mg, 2.64 mmol) with [9-^{2}H]-9-BBN (0.628 M solution in THF, 4.67 mL, 2.93 mmol) and (+)-α-pinene (0.498 mL, 3.14 mmol). The product (425 mg, 95.3%) upon conversion of an aliquot to the (-)-camphanic acid ester showed a signal for the benzylic *pro-S* hydrogen and a small signal (doublet, 0.05–0.1 H) for the *pro-R* hydrogen.

3,5-Dimethoxy-[7-^{2}H]benzyl Tosylate. To a suspension of NaH in mineral oil (87 mg, 60% dispersion, 2.1 mmol) in 8 mL of dry ether was added 333 mg (2.0 mmol) of 3,5-dimethoxy-[7-^{2}H]benzyl alcohol under argon. After being stirred for 15 h at 50 °C, the mixture was cooled to −60 °C, and a solution of *p*-toluenesulfonyl chloride (381 mg, 2.0 mmol) in 2 mL of dry ether was added dropwise with stirring. The mixture was stirred for 1 h at −30 to −10 °C and then for 2 $^1/_2$ h at 4 °C. Insoluble material was filtered off and washed with a small amount of dry ether. The combined ether solution was cooled to −60 °C to give colorless needles of the tosylate (330 mg, 51.1%).

3,5-Dimethoxy-[7-^{2}H,7-^{3}H]toluene. A. By Reduction with [^{3}H]LiAlH$_4$. To a solution of 3,5-dimethoxy-(7*S*)-[7-^{2}H]benzyl tosylate (161 mg, 0.50 mmol) in 5 mL of dry ether was added $LiAlH_4$ (1.0 mg) at 0 °C under argon, followed after several minutes by [^{3}H]$LiAlH_4$ (1.0 mg, 5.0 mCi). After stirring for 3 h at room temperature, excess $LiAlH_4$ (25 mg) was added and stirring was continued for 3 h. Excess reagent was decomposed with water at 0 °C, and 1 N HCl was added to dissolve the precipitate. The mixture was extracted with ether, and the ether phase was washed with 5% $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated in vacuo to give crude 3,5-dimethoxy-(7*S*)-[7-^{2}H,7-^{3}H]toluene (455 μCi), which was purified by preparative-layer chromatography (silica gel, *n*-hexane/ether, 4:1) with almost quantitative recovery of radioactivity; radiochemical yield 9.1%.

B. By Reduction with Supertritide. To a solution of 3,5-dimethoxy-(7*S*)-[7-^{2}H]benzyl tosylate (138 mg, 0.428 mmol) in dry THF (0.4 mL) was added Superhydride (Aldrich; 43 μL of a 1 M solution in THF, 0.043 mmol) at room temperature under argon, followed, after 10 min of stirring, by ^{3}H Superhydride (286 μL of a 1 M solution in THF, 0.286 mmol, 10 mCi). Stirring was continued for 1$^1/_2$ h, excess nonlabeled Superhydride (526 μL, 0.526 mmol) was then added followed by stirring for another 2 h. Excess reagent was destroyed with water, and the mixture was extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative-layer chromatography (silica gel, $CHCl_3$) to give 3,5-dimethoxy-(7*S*)-[7-^{2}H,7-^{3}H]-toluene (7.34 mCi, 0.42 mmol, 73% radiochemical yield). Under identical conditions the *R* isomer was obtained from 3,5-dimethoxy-(7*R*)-[7-^{2}H]benzyl tosylate in 81% radiochemical yield.

Sodium (*R*)- and (*S*)-[2-^{2}H,2-^{3}H]acetate.[18] To a solution of 3,5-dimethoxy-(7*S*)-[7-^{2}H,7-^{3}H]toluene (0.42 mmol, 7.34 mCi) in 2 mL of *n*-pentane was added SiO_2 (4.5 g, 100 mesh), and the solvent was evaporated in vacuo. The resulting SiO_2 carrying the labeled material was stirred for 4 h at −78 °C under a stream of ozone. After standing at room temperature for 1 h, the ozonolysis was repeated for 4 h at −78 °C. The mixture was then warmed to 4 °C, 10 mL of water was added, and after the mixture stood at 4 °C overnight, it was subjected to steam distillation, replacing the water as necessary. The distillate (250 mL) was neutralized with 0.1 N NaOH and evaporated to dryness. The residue (5.77 mCi) was dissolved in 90 mL of water, mixed with 1.8 g of $HgSO_4$ and 3 mL of concentrated H_2SO_4, and subjected to steam distillation. Neutralization of the distillate and evaporation to dryness gave sodium (*S*)-[2-^{2}H,2-^{3}H]acetate (0.30 mmol, 5.16 mCi) in 70% radiochemical yield. The *F* value of this material was determined to be 21.

Similary, 3,5-dimethoxy-(7*R*)-[7-^{2}H,7-^{3}H]toluene (0.42 mmol, 8.09 mCi) gave sodium *R*-[2-^{2}H,2-^{3}H]acetate (5.78 mCi) of *F* = 76.5 in 71.4% radiochemical yield.

Acknowledgment. We thank Professor R. M. Coates and Dr. C. J. Pearce, University of Illinois, Urbana, for providing the tritiated Superhydride. They as well as Professor H. C. Brown, Purdue University, are also thanked for allowing us to use some of their facilities. Financial support of this work by the U.S. Public Health Service (NIH Grant GM 18852) and the American Cancer Society is gratefully acknowledged. T.M.Z. is the recipient of a postdoctoral fellowship from the National Cancer Institute.

