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Salarins A and B and Tulearin A: New Cytotoxic Sponge-Derived Macrolides

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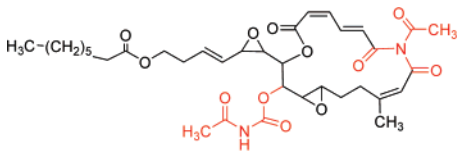
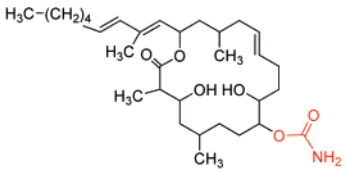
ABSTRACT



Salarin A



Tulearin A

Three novel nitrogenous macrolides designated salarin A and B (1 and 2) and tulearin A (3) were isolated from the Madagascar *Fascaplysinopsis* sp. sponge. The structures of the compounds were elucidated by interpretation of MS and 1D and 2D NMR spectra. Both salarins carry an acetylcarbamate moiety, and in addition, 1 contains a triacylamine group and 2 a methoxymethylketone lactam. Tulearin A carries the naturally rare carbamate ester. The compounds were found to be toxic to brine shrimp larvae, and salarin A and tulearin A were also cytotoxic to leukemia cells.

In connection with our long-standing interest in the chemistry of marine sponges,[1,2] we have investigated the Madagascar *Fascaplysinopsis* sp. sponge collected in Salary Bay ca. 100 km north of Tulear.[3]

Bioguided (brine shrimp test) separation of the $CHCl_3$−CH_3OH (1:1) extract afforded several new compounds, the structure of three of them, designated salarin A (**1**, 5.5 mg, 0.016 wt %), salarin B (**2**, 2.5 mg, 0.008 wt %), and tulearin A (**3**, 6.6 mg 0.019 wt %) follow.

The mass spectrometric analysis of **1**[4] provided a molecular formula of $C_{35}H_{46}N_2O_{12}$ (HR-MALDIMS (TOF) *m/z* 709.2991 for $[M + Na]^+$), with 14 degrees of unsaturation. The ^1H, ^{13}C (Table 1), COSY, HSQC, TOCSY, HSQC-TOCSY, and HMBC spectra revealed the presence of the following moieties (a) two epoxides [δ 55.7d and 53.6d (*E*); δ 57.3d and 57.0d (*Z*)]; (b) two double bonds (δ 125.9d and 134.2d as well as a conjugated one δ 123.3d, and 155.4s); (c) an octanoate ester (δ 173.5s, 34.8t, additional five methylenes and 14.7q); (d) an 6-oxohexa-2,4-dienoate (δ 164.6s, 125.7d, 141.6d, 140.8d, 135.5d, and 171.9s); (e) an *N*-acetyl carbamate (δ 152.5s, 171.5s, and 24.3q); and (f) a triacylamine (δ 171.9s, 172.4s, 25.4q, and 167.9s). While the determination of moieties a−c was straightforward each one of the other three groups required clarifications. Outstanding in moiety (d) was the low field double doublet at δ 8.31 (H-4, J = 15.7 and 11.3 Hz), the latter value together with the

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(1) Kashman, Y.; Rudi, A.; Pappo, D. *Pure Appl. Chem.* **2007**, *79*, 491−505.
(2) Bishara, A.; Rudi, A.; Goldberg, I.; Benayahu, Y.; Kashman, Y. *Tetrahedron* **2006**, *62*, 12092−12097.

(3) The identification of the spicule-less sponge genus was not straightforward. It seems to be closest to *Fascaplysinopsis* (Demospongiae, order Dictyoceratid, family Thorectidae), a genus described thus far only from Australia and Indonesia.

