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Salarin C, a new cytotoxic sponge-derived nitrogenous macrolide

Ashgan Bishara [a], Amira Rudi [a], Maurice Aknin [c], Drorit Neumann [b], Nathalie Ben-Califa [b], Yoel Kashman [a,*]

[a] School of Chemistry, Tel Aviv University, Ramat Aviv 69978, Israel
[b] Department of Cell and Developmental Biology, Tel Aviv University, Ramat Aviv 69978, Israel
[c] Laboratorie de Chimîe des Substances Naturelles et des Aliments, Faculté des Sciences et Techniques, Université de Réunion, 15 Avenue Rene Cassin, B.P. 7151, 97715 Saint Denis, Cedex 9, France

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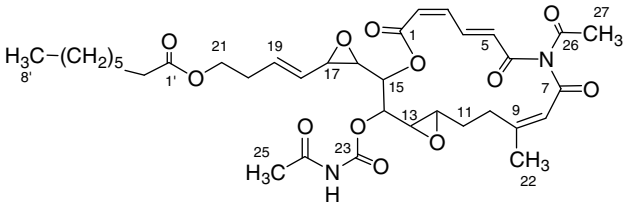
ABSTRACT

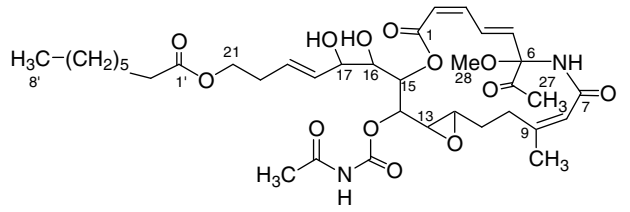
A novel nitrogenous macrolide, designated salarin C (**3**), was isolated from the Madagascan sponge *Fascaplysinopsis* sp. The structure of the compound was elucidated by interpretation of MS and 1D and 2D NMR spectra. Salarin C is closely related to salarin A and is considered to be the precursor of salarins A and B (**1**,**2**). Air oxidation was found to transform **3** to **1**. Salarin C was found to inhibit cell proliferation of human leukeamic cell lines UT-7 and K562 and the murine pro-B cell line Ba/F3 at concentrations of 0.0005–0.5 mg/ml. A possible biogenesis is discussed.

We reported recently the isolation and structure determination of two cytotoxic nitrogenous macrolides from the Madagascan sponge *Fascaplysinopsis* sp. collected in Salary Bay, ca. 100 km north of Tulear.[1] From yet another collection of the sponge, we isolated several additional compounds, including one, bright orange in colour, which was closely related to salarins A (**1**) and B (**2**) and was designated salarin C (**3**) (Fig. 1).

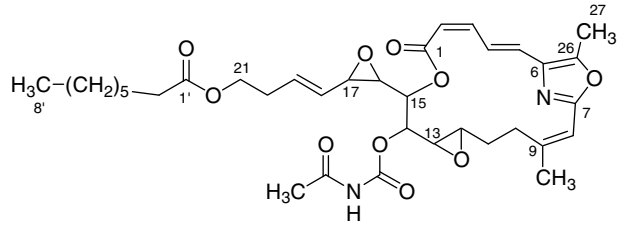
The mass spectroscopic analysis of salarin C (**3**) provided a pseudo molecular formula of $C_{35}H_{46}N_2O_{10}Na$ HR-ESIMS (TOF) *m/z* 677.3035 for $[M+Na]^+$ (calcd 677.3044), with 14 degrees of unsaturation.[2] The ^1H, ^{13}C (Table 1), COSY, HSQC, TOCSY and HMBC spectra (Fig. 2) revealed the presence of the following moieties: (a) two epoxides [δ 56.8 d and 54.9 d (*E*); δ 56.1 d and 56.7 d (*Z*)]; (b) an isolated double bond (δ 124.6 d and 133.7 d); (c) another double bond conjugated to a heterocycle (δ 110.6 d and 150.6 s); (d) an α,β,γ,δ-dienoate group [δ 164.9 s; 117.5 d and 142.9 d (*Z*); and δ 126.0 d and 128.7 d (*E*)]; (e) an octanoate ester (δ 172.7 s, 34.0 t, an additional five methylenes and a methyl 14.0 q); (f) a 5-methyl 3-substituted oxazole (δ 128.7 s, 159.3 s, 145.6 s and 9.3 q) and (g) an *N*-acetyl carbamate (δ 151.2 s, 170.3 s and 23.3 q). The COSY experiment established three significant spin systems, as depicted in Figure 2, which were correlated by the HMBC experiment. Comparison of the ^1H and ^{13}C NMR data of salarin C with that of salarin A (**1**)[1] indicated high similarity. A major change was replacement of the triacylamine subunit of salarin A by an oxazole ring in salarin C. Strong support for the oxazole ring came from the ^{15}N resonance, measured from the $^3J(CH–N)$ HMBC correlation, of δ 245.0 ppm[3] (in addition to the δ 143.0 ppm shift of the acetyl carbamate nitrogen atom)[1] (Fig. 2). Assembling moieties a–g via three unaccounted for pairs of carbon atoms (C-10, 11 and 20, 21, methylenes, and C-14, 15 oxymethines) from COSY, HSQC, TOCSY and HMBC data (Fig. 2) afforded the gross structure of salarin C. The



