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Zampanolide, a New Cytotoxic Macrolide from a Marine Sponge

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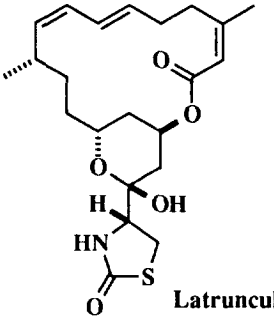
Abstract: A novel 20-membered macrolide, zampanolide (**3**), has been isolated together with latrunculin A (**1**) from a different collection of the sponge which previously yielded **1** and laulimalide (**2**). The gross structure of **3** was determined by 2D NMR and partial stereochemistry by NOE analysis. It is a highly unsaturated 20-membered macrolide containing an uncommon carbinol amine functionality. Zampanolide showed potent cytotoxicity against several tumor cell lines.

Recently, we reported the X-ray structures of the known latrunculin A (**1**) and laulimalide (**2**) which were isolated as major cytotoxic constituents of a sponge collected at Shimoji Island, Okinawa.[1] The same specimen subsequently yielded two new related compounds, latrunculin S and neolaulimalide as minor constituents.[2] Another specimen of the same sponge (*Fasciospongia rimosa*[3]) was collected at Cape Zampa in the island of Okinawa. Investigation of this sample furnished a novel macrolide, zampanolide (**3**) as a minor constituent in addition to a substantial amount of **1**. Zampanolide (**3**) exhibited potent cytotoxicity (IC_{50} 1-5 ng/mL) against P388, A549, HT29, and MEL28 cell lines. In this paper we report the isolation and structure elucidation of **3**.

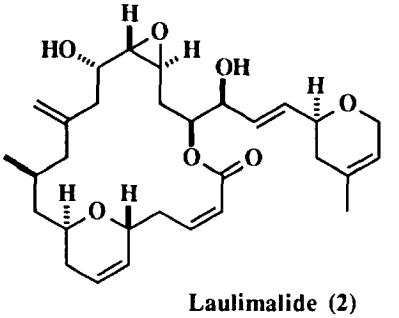
A sample (480 g) of the sponge from Cape Zampa was extracted with acetone. An ethyl acetate soluble portion (2.87 g) of the extract was separated on a silica gel column into nine fractions by elution with a step-gradient of heptane and EtOAc. The sixth fraction gave a nearly pure sample (1.01 g) of latrunculin A (**1**). Repeated chromatography of the seventh fraction by silica (heptane/EtOAc 1:1) and ODS HPLC (MeCN/H_2O 7:1) furnished 3.9 mg of zampanolide (**3**) and 13.7 mg of **1**.

Zampanolide (**3**) obtained as an amorphous solid, $[\alpha]_D^{29}$ -101° (c 0.12, CH_2Cl_2), was analyzed for $C_{29}H_{37}NO_6$ by HR-FABMS.[4] The ^{13}C NMR spectrum[5] showed signals for 29 carbons of which 17 were due to sp^2 carbons including three carbonyls (δ 197.2, 165.5, 165.2). This observation together with the unsaturation requirement suggested the molecule to have two rings. Connectivity studies by COSY, TOCSY, HMQC, and HMBC experiments enabled us to depict the gross structure of **3** (Figure 1). Assignment of the ^1H and ^{13}C NMR signals and the observed connectivity are shown in Table 1. Observation of the HMBC cross peaks for H19/C1, H6ab/C7 and H9/C7, and H20/C1' and NH/C1' allowed us to locate the carbonyls, a lactone, ketone, and amide, respectively, in the molecule. The presence of a carbinol amine moiety at C20 was secured by observing coupling of the hydroxyl proton (δ 6.11, d, J = 5.2 Hz) with H20 (δ 5.33) which in turn coupled to NH at δ 8.29 (d, J = 9.2 Hz) and H19 (δ 4.96). The carbon chemical shift (δ 72.9) of C20 is comparable with that (δ 74.9) observed for the methine carbon bearing both nitrogen and oxygen atoms in