(4) **Salarin A:** pale yellow oil; $[\alpha]^{23}_D$ −57 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3690, 3028, 3010, 1728, 1602, 1370 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HR-MALDIMS (TOF) *m/z* 709.2991 $[M + Na]^+$ (calcd for $C_{35}H_{46}N_2O_{12}Na$ 709.2943); (negative) FABMS *m/z* 686 $[M - H]^-$ (100), 643 ($[M - H]^- - C_2H_3O$) (10); 601 ($[M - H]^- - C_3H_3O_2N$) (40), 558 ($[M - H]^- - C_5H_6O_3N$) (10).

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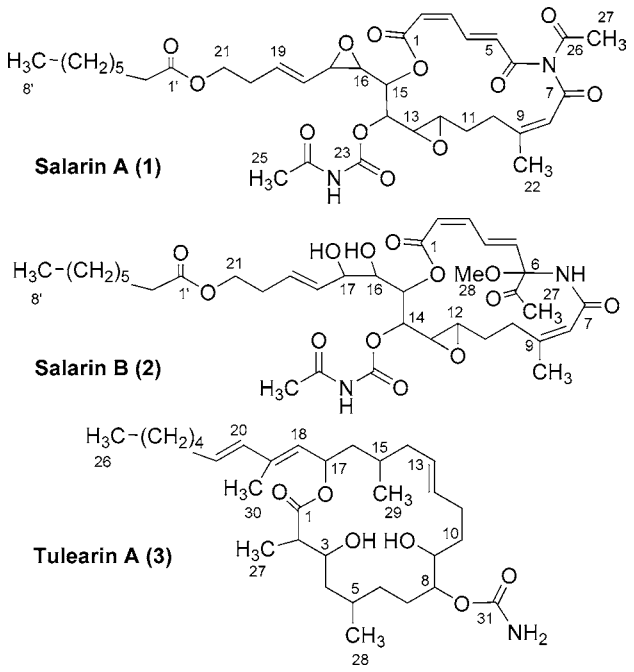

other NMR data, including COSY and HMBC experiments, established the 2*Z*,4*E* geometry of this moiety.[5] The exceptionally low field signal, of H-4, agrees only with the 2*Z*,4*E* isomer and requires a carbonyl at position C-6.[5]

An NOE between Me-22 and H-8 and $J_{18,19}$ = 15.6 Hz coupling determined the *Z* and *E* geometry of the 8(9) and 18(19) double bonds. Furthermore, a 12(13) *E* and 16(17) *Z* geometry of the two epoxides was established from the $J_{12,13}$ = 2.2 Hz and $J_{16,17}$ = 3.8 Hz values, respectively.

The naturally unique *N*-acetylcarbamate group was suggested following CH- and NH-HMBC experiments (δ_N 143 ppm) and was in agreement with the acidity of the imide proton, among the two carbonyls, which could be methylated with CH_3I in the presence of K_2CO_3 in acetone to afford the N-CH_3 derivative (δ_H 3.23s, δ_C 30.3q). Crucial were HMBC correlations from the newly introduced N-CH_3 group to the vicinal CO(23), δ 152.2s and CO(24), δ 171.1s groups.

Assembling groups a−e via three unaccounted for pairs of carbon atoms (C-10,11 and 20,21, methylenes, and 14,15-oxymethines) by COSY and HMBC data (Figure 1) afforded the structure of **1** lacking only a $NCOCH_3$ functionality (N−$C_{26,27}$), proving, as a result, the three acylamine moiety (f). Strong support for the latter functionality came from the ^{15}N resonance, measured from the ^3J(C**H**$_3$CO**N**) HMBC correlation of 202.9 ppm in excellent agreement with the correrponding resonance of 203.5 ppm measured for *N*-acetylsuccinimide, consequentially completing the gross structure of **1**.[6,7]

Salarin B (**2**) analyzed for $C_{36}H_{52}N_2O_{13}$ with 12 degrees of unsaturation from the FABMS *m/z* 703.2 [M + H − H_2O]$^+$ and the HR-ESIMS (*m/z* 741.3028 [M − H_2O + K]$^+$).[8] Loss of water in the MS of **2** became clear from the

