A New Dimension to the Biosynthetic Products Isolated from the Sponge *Negombata magnifica*

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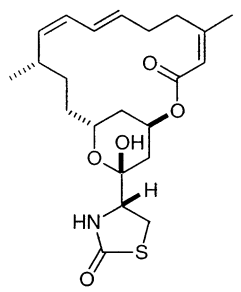
Latrunculeic acid (**3**), a novel analogue of latrunculin B (**2a**), was isolated from the Red Sea sponge *Negombata magnifica* and characterized. Several known compounds were also isolated, including latrunculin B (**2a**), 15-methoxylatrunculin B (**2b**), 16-*epi*-latrunculin B (**4**), and latrunculin C (**5**). In contrast to the other members of the latrunculin family, the novel compound **3** is a polyketide devoid of the normal macrocyclic and thiazolidinone rings present in previously identified members of this family.

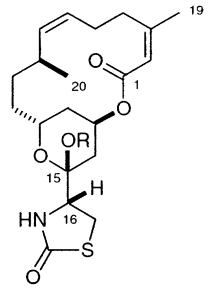
The latrunculins are a class of macrolides that have been isolated from sponges[1] and nudibranchs.[2] Since the original report of latrunculin A (**1**) and latrunculin B (**2a**),[1b] two congeners of the former[1f,3] and four of the latter[3−5] have been described. Strikingly, all of these contain the rare thiazolidinone ring, which is also present in each of the synthetic analogues studied to date.[3,6,7] While **1** has been isolated from materials collected throughout the Indo-Pacific, the Red Sea organisms appear to be the only taxa capable of the production of both **1** and **2a** and their congeners, yet not simultaneously.[8d]

In connection with a comprehensive reexamination of sponges and associated nudibranchs that contain the latrunculins, we chose to further examine a sponge that produces the latrunculin B series. Taxonomy changes over the years caused minor complications in nomenclature. Some texts describe this organism as *Latrunculia cortica*[9a] or *L. magnifica*.[1a] The current designation is *Negombata magnifica*.[9b,c] The *N. magnifica* (coll. no. 88115, Gulf of Eilat) used in this study was stored in 1988 in our repository, and an early cursory study showed **2a** to be the major metabolite. Recent scrutiny of the original ^{13}C NMR data (taken in 1989) of several crude extract fractions indicated the presence of additional metabolites. We now describe the isolation and structure elucidation of a new analogue, latrunculeic acid (**3**), from this extract along with known compounds, latrunculin B (**2a**),[1b] 15-methoxylatrunculin B (**2b**),[3] 16-*epi*-latrunculin B (**4**),[5] and latrunculin C (**5**).[4] Compound **2b** has been previously synthesized[3] and assessed for bioactivity[6] but never reported as a natural product.

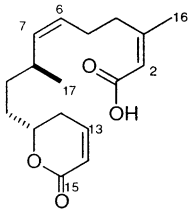
Flash column chromatography (reversed-phase) of the sponge methanol extract provided several fractions. Extensive chromatography afforded **2a** and **4** as the major components and **5**, **2b**, and the new compound (**3**) as minor metabolites. The structure elucidation of **3** began with an analysis of its HRMS data that featured a *m*/*z* peak of 315.1570 (M + Na). This indicated a molecular formula of $C_{17}H_{24}O_4$, representing six units of unsaturation, equivalent to **2a** without the thiazolidinone ring (C_3H_4ONS). Also, the ^1H NMR signals of a thiazolidinone ring were not observed. The ^{13}C NMR data confirmed the presence of 17



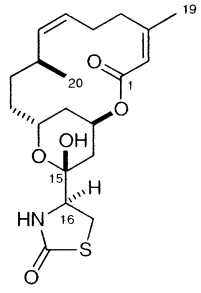
1 latrunculin A



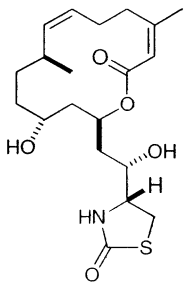
2a R = H latrunculin B
2b R = CH_3 15-methoxylatrunculin B



