infra, and at last, unequivocally, the HR-ESIMS spectrum resulted in the **2** + slightly modified **3** combined structure.

The mass spectroscopic analysis and ^{13}C resonance values of compound **1** provided a pseudo molecular formula of $C_{66}H_{95}N_5O_{19}Na$ in the positive HR-ESIMS mode, *m/z* 1284.6544, Δ = 1.9 mmu, for $[M + Na]^+$, and in the negative mode suitable $[M - H]^-$ and $[M + Cl]^-$ peaks at *m/z* 1260.6586 and 1296.6329, Δ = 1.5 mmu, respectively, implying 22 degrees of unsaturation. The ^1H, ^{13}C (PND and DEPT), COSY, HSQC, TOCSY, and HMBC spectra (Table 1 and Supporting Information) revealed the presence in **1** of two major parts with almost the same resonance values as the corresponding atoms in salarin A (**2**)[2] and taumycin A (**3**) ($\Delta\delta_C$ values of less than 1 ppm).[4] Observed changes in the NMR spectra of **1** were in the C-15 to C-18 segment of the salarin A part[12] and in the C-12″ to C-14″ and C-19″ segment of the taumycin A part. It became clear that the 16,17-epoxide of **2** opened up, and C-13″ changed to a ketone (δ_C 196.7s ppm, likely unsaturated) adjoined with an α-methylene (C-14″) carrying an *N*-formyl group (C-19″, δ_C 163.5 d, δ^{15}_N 123.2 ppm). A bond between the latter nitrogen atom to C-17 of the opened epoxide moiety of salarin A connected the two parts of the molecule one to the other as established by the HMBC and NOE correlations (Figure 1), thus completing the gross structure of tausalarin C (**1**).

Table 1. NMR Spectroscopic Data for the Connection Site of Tausalarin C[a]

P.	δ_C (mult)[b]	δ_H, mult (J in Hz) [b,c]	NOESY	HMBC (H−C)
14	71.4 d	5.23 t (3.0)	13, 17	13, 15, 16, 23
15	74.6 d	5.30 t (3.0)	17	1, 13, 14, 16
16	70.8 d	3.78 dt (8.8, 3.0)	17, 18, 19	14, 15, 17
17	62.9 d	4.27 t (8.8)	15, 16, 18, 19, 19″	16, 18, 19, 14″, 19″
18	125.4 d	5.56 dd (15.5, 8.8)	16, 17	17, 20
19	134.6 d	5.86 dt (15.5, 6.7)	16, 17, 21	18, 20, 21
11″	165.2 s			
12″	120.8 d	6.09 s	10b″, 17″	10″, 11″, 13″
13″	196.7 s			
14″	50.8 t	4.40 d (17.8)	14b″	17, 19″
		3.64 d (17.8)	14a″	
18″	20.3 q	2.16 s		10″, 11″, 12″
19″	163.5 d	8.16 s	17	17, 14″

[a] Carbon numbers of the two halves of tausalarin C are according to the numbers of salarin A and taumycin A. $\Delta\delta_C$ values for the rest of the carbon atoms are less the 1 ppm. [b] Data recorded in $CDCl_3$ on Bruker Avance 500 MHz instruments and Bruker Avance 400 MHz for ^{13}C (100 MHz). [c] The CH correlations were assigned by an HSQC experiment.

(6) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *J. Nat Prod.* **1999**, *62*, 934–936.

(7) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *Tetrahedon Lett.* **1996**, *37*, 7519–7520.

(8) Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Org. Chem.* **1994**, *59*, 7219–7226.

(9) Ratnayake, R.; Fremlin, L. J.; Lacey, E.; Gill, J. H.; Capon, R. J. *J. Nat. Prod.* **2008**, *71*, 403–408.

(10) Seo, C.; Yim, J. H.; Lee, H. K.; Park, S. M.; Sohn, J. H.; Oh, H. *Tetrahedron Lett.* **2008**, *49*, 29–31.