Salarin A (1)



Salarin B (2)



Salarin C (3)

Figure 1. Salarins A–C (**1**–**3**).

* Corresponding author. Tel.: +972 36408419; fax: +972 3 6409293.
E-mail address: kashman@post.tau.ac.il (Y. Kashman).

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Table 1
NMR spectroscopic data for salarin C (**3**)

Position	δ_C (mult)[a]	δ_H, mult (J in Hz)[b]	^1H–^1H COSY[c]	NOESY	HMBC (H–C)
1	164.9 s				
2	117.5 d	5.72 d (12.1)	3	3	1, 3
3	142.9 d	6.47 t (12.1)	2, 4	2, 5	1, 4, 5
4	126.0 d	8.50 dd (15.0, 12.1)	3, 5	3, 10a, 16	2, 3, 5, 6
5	128.7 d[e]	6.22 d (15.0)	4	3, 27	3, 4, 6, 26
6	128.7 s[e]				
7	159.3 s				
8	110.6 d	5.87 s	22	22	7, 9, 10, 22
9	150.6 s				
10	29.7 t	3.72 m[d]	10b, 11a, 11b	4, 10b, 11a, 22	8, 9, 11, 12, 22
		2.00 m	10a, 11a	10a, 12	
11	29.5 t	1.51 m	10, 11b, 12	10a, 10b, 12	10, 12, 13
		1.30 m	10, 11a, 12	11a	9, 10, 12, 13
12	56.8 d	3.23 dt (9.0, 2.2)	11a, 11b, 13	10a, 10b, 11a, 14, 16	11, 13, 14, 15
13	54.9 d	3.75 dd (9.0, 2.2)	12, 14	10b, 11a, 11b, 14	12, 14, 15
14	78.0 d	4.95 dd (9.0, 2.9)	13, 15	12, 13, 15	13, 15, 16, 23
15	67.9 d	5.88 m[d]	14, 16	14, 18	1, 13, 14, 16, 17
16	56.1 d	3.40 dd (7.8, 3.9)	15, 17	12, 17	14, 15, 17
17	56.7 d	3.09 dd (6.4, 3.9)	16, 18	16, 18, 19	16, 18, 19
18	124.6 d	5.50 dd (15.3, 6.4)	17, 19	15, 17, 20	17, 20
19	133.7 d	5.75 dt (15.3, 6.4)	18, 20	17, 20, 21	17, 20, 21
20	31.8 t	2.14 m[d] (2H)	19, 21	18, 19, 21	18, 19, 21
21	62.7 t	4.04 td (6.9, 2.9) (2H)	20	19, 20	19, 20, 1′
22	24.6 q	1.52 s	8	8, 10b	8, 9, 10
23	151.2 s				
NH		6.94 s		25	25
24	170.3 s				
25	23.3 q	2.13 s		NH	24
26	145.6 s				
27	9.3 q	1.73 s		5	6, 26
1′	172.7 s				
2′	34.0 t	2.19 t (7.8) (2H)	3′	3′, 4′	1′, 3′, 4′
3′	25.0 t	1.59 m (2H)	4′		2′, 4′
4′	29.0 t	1.22 m (2H)	3′, 5′		3′, 5′
5′	29.1 t	1.22 m (2H)	4′, 6′		4′, 6′
6′	31.7 t	1.19 m (2H)	5′, 7′		5′, 7′
7′	22.7 t	1.22 m (2H)	6′, 8′		5′, 6′, 8′
8′	14.0 q	0.88 t (6.8)	6′, 7′		6′, 7′