Table 1. NMR Spectroscopic Data for Salarin **A** and **B**

	1		**2**	
p	δ_C (mult)[a]	δ_H, mult (*J*, Hz)[b]	δ_C (mult)[a]	δ_H, mult (*J*, Hz)[b]
1	164.6 s		164.7 s	
2	125.7 d	5.63 d (11.3)	117.5 d	5.71 d (11.2)
3	141.6 d	5.93 t (11.3)	146.2 d	6.50 t (11.2)
4	140.8 d	8.31 dd (15.7, 11.3)	127.8 d	7.71 dd (15.4, 11.2)
5	135.5 d	6.10 d (15.7)	142.4 d	6.42 d (15.4)
6	171.9 s		89.8 s	
NH				6.94 s
7	167.9 s		165.7 s	
8	123.3 d	5.98 s	119.8 d	5.33 s
9	155.4 s		153.8 s	
10	28.1 t	3.10 m[d]	30.6 t	2.10 m (2H)[d]
		2.05 m[d]		
11	28.3 t	1.67 m[d]	34.5 t	1.96 m[d]
		1.00 m[d]		1.41 m[d]
12	55.7 d	2.89 dd (7.1, 2.2)	55.5 d	2.79 m
13	53.6 d	3.15 dd (4.4, 2.2)	59.5 d	3.08 dd (8.0, 1.9)
14	73.1 d	5.17 t (4.4)	83.4 d	3.63 t (8.0)
15	71.1d	5.29 dd (7.3, 4.4)	70.7 d	5.40 m[d]
16	57.3 d	3.16 dd (7.3, 3.8)	77.6 d	4.98 dd (6.4, 2.9)
17	57.0 d	3.34 dd (6.5, 3.8)	83.5 d	4.51 m
18	125.9 d	5.60 dd (15.6, 6.5)	130.6 d	5.41 m[d]
19	134.2 d	5.87 dt (15.6, 6.5)	131.5 d	5.89 dt (14.9, 6.8)
20	32.6 t	2.23 m (2H)[d]	32.5 t	2.22 m (2H)[d]
21	63.4 t	4.11 t (6.7) (2H)	63.3 t	4.09 m (2H)[d]
22	23.9 q	1.46 s	24.6 q	1.52 s
23	152.5 s		152.1 s	
NH′		8.56 s		7.89 s
24	171.5 s		171.4 s	
25	24.3 q	2.27 s	24.3 q	2.26 s
26	172.4 s		202.1 s	
27	25.4 q	2.10 s	24.0 q	1.90 s
28			51.0 q	
1′	173.5 s		173.6 s	
2′	34.8 t	2.21 m (2H)[d]	34.8 t	2.28 m (2H)[d]
3′	25.7 t	1.64 m (2H)[d]	25.8 t	1.68 m (2H)[d]
4′	29.8 t	1.28 m (2H)[d]	29.9 t	1.29 m (2H)[d]
5′	29.9 t	1.25 m (2H)[d]	29.8 t	1.29 m (2H)[d]
6′	32.5 t	1.29 m (2H)[d]	32.4 t	1.28 m (2H)[d]
7′	23.4 t	1.30 m (2H)[d]	23.4 t	1.33 m (2H)[d]
8′	14.7 q	0.95 t (6.9)	14.2 q	0.97 t (6.8)

[a] Data recorded in C_6D_6 on Bruker Avance 500 and 400 MHz instruments (100 MHz for ^{13}C). [b] The CH correlation were assigned by an HSQC experiment. [c] a, b, a geminal pair, denote the upper (a) and lower (b) fields protons. [d] Multiplicities were not determined because of overlapping with other signals.

(5) Feliu, A; Seltzer, S. *J. Org. Chem.* **1985**, *50*, 447−451; $J_{2,3}$ = 11.3 Hz (*Z*), $J_{4,5}$ = 15.7 Hz (*E*).