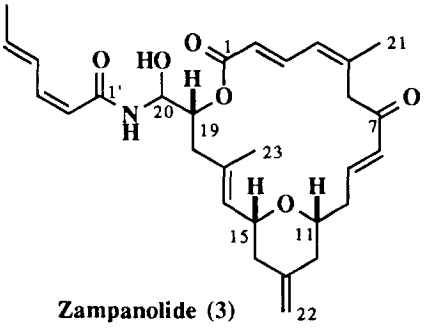
onnamide A.[6] The configurations of the double bonds at C2, C8, C2', and C4' were determined to be *E*, *E*, *Z*, and *E*, respectively, by the coupling constants of their respective proton NMR signals (Table 1). The configurations of 4*Z* and 16*E* were secured by carbon chemical shifts of the methyl groups at C5 (C21: δ 23.6) and C17 (C23: δ 16.6), respectively. NOE observation between H3 and H6 and between H2' and H3' also confirmed 4*Z* and 2'*Z* configurations.



Latrunculin A (1)



Laulimalide (2)



Zampanolide (3)

The relative configurations at the chiral centers C11, C15, and C19 were proposed by NOE analysis (Figure 1) and coupling constants ($J_{14a,15}$ = 11.3 Hz, $J_{15,16}$ = 7.6 Hz, $J_{18a,19}$ = 10.0 Hz, $J_{18b,19}$ = 1.8 Hz). The axial orientation of the H15 (δ 3.87, ddd, *J* = 11.3, 7.6, 2.7 Hz) in the tetrahydropyran ring was suggested by a large coupling constant (*J* = 11.3 Hz). A cross peak between the signal of H15 and that (δ 3.27) of H11 in a PSNOESY spectrum indicated the diaxial orientation of the both protons. NOEs were also observed between H15 and H23 (δ 1.61) and between H23 and H19 (δ 4.96), suggesting that in a preferred conformation the C16,17 double bond was tilted to have the methyl group (C23) pointing to the same direction with H15 and H19 as shown in Figure 1. Further support of this conformational preference is provided by relatively large coupling constants observed between H15 and H16 (*J* = 7.6 Hz) and between H18a and H19 (*J* = 10 Hz). Thus, the relative configuration of C19 can be determined in relation to that of C15, and the configurations at the three chiral centers are assigned to be 11*R**, 15*R**, and 19*R**. Although NOEs were also observed between H19 and H20 (δ 5.33) and between H19 and NH (δ 8.29), no conclusion has yet been drawn on the configuration of C20. Zampanolide (**3**) is a unique 20-membered macrolide having high unsaturation and an uncommon carbinol amine functionality. The latter functional group has been encountered with such highly bioactive marine metabolites as onnamide and related compounds[7] and ecteinascidins.[8] Further refinement of the stereochemistry and biological studies of this novel macrolide are now under way.