3 latrunculeic acid



4 16-*epi*-latrunculin B



5 latrunculin C

carbons. Additional structural features included an α,β unsaturated ester moiety as evidenced by vinylic proton signals at δ_H 6.87 (H-13) and 6.02 (H-14) and a carbonyl carbon at δ_C 165 (C-15). An α-pyrone could account for three units of unsaturation, which further required that the two double bonds were part of an acyclic chain capped by the carboxylic acid group. The connectivities shown in the final structure for this chain beginning at C-1 and

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Table 1. ^{13}C (125 MHz), ^{1}H (500 MHz), and HMBC NMR Spectral Data of Latrunculeic Acid (**3**) in $CDCl_3$

no.	^{13}C	^{1}H (mult.)	HMBC (H to C)
1	170.0		
2	115.8	5.70 (1H, d, 1.5)	1, 3, 4, 16
3	162.7		
4a	33.9	2.72 (1H, ddd, 12.2, 8.1, 8.1)	2, 3, 5, 6
4b		2.60 (1H, ddd, 12.2, 7.8, 7.8)	2, 3, 5, 6
5	26.5	2.21 (2H, m)	4, 6, 7
6	127.8	5.36 (1H, ddd, 10.8, 7.5, 7.5)	4, 5, 7, 8
7	136.5	5.15 (1H, dd, 10.7, 10.0)	6, 8
8	31.5	2.48 (1H, m)	6, 7, 17
9	32.4	1.43 (2H, m)	7, 8, 17
10a	32.9	1.77 (1H, m)	9, 11, 12
10b		1.61 (1H, m)	9, 11, 12
11	78.3	4.40 (1H, m)	
12	29.6	2.33 (2H, m)	13, 14
13	145.4	6.88 (1H, ddd, 9.8, 4.5, 4.5)	
14	121.5	6.20 (1H, ddd, 9.8, 2.0, 2.0)	12, 15
15	165.0		
16	25.8	1.93 (3H, d, 1.5)	
17	21.4	0.96 (3H, d, 6.8)	

extending to C-11 along with two methyl groups (at C-3 and C-8) were verified by a series of 2D NMR experiments (COSY, HMBC, HMQC), and the data set is summarized in Table 1. Several of the stereochemical elements of **3** were expected to be analogous to those of **2a**. Consistent with this assumption was the C-6−C-7 double bond assigned as *Z* on the basis of a coupling constant of $^{3}J = 10.7$ Hz. Likewise, the C-2−C-3 double bond was also assigned as *Z* by the diagnostic ^{13}C shift of Me16 (δ_C 25.8) versus that of latrunculin B (δ_C 25.8) and 16-*epi*-latrunculinB (δ_C 24.4) (see Table S1). The stereochemical assignment at C-8 and C-11 could not be done directly using the NMR data collected. Alternatively, on the basis of the observation that all the known latrunculin analogues have the 8*S*, 11*R* configuration (or the equivalent as a function of differing numbering schemes), this same absolute configuration is provisionally suggested for **3**.

Unlike all the previously identified latrunculins, which are ketide amino acids, **3** is a polyketide devoid of the macrocyclic and thiazolidinone rings. The biosynthetic process responsible for its production, while unclear at this point, deserves brief comment, as we believe **3** is not an artifact of isolation. The overall core structures of latrunculin A (**1**) and B (**2**) are chemically very stable, with the hemiacetal moiety being the most sensitive region for any further structural modifications. In this context, latrunculin B (**2a**) provides one possible precursor to **3** via a double elimination process involving extrusion of the thiazolidinone ring at C-15 and cleavage of the lactone oxygen at C-13. Alternatively **3** could be directly assembled via a polyketide route not involving the intermediacy of **2a**. Overall, the latrunculins are of great importance as potential therapeutics and molecular probes in cytoskeletal research.[8] The latrunculins cause unique microfilament inhibition due to a one-to-one complex formed with monomeric actin.[6] The 16-membered macrocycle **1** is more potent than the 14-membered **2a**.[8b,c] The results of the actin-disruption assay (Table 2) agree with the hypothesis that the NH, OH, and macrolide structures are all needed for cytoskeletal activity.[6a] Interestingly, latrunculin B was more potent than its 16-epimer in our assay (Table 2). A comprehensive structure−activity study for the latrunculins has yet to be reported, and it would be relevant in the future to examine more analogues in this context. The biogeographical variation among the latrunculins has been previously discussed. Sponges from the northern gulf of Eilat are a source of just latrunculin B, while those from the western side of the Sinai Peninsula contain only latrunculin A.[8d] Our observations are consistent with this previous pattern found among *Negombata* sponges.