(6) Although transannular NOEs were observed, the high molecule flexibility avoided conclusions regarding the chiral centers of the molecule. More material will have to be gained for attempting derivatization of **1**, which thus far has failed to give crystals. All three new compounds are unstable, especially under acidic or basic conditions. In the case of **1** and **2**, unstability, most likely, is due to the oxohexa-2,4-dienoate moiety that readily isomerizes.

(7) An alternative acyl iminoanhydride group [H_3CCO_2C(=NCO−)−] was excluded because of the instability of this group that readily rearranges to the triacyl amine structure. Heard, R. D. H.; Ryan, M. T.; Bolker, H. I. *J. Org. Chem.* **1959**, *24*, 172−175. Hassner, A.; Wentworth, W. A.; Pomerantz, I. H.; *J. Org. Chem.* **1963**, *28*, 304−306. Iesce, R.; Graziano, M. L.; Cimminiello, G.; Cermola, F.; Parrilli, M.; Scarpati, R. *Chem. Soc., Perkin Trans. 2* **1991**, 1085−1089.

(8) Salarin B: colorless oil; $[\alpha]^{23}_D$ −130 (*c* 0.12, $CHCl_3$); HR-ESIMS (QqTOF) *m/z* 741.3028 [M − H_2O + K]$^+$ (calcd for $C_{36}H_{50}N_2O_{12}K$ 741.2995).

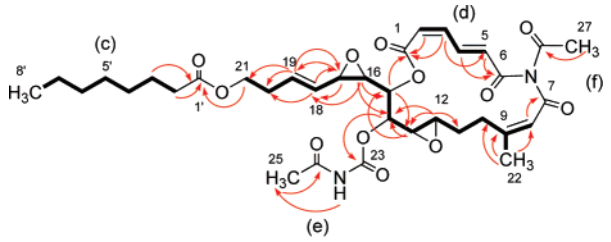

Figure 1. COSY (—) and key HMBC correlations (→) of salarin **A** (**1**).

^{13}C NMR spectrum requiring thirteen oxygen atoms in the molecule (one COCH3, three esters, one NHCO, one NCOCH3, two methinoxy groups, one epoxide, and a methoxyl, Table 1). The NMR data suggested a close relationship with salarin A (**1**), differing only in two functional groups. That is, **2** lacks the 16,17-epoxide of **1**, being replaced by a 16,17-diol, and the triacylamine moiety (f). Instead of the latter functionality, **2** possesses a lactam moiety adjacent to a carbon (C-6) carrying a methoxyl and a methyl ketone (Figure 2). The latter unique moiety

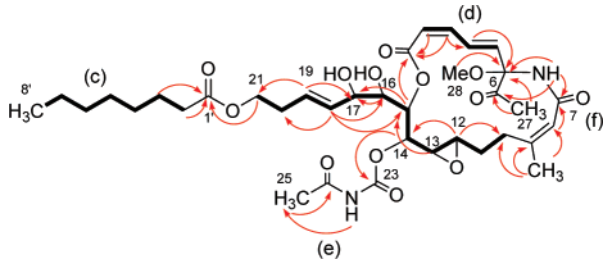

Figure 2. COSY (—) and key HMBC correlations (→) of salarin **B** (**2**).

resembles a similar rare functionality in the *Aspergillus* metabolite synerazol.[9] The structure of the C5−C9 segment (f) was suggested on the basis of 2D NMR (Figure 2) and MS data.[10]

Both salarin A and B (**1** and **2**) possess novel macrolide structures, not only in the triacylamine and the substituted lactam functionalites of **1** and **2**, respectively, but also in the construction of the macrolide from two carbon chains (a 6-amidohexa-2,4-dienoic acid and a functionalized C_{15}-acid). It is also feasible that the nitrogenous macrolide is obtained by a Beckmann rearrangement of an α-ketooxime of a single chain. A similar combination of two chains can be found in the two nitrogenous macrolides madangolide[11] and laingolide A[12] isolated from the cyanobacteria *Lyngbia bouillonii*.

The HR-ESIMS (QqTOF) of tulearin A (**3**)[13] exhibited a molecular ion $[M + Na]^+$ at *m/z* 558.3757, proving a formula of $C_{31}H_{53}NO_6Na$ with six degrees of unsaturation.