[a] Data recorded in C_6D_6 on Bruker Avance 400 MHz instruments (100 MHz for ^{13}C).
[b] The CH correlations were assigned by an HSQC experiment.
[c] a, b, a geminal pair, denote the upper (a) and lower (b) protons.
[d] Multiplicities were not determined because of overlapping with other signals.
[e] The two 128.7 resonances separate well in $CDCl_3$; δ_c (128.0 d, C-5; 134.3 s, C-6).

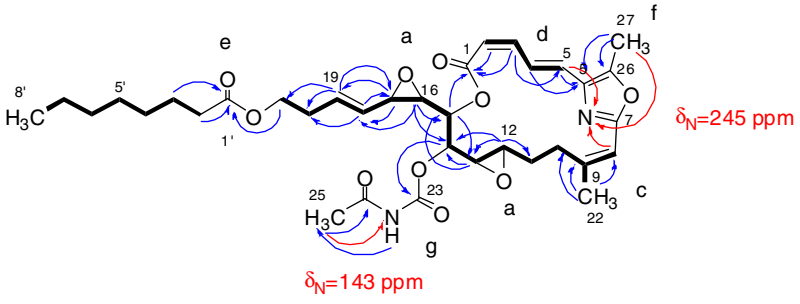
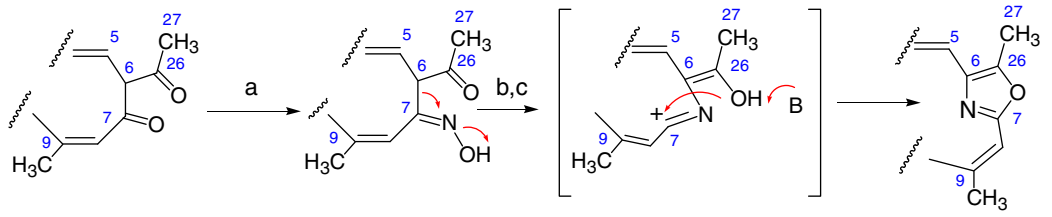


Figure 2. COSY (▬), and key HMBC correlations (↷) and ^{15}N-HMBC (↷) of salarin C.