Table 2. Microfilament-Disrupting Activity[a]

compound	activity observed (μg/mL)[a]
latrunculin A (**1**)	0.5−1.0
latrunculin B (**2a**)	0.5−10.0
15-methoxylatrunculin B (**2b**)	no activity
latrunculeic acid (**3**)	no activity
16-*epi*-latrunculinB (**4**)	5−10

[a] Latrunculin concentrations where microfilament loss was detected. Concentrations up to 10 μg/mL were evaluated.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.7 MHz, respectively. Mass spectrometry data were obtained with an electrospray ionization time-of-flight (ESI-TOF) mass spectrometer. Initial fractionation was done using a flash system with a C_{18} column. Preparative HPLC was performed via a C_{18} 6 μm column with UV detection at 254 nm in conjunction with light-scattering detection. Semipreparative HPLC was performed using a C_{18} 4 μm column with UV detection at 230 nm.

Biological Material, Collection, and Identification. The *Negombata magnifica* (coll. no. 88115) was collected by scuba off the coast of Eilat in the Red Sea. It was identified by comparison of the underwater photos and physical features to those previously published.[9]

Extraction and Isolation. The sponge methanol extract (2.7 g), from our repository, was subjected to low-pressure reversed-phase liquid chromatography on a flash column in portions of about 1 g each, in fractions of increasing organic content beginning with 50% methanol in water, resulting in nine fractions. A 140 mg portion of the fourth flash fraction was subjected to preparative HPLC over a gradient of 30%−70% acetonitrile in water, resulting in four fractions. Semipreparative HPLC (55% aqueous acetonitrile) of the second preparative HPLC fraction afforded **2a**[1b] (39.5 mg) and **4**[5] (8.9 mg). Semipreparative HPLC of the third preparative HPLC fraction (65% acetonitrile) afforded **2b**[3] (1.6 mg). The third flash fraction was subjected to preparative HPLC over a gradient of 40%−60% acetonitrile in water, resulting in five fractions. Semipreparative HPLC of the second of these fractions (40% acetonitrile) afforded **5**[4] (2.0 mg). Semipreparative HPLC of the fourth of these fractions (45% acetonitrile) afforded **3** (4.0 mg). The water used for all HPLC separations contained 0.1% formic acid. The known compounds were identified by a comparison of their properties to those in the literature.

Latrunculeic Acid (3): clear colorless oil; $[\alpha]_D$ −50.9° (*c* 0.05, CH_3OH); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), in Table 1; ESITOFMS *m*/*z* 607 $[2M + Na]^+$ (22), 315 $[M + Na]^+$ (100), 293 $[M + H]^+$ (10), 275 $[M + H - H_2O]^+$ (18); HRESITOFMS *m*/*z* 315.1570 (calcd for $C_{17}H_{24}O_4$Na 315.1567).

Bioassay. The microfilament-disrupting effects of the latrunculins were evaluated in A-10, rat aortic smooth muscle cells. Cells were treated with the compounds and then fixed, and microfilaments visualized using rhodamine-phalloidin. Microfilament disruption was defined as loss of microfilament structures.

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Supporting Information Available: The isolation scheme, comparative NMR table for **3** versus **2a** and **4**, and ^{1}H and ^{13}C NMR spectra of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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