(11) **Tausalarin C:** Pale yellow oil; $[\alpha]_D^{24}$ −6 (*c* 0.64, $CHCl_3$); IR ($CHCl_3$) n_{max} 3679, 3054, 2986, 1722, 1605, 1421 cm^{-1}. UV (MeOH) λ_{max} 244 nm (ε 28 500). FABMS *m/z* 1262.4 $[M + H]^+$ (100), 1284.4 $[M + Na]^+$ (70). Positive HR-ESIMS *m/z* 1284.6544 $[M + Na]^+$ (Calcd for $C_{66}H_{95}N_5O_{19}Na$, 1284.6519) and negative HR-ESIMS *m/z* 1296.6329 $[M + Cl]^-$ (Calcd for $C_{66}H_{95}N_5O_{19}Cl$, 1296.6310). For NMR data (^1H, ^{13}C, COSY, TOCSY, HSQC, and HMBC spectra), see the Supporting Information.

(12) Carbon numbers of the two halves of tausalarin C are according to the numbers of salarin A and taumycin A. The configuration of C-17, -9″ and the mutual relative configuration of the two halves are unknown.

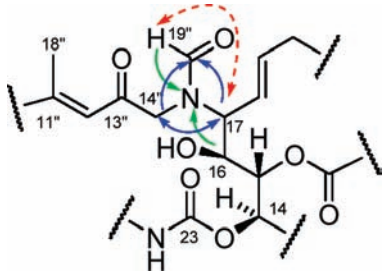


Figure 1. Key HMBC correlations (blue arrows), ^{15}NH-HMBC correlations (green arrows), and selective NOE correlation (red arrow) for the connection site of tausalarin C.

It is suggested that a common precursor of **1** and **3** (pretaumycin A, Scheme 1) undergoes either (a) N-formylation to afford after ring cyclization and water elimination the oxazole ring of taumycin A (**3**) or (b) attack by the primary amine group (before formylation) of pretaumycin A, in an alternate route, to open up the vinyl 16,17-epoxide of salarin A to yield pretausalarin C which after successive N-formylation affords compound **1**. The configuration of the substituted allylic C-17 atom ($J_{16,17}$ = 8.8 Hz), due to possible rotamers around the 16, 17 bond, remains ambiguous.

Whereas the relative configurations of six out of the seven centers of taumycin A were suggested,[4,13] no configurational assignment of the six asymmetric centers of salarin A was achieved previous to this work.[2−4] After lengthy crystallization trials, a crystalline structure of salarin A (**2**) suitable for X-ray diffraction analysis was obtained and the relative configuration of the six chiral centers achieved.

The X-ray measurements of compound **2** were carried out on a Nonius KappaCCD diffractometer at a low temperature to optimize the precision of the crystallographic determination, with MoKα radiation.[14]

The molecule geometry reveals common bond lengths and bond angle characteristics, and the noncentrosymmetric space group of the crystal is consistent with the chiral nature of this compound (Figure 2). There are six asymmetric carbons in the molecule, and their relative absolute configurations are *R* at C-12 and C-14 and *S* at C-13, C-15, C-16, and C-17. Despite this, the absolute configuration of the entire structure could not be determined from diffraction data due to the lack of strong anomalous X-ray scatterers. The macrocyclic fragment O1−C15 has a shape of a "cradle". The C14−C15 and C7−N28 are at the base of this cradle. The three acyclic residues extend from the base atoms C14, C15, and N28. The long side chain, C16 through O11 combined with C1′ through C8′, folds into a twisted U-shape to make the molecular structure as compact as possible. A similar structure can also be expected in the salarin A half of **1**.