The 1D and 2D NMR data (Table 2) revealed the presence of (a) an *E,E* $\Delta^{18,20}$-diene (δ 129.5d, 136.6s, 134.6d, and 131.2d) and a nonconjugated *E* double bond (δ 131.9d and 130.5d); (b) two secondary alcohol groups (δ 70.8d, 69.9d);

Table 2. NMR Spectroscopic Data for Tulearin **A** (**3**)

p	δ_C (mult)[a]	δ_H, mult (*J*, Hz)[b]	COSY[c]	HMBC (H−C)
1	174.9 s			
2	46.4 d	2.50 qd (7.6, 2.8)	3, OH	1, 3, 4, 28
3	70.8 d	3.77 m	2, 4a, 4b, OH	2, 5, 28
OH		3.35 d (7.6)	2, 3	2, 3, 4
4	43.8 t	1.54 td (13.7, 4.4)	3, 5	2, 3, 5, 29
		1.17 br t (13.7)		2, 3
5	28.0 d	1.67 m[d]	4b,6b, 29	6, 29
6	34.9 t	1.32 m[d]	6b, 7	4, 5, 7, 29
		1.22 m[d]	5, 6a, 7	4, 5, 7, 29
7	28.8 t	1.62 m (2H)[e]	6a, 6b, 8	4, 5, 8, 9
8	75.5 d	4.60 td (5.5, 3.9)	7, 9, OH	6, 7, 31
9	69.9 d	3.69dtd (7.2, 6.7, 3.9)	8, 10, OH	10
OH		3.38 d (7.2)	8, 9	9, 10
10	32.7 t	1.51 m (2H)[d]	9, 11	8, 9, 11, 12
11	27.8 t	2.13 q (6.0) (2H)	10, 12	9, 10, 12, 13
12	131.9 d	5.40 m[d]	11	11, 13, 14
13	130.5 d	5.44 m[d]	14a, 14b	12, 14
14	41.3 t	1.99 m[d]	13, 14b	13, 15, 16, 30
		1.78 m[d]	13, 14a, 15	15, 30
15	29.7 d	1.62 m[d]	14b, 16b, 30	17, 30
16	42.8 t	1.83 td (13.1, 4.4)	16b, 17	14, 15, 17, 30
		1.24 td (13.1, 4.4)	15, 16a, 17	15
17	69.6 d	5.80 td (9.1, 4.4)	16a, 16b, 18	15, 18, 19
18	129.5 d	5.28 d (9.1)	17, 27	20, 27
19	136.6 s			
20	134.6 d	6.04 d (15.5)	21, 22	18, 19, 22, 27
21	131.2 d	5.75 dt (15.5, 6.9)	20, 22	19, 22, 23
22	33.6 t	2.08 q (7.0) (2H)	21, 23	20, 21, 23, 24
23	29.4 t	1.40 quin (7.0)	22, 23b	22, 24, 25
		1.29 m[d]	23a	25
24	31.3 t	1.28 m (2H)[d]	23a, 25	23, 25, 26
25	23.0 t	1.30 m (2H)[d]	24, 26	24, 26
26	14.1 q	0.87 t (7.0)	24	24, 25
27	12.9 q	1.85 s	18	18, 19, 20
28	14.0 q	1.15 d (6.5)	2	1, 2, 3
29	18.6q	0.90 d (7.1)	5	4, 5, 6
30	18.2 q	0.94 d (6.7)	15	14, 15, 16
31	157.7 s			
NH_2		6.26 br s[e]		

[a] Data recorded in acetone-d_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] a, b, a for a geminal pair, denote the upper (a) and lower (b) protons. [d] Multiplicities were not determined because of overlapping with other signals. [e] The shift of N**H**$_2$ is given in DMSO-d_6.