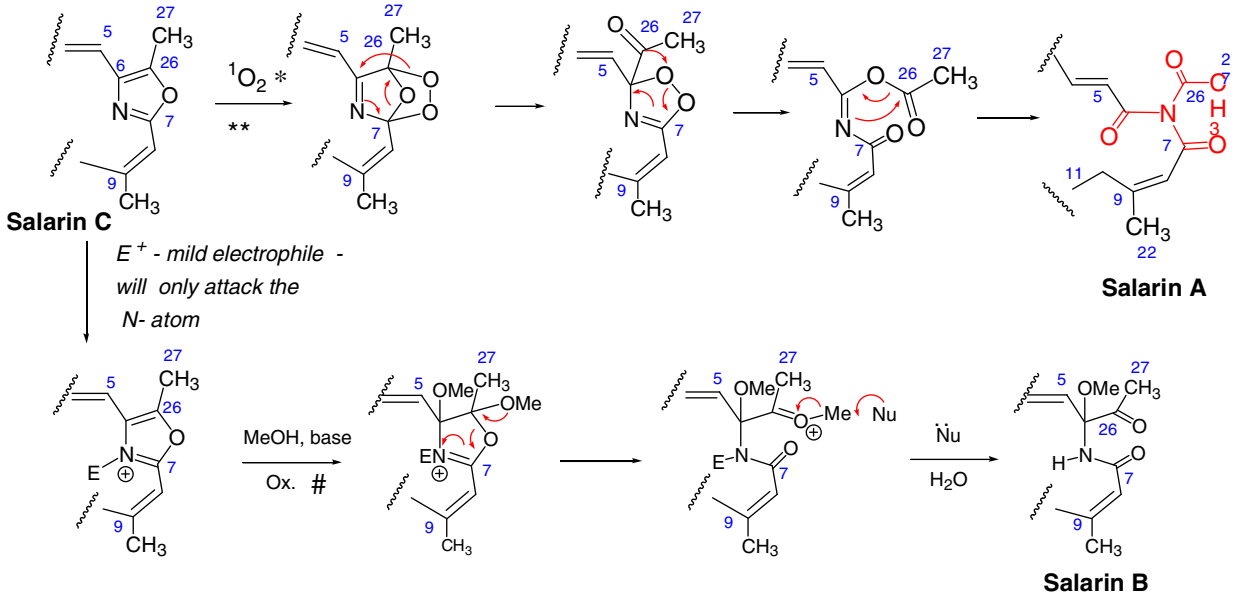
Z, E, Z and *E* configurations of double bonds 2(3), 4(5), 8(9) and 18(19), respectively, and the *E* and *Z* configurations of the two epoxides, 12(13) and 16(17), respectively, of compound **3** were determined from the *J* values, measured NOEs and by comparison with the appropriate counterpart values in compounds **1** and **2**.[1]

Oxazoles are present widely in biologically active natural compounds.[4,5] It is believed that the oxazole rings are biosynthesized from amino acids, namely from serine or threonine.[6] The amino acid origin of oxazoles in cyclic peptides such as bistratamide[7] is most likely, and in epothilone D, for example, it has even been proven.[8] However, oxazole precursors in the marine natural compounds calyculins,[9] phorbazoles[10] and mycalolide[11] were proposed recently by Uemura to be obtained via a different route involving Beckman rearrangement of α-formyl ketoximes.[12] Similarly, it can be suggested that the oxazole ring of salarin C is obtained from an α-acetyl ketoxime as depicted in Scheme 1.

Of prime interest was the finding in the literature that oxazoles ring-open under oxidative conditions to afford, with $^1O_2$ triacylam-



Scheme 1. Suggested biogenesis of the oxazole ring of salarin C (**3**).[12] Reagents: (a) H_2NOH; (b) Beckmann rearrangement; (c) enolization together with rearrangement and closure of the oxazole.



Scheme 2. Suggested transformation of salarin C to salarins A and B. ∗: Suggested mechanism according to Wasserman (1981) and Scarpati (1991).[13] ∗∗: The same oxidation takes place by air oxidation. # Suggested mechanism according to Hassner (1989), (Br_2 Ox., MeOH).[14]

ines,[13] and under mild basic bromine-oxidation, an amidomethoxy ketone.[14] Salarins A and B are precisely the expected products from salarin C under analogous biosynthetic oxidations. Detailed suggested mechanisms leading to salarins A and B from C are depicted in Scheme 2.

Unexpectedly, stirring salarin C (**3**) in chloroform slowly afforded salarin A (**1**) (ca. 50% in 3 days), over a longer period, isomerization of the α,β,γ,δ-dienoate takes place.

Thus far, we have failed to crystallize salarin C, most likely, because of the high conformational flexibility of the macrolide and the side chain. The latter flexibility is also responsible for the failure to determine the relative configuration of **3**. Transannular NOEs could be measured between H-4 and H-10; H-12 and H-16 and H-4 and H-16, nevertheless, the existence of more than a single ring-conformer was clear from a NOE between H-3 and H-4, which in the major conformation have to be *anti* to each other ($J_{3,4}$ = 12.1 Hz). Derivatization and/or degradation of **3** seems to be essential for configuration assignment.