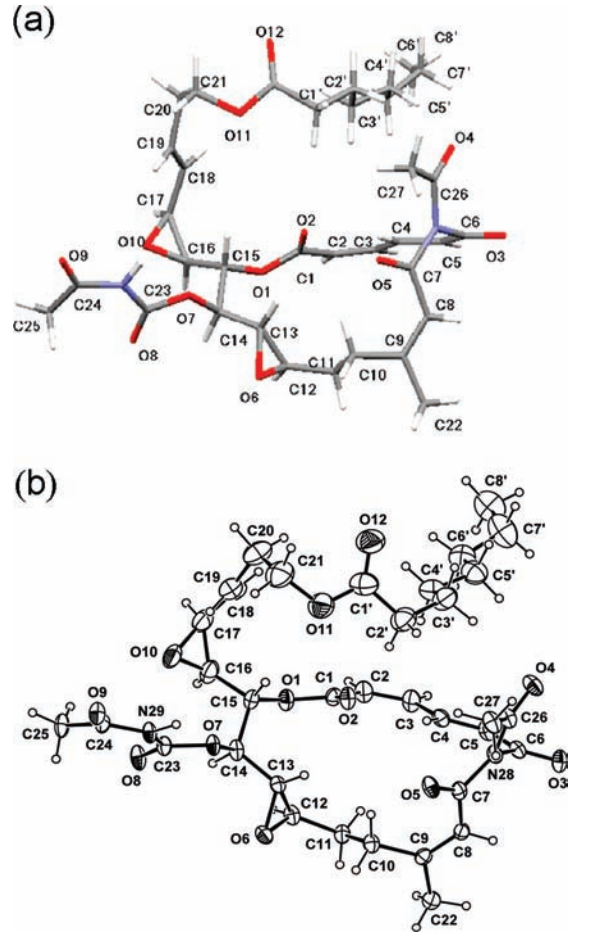


Figure 2. (a) Wire frame model and (b) ORTEP representation of compound **2** obtained by X-ray diffraction analysis.

On the basis of the suggested biogenesis of compound **1** (Scheme 1), the relative stereochemistry of the corresponding five stereogenic centers in **1** (C-12 to C-16) have to be the same as in salarin A and the chiral centers in the second half of **1** the same as in taumycin A.[13] The distance between the two halves of the molecule and no observed NOEs prevented the determination of the relative mutual stereochemistry between the two parts of the molecule.

The effect of tausalarin C on cell proliferation was determined in two different human leukemic cell lines, K562[15] and UT7,[16] using the colorimetric methylthiazole tetrazolium bromide (MTT) assay.[17] Tausalarin C at 1 μM inhibited 35%, 65%, and 74% of K562 growth after 24 h, 48 h, and 72 h, respectively, higher activity than measured for the individual compounds.[2−4] Notably, tausalarin C did not significantly inhibit proliferation of the UT7 cells.

(13) One D-leu and one L-leu, determined by the Marfey analysis, only enabled the assignment of the relative chirality of the taumycin half.

(14) Crystal data: $C_{35}H_{46}N_2O_{12}$, M = 686.74, monoclinic, space group $P2_1$, a = 13.7888(4), b = 9.6310(3), c = 13.7835(4) Å, β = 90.689(2)°, V = 1830.32(9) Å^3, Z = 2, T = 110(2) K, D_c = 1.246 g·cm^{-3}, μ(MoKα) = 0.094 mm^{-1}, 20 001 collected and 3152 unique reflections to $2\theta_{max}$ = 55.7° (R_{int} = 0.058), 447 refined parameters, R_1 = 0.060 for 6206 observations with $I > 2\sigma(I)$, R_1 = 0.086 (wR_2 = 0.134) for all unique data. CCDC-736201.

(15) Lozzio, C. B.; Lozzio, B. B. *Blood* **1975**, *45*, 321–323.

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(16) Komatsu, N.; Nakauchi, H.; Miwa, A.; Ishihara, T.; Eguchi, M.; Moroi, M.; Okada, M.; Sato, Y.; Wada, H.; Yawata, Y.; Suda, T.; Niura, Y. *Cancer Res.* **1991**, *51*, 341–348.
(17) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

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

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