Table 1. ^{1}H and ^{13}C NMR Data for Zampanolide (3) in DMSO-d_6

No.	^{13}C	^{1}H	HMBC	COSY	TOCSY[1]
1	165.5 s	-	-	-	-
2	120.6 d	5.93 d, J = 15.0 Hz	C1,4	H3	H3,21
3	139.5 d	7.52 dd, J = 15.0, 11.6 Hz	C1,2,5	H2,4	H2,4,21
4	125.1 d	6.20 d, J = 11.6 Hz	C2,3,6,21	H3,21	H3,21
5	143.0 s	-	-	-	-
6	44.8 t	a3.01 d, J = 14.4 Hz	C4,5,7,21	H6b,21	H6b,21
		b4.12 d, J = 14.4 Hz	C4,5,7,21	H6a	H6a,21
7	197.2 s	-	-	-	-
8	130.8 d	5.95 d, J = 15.3 Hz	C10	H9	H9
9	145.8 d	6.74 ddd, J = 15.3, 8.6, 5.5 Hz	C7	H8,10	H8,10,11
10	39.2 t	2.30 m (2H)	C8,9	H9,11	H9,11,12a
11	76.0 d	3.27 m		H10,12ab	H9,10,12ab
12	40.3 t	a1.84 m	C11,13,22	H11,12b	H10,11,12b,22
		b2.17 brd, J = 12.8 Hz	C13,14,22	H11,12a	H11.12a,22
13	143.7 s	-	-	-	-
14	40.3 t	a1.88 m	C13,15,16,22	H14b,15	H14b,15,16,22
		b2.07 m	C12,13,22	H14a,15	H14a,15,16,22
15	75.1 d	3.87 ddd, J = 11.3, 7.6, 2.7 Hz	C17	H14ab,16	H14ab,16,22,23
16	129.0 d	5.10 d, J = 7.6 Hz	C18,23	H15,23	H14ab,15,23
17	132.4 s	-	-	-	-
18	40.8 t	a2.09 m	C16,17,19,23	H18b,19	H18b,19,20,23,OH,NH
		b2.33 m	C16,17,23	H18a,19	H18a,19,OH,NH
19	71.9 d	4.96 ddd, J = 10.0, 6.0, 1.8 Hz	C1,20	H18ab,20	H18ab,20,OH,NH
20	72.9 d	5.33 ddd, J = 9.2, 6.0, 5.2 Hz	C1'	H19,NH,OH	H18a,19,OH,NH
21	23.6 q	1.75 s	C4,5,6	H4,6a	H2,3,4,6ab
22	108.9 t	4.73 s (2H)	C12,13,14		H12ab,14ab,15
23	16.6 q	1.61 s	C16,17,18	H16	H15,16,18a
1'	165.2 s	-	-	-	-
2'	119.2 d	5.65 d, J = 11.3 Hz	C1',4'	H3'	H3',4',5',6'
3'	140.6 d	6.37 t, J = 11.3 Hz	C1',5'	H2',4'	H2',4',6'
4'	128.6 d	7.44 ddq, J = 14.7, 11.3, 1.2 Hz	C6'	H3',5',6'	H2',3',6'
5'	137.1 d	5.97 m	C3',6'	H4',6'	H2',6'
6'	18.3 q	1.79 dd, J = 6.7, 1.2 Hz	C4',5'	H4',5'	H2',3',4',5'
NH	-	8.29 d, J = 9.2 Hz	C1'	H20	H18ab,19,20,OH
OH	-	6.11 d, J = 5.2 Hz	C19,20	H20	H18ab,19,20,NH

1. Mixing time = 80 ms.

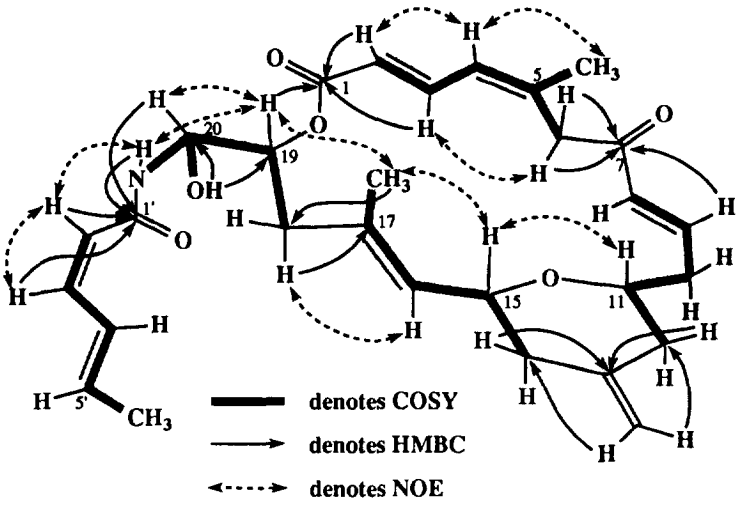


Figure 1. Selected connectivity and stereochemical correlation for zampanolide (**3**)

Acknowledgment

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References and Notes

1. Jefford, C. W.; Bernardinelli, G.; Tanaka, J.; Higa, T. *Tetrahedron Lett.* **1996**, *37*, 159-162.
2. Tanaka, J.; Higa, T.; Bernardinelli, G.; Jefford, C. W. *Chem. Lett.* **1996**, 255-256.
3. Since the original taxonomic identification of the sponge as *Fasciospongia rimosa* has been questioned, a specimen has been submitted for reexamination.
4. Zampanolide (**3**): IR (CCl_4) 3450, 2950, 1720, 1680, 1645 cm^{-1}; UV (MeOH) λmax 264 nm (ε $3x10^4$), 230 nm (ε $2.5x10^4$); CD (MeOH) Δε -30 (260 nm), +45 (227 nm); HR-FABMS obsd 496.2683 (M+H), calcd for $C_{29}H_{38}NO_6$ 496.2699.
5. Due to the instability of zampanolide in $CDCl_3$, all NMR spectra (^1H at 500 MHz, ^{13}C at 125 MHz) were recorded in DMSO-d_6.
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