Salarin C was found to be cytotoxic to several cells. To investigate the potential effects of salarin C on cell proliferation, the human leukeamic cell lines UT-7[15] and K562,[16] and the murine pro-B cell line Ba/F3 which stably expresses the EPO receptor (EPO-R)[17] were used as targets. The anti-proliferative activity of salarin C was dose-dependent; salarin C at concentrations of 0.0005-0.5 mg/ml was added to the cells for 24 hours, and cell viability was determined by MTT assay.[18] Salarin C was by far more potent than salarins A and B, and tulearin A, ~50% inhibition of cell proliferation was obtained at 0.5 mg/ml (1 mM) of salarin C for the UT-7 cell line and at 0.05 mg/ml (0.1 mM) for the K562 cell line. The sensitivity of the Ba/F3 cells was much higher as their proliferation was arrested completely at 0.1 mM of salarin C. The effective concentration range of salarin C (IC_{50} 0.1–1 mM) is comparable, and even lower than those of other chemotherapeutic agents (e.g., geldanamycin).[19]

The finding that salarin C inhibits cell proliferation in a dose-dependent manner renders it an attractive candidate for basic science, as well as for potential clinical applications. This may reach beyond unregulated proliferation to applications that include infectious diseases, neurological and autoimmune manifestations.

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References and notes

1. Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 153–156.
2. *Salarin C*: bright orange oil; $[\alpha]_D^{23}$ −64 (*c* 0.34, $CHCl_3$): IR ($CHCl_3$) ν_{max} 3648, 3054, 2986, 1717, 1421, 1272 cm^{-1}. UV (MeOH) affords three absorptions at 206, 270 and 347 nm. ^1H and ^{13}C NMR see Table 1. HR-ESIMS (QqTOF) *m/z* 677.3035 $[M+Na]^+$(calcd for $C_{35}H_{46}N_2O_{10}Na$, 677.3044). FABMS *m/z* 655.3 $[M+H]^+$ (100), 677.3 $[M+Na]^+$ (10); 570.3 $[M-H-C_3H_3O_2N]^+$ (15).
3. Martin, G. E.; Hadden, C. E. *J. Nat. Prod.* **2000**, *63*, 543–585.
4. Jin, Z. *Nat. Prod. Rep.* **2003**, *20*, 584–605.

5. Munro, M. H. G.; Blunt, J. W. *Marine Literature Database*; Department of Chemistry, University of Canterbury: New Zealand, 2007.
6. Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. *Nat. Prod. Rep.* **1999**, *16*, 249–263.
7. Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671–6675.
8. Schneider, T. L.; Walsh, C. T.; O'Conner, S. E. *J. Am. Chem. Soc.* **2002**, *124*, 11272–11273.
9. Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuta, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780–2781.
10. Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126–8131.
11. Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1989**, *30*, 2809–2812.
12. Ichino, T.; Arimoto, H.; Uemura, D. *Chem. Commun.* **2006**, 1742–1744.
13. (a) Wasserman, H. H.; Gamble, R. J.; Pulwer, M. J. *Tetrahedron* **1981**, *37*, 4059–4067; (b) Iesce, R.; Graziano, M. L.; Cimminiello, G.; Cermola, F.; Parrilli, M.; Scarpati, R. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1085–1089.
14. Hassner, A.; Fischer, B. *Tetrahedron* **1989**, *45*, 6249–6262.
15. Komatsu, N.; Nakauchi, H.; Miwa, A.; Ishihara, T.; Eguchi, M.; Moroi, M.; Okada, M.; Sato, Y.; Wada, H.; Yawata, Y.; Suda, T.; Miura, Y. *Cancer Res.* **1991**, *51*, 341–348.
16. Lozzio, C. B.; Lozzio, B. B. *Blood* **1975**, *45*, 321–334.
17. Neumann, D.; Wikstrom, L.; Watowich, S. S.; Lodish, H. F. *J. Biol. Chem.* **1993**, *268*, 13639–13649.
18. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
19. Jeon, Y. K.; Park, C. H.; Kim, K. Y.; Li, Y. C.; Kim, J.; Kim, Y. A.; Paik, J. H.; Park, B. K.; Kim, C. W.; Kim, Y. N. *J. Pathol.* **2007**, *213*, 170–179.