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(10) MS fragmentations of **2** confirmed conclusively the suggested structure, e.g., peaks of M − OCH_3, M − $COCH_3$, and *m/z* 257 for the C-15 side chain ($C_{14}H_{25}O_4$).
(11) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *J. Nat Prod*. **1999**, *62*, 934−936.
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(13) Tulearin A: colorless oil; $[\alpha]^{23}_D$ −45 (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3680 3430, 3020, 2960, 1729, 1602, 1582 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HR-ESIMS (QqTOF) *m/z* 558.3757 $[M + Na]^+$ (calcd for $C_{31}H_{53}NO_6Na$ 558.3765).

(c) a lactone (δ 174.9s, 69.6d); (d) five methyl groups (one triplet, one singlet, and three doublets (δ 14.1q, 12.9q, 14.0q, 18.6q, 18.2q); and (e) a carbamate ester (δ 75.5d, 157.7s). The *E*,*E* configuration of the diene was established from the J_{20-21} (15.5 Hz) coupling and NOEs between H-18 and H-20 and between CH_3-30 and H-21 and the *E* geometry of the 12(13) double bond from line shape simulation of H-12 and 13 in the *E* and *Z* configuration (GNMR 4.1, Adept UK).

COSY and HMBC correlations (Table 2 and Figure 3) determined the complete planar structure of tulearin A (3).

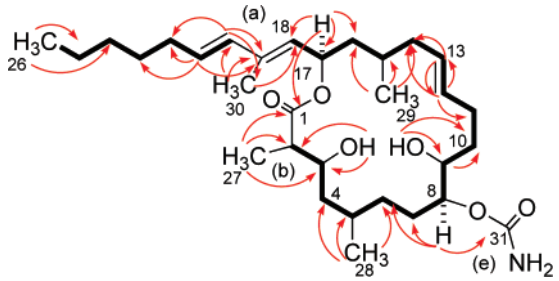

Figure 3. COSY (—) and key HMBC correlations (→) of tulearin **A** (**3**).

The core of tulearin A is a 2,4,15,19-tetramethylated hexaeicosanoic polyketide acid forming an 18-membered lactone (from C-1 to -17), carrying on the macrolide chain, besides two hydroxyls (on C-3 and -9) a carbamate (on C-8). The carbamate function is rare in nature, known, e.g., in palmerolide A, recently isolated from a tunicate,[14] in the microorganism-derived macrolide geldanamycin[15] and in saxitoxin, a neurotoxin isolated from butter clams.[16]

Thus far, we have failed to crystallize the compound; although transannular NOEs could be measured, because of the high conformational flexibility of the macrolide, the chirality of the seven asymmetric centers could not be determined.[17]

The effects of salarin A and tulearin A on cell proliferation were determined in two different human leukemic cell lines, K562[18] and UT7,[19] using the colorimetric methylthiazole tetrazolium bromide (MTT) assay.[20] Tulearin A inhibited cell growth of both cell lines, in a dose- and time-dependent manner. K562 cells displayed a greater sensitivity to tulearin A, as compared to UT7. Namely, after 3 days culture in the presence of 0.5 μg/mL of **3**, a ~60% inhibition of proliferation was observed for K562 cells and 35% inhibition of proliferation for the UT7 cells. Salarin A also inhibited cell proliferation, yet its activity profile was different. The UT7 cells displayed less sensitivity to salarin A as compared to tulearin A. Namely, at 0.5 and 1 μg/mL it inhibited proliferation of UT7 cells to an extent of ~20 and 45%, respectively. Notably, salarin A did not inhibit proliferation of the K562 cells.

In view of the new and interesting structures of the three compounds, we plan to recollect a larger sample of the sponge so as to be able to determine the relative configurations and to evaluate their biological properties.

Acknowledgment. We thank Prof. J. Vacelet (Centre d'Oceanologie de Marseille, France) for identification of the sponge, Dr. L. Fisher for the NMR simulation, and Dr. A. Sacher (of the Maiman Institute for Proteome Research, Tel Aviv University) for performing the electrospray and MALDI mass measurements.

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

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