Registry No. Propane-1,3-dithiol, 109-80-8; 3,5-dimethoxybenzaldehyde, 7311-34-4; 2-(3,5-dimethoxyphenyl)-1,3-dithiane, 57009-72-0; 2-(3,5-dimethoxyphenyl)[2-^{2}H]-1,3-dithiane, 86728-49-6; 3,5-dimethoxy[7-^{2}H]benzaldehyde, 86728-50-9; 3,5-dimethoxy-(7*S*)-[7-^{2}H]benzyl alcohol, 86728-51-0; (+)-α-pinanyl-9-borabicyclo[3.3.1]nonane, 64106-79-2; 3,5-dimethoxy-(7*S*)-[7-^{2}H]-benzyl alcohol (-)-camphanate, 86728-52-1; (+)-α-(pinan[2-^{2}H]-3-yl)-9-borabicyclo[3.3.1]nonane, 70738-23-7; 3,5-dimethoxy-(7*S*)-[7-^{2}H]benzyl tosylate, 86747-48-0; 3,5-dimethoxy-(7*S*)-[7-^{2}H,7-^{3}H]toluene, 86728-53-2; 3,5-dimethoxy-(7*R*)-[7-^{2}H,7-^{3}H]toluene, 86728-54-3; 3,5-dimethoxy-(7*R*)-[7-^{2}H]benzyl tosylate, 86747-49-1; sodium (*S*)-[2-^{2}H,2-^{3}H]acetate, 62678-90-4; sodium (*R*)-[2-^{2}H,2-^{3}H]acetate, 62678-94-8.

(18) Klein, H.; Steinmetz, H. *Tetrahedron Lett.* **1975**, 4249–4250.

Marine Toxins of *Latrunculia magnifica*

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Latrunculins A (1) and B (2) are the major extractable toxins of *Latrunculia magnifica*, constituting up to 0.35% of the dry weight of the sponge. The structure of the compounds was determined by detailed spectral analysis, some minor chemical transformations, and X-ray crystallography. The latrunculins are the first-known 2-thiazolidinone-bearing marine macrolides. They were found to cause major alterations in specific cytoskeletal proteins. A retrosynthesis of the new compounds is suggested.

Among the shallow-water coral reefs of the northern Red Sea, many sponge species grow below or beneath coral plates and rocks and, if artificially exposed, are immediately devoured by various fish. Only a few sponge species grow exposed, and among them, the most prominent are colonies of the branching red-colored *Latrunculia magnifica* (Keller). Colonies of *L. magnifica* have never been observed to be damaged or eaten by fish.[1] When squeezed manually, these sponges exude a reddish fluid accompanied by a strong odor. In the sea this "juice" causes fish to flee immediately. Squeezing *L. magnifica* into an aquarium

(1) I. Neeman, L. Fishelson, and Y. Kashman, *Mar. Biol. (Berlin)*, **30**, 293 (1975).

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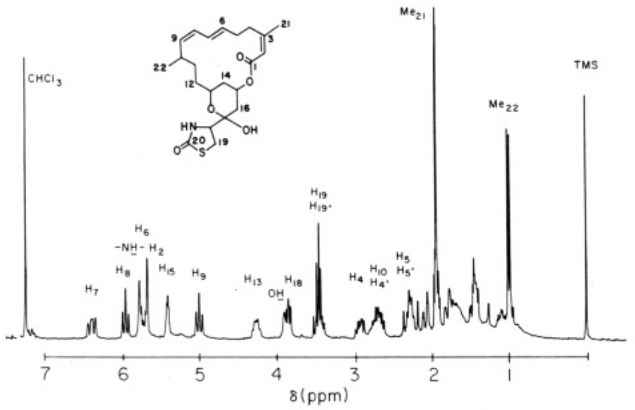


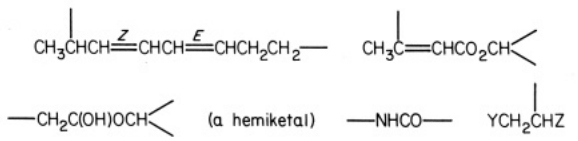
Figure 1. 270-MHz ^1H NMR spectrum of latrunculin A (1).

with fish causes poisoning and death within minutes. The toxic component causes excitation of the fish in seconds, followed by hemorrhage, loss of balance, and, finally, after a few minutes, death.[1] Similar hemorrhages could also be observed in cat and mice upon injection of the red "juice" or the pure toxin.[2] Like other secondary metabolites that were isolated from various marine organisms and are conceived to function, at least in part, in the defense mechanism of these marine organisms, we assume that the *Latrunculia* toxins play a similar role in sponge protection.

It was the toxicity to fish that served to monitor the isolation and purification of the toxins.[1,3] Toxicity was demonstrated in the petroleum ether extract of the lyophilized sponge as well as in more polar extracts (CH_2Cl_2 and CH_3OH). Repeated silica gel and Sephadex LH-20 chromatography of the crude extract resulted in the purification of two toxins, designated latrunculin A (1) and B (2). [The structure of a third toxin, discovered only in one extract in minute amounts, is as yet uncertain.] The relative amounts of the toxins vary remarkably.

About 20 different animal collections were performed between 1977 and 1981 in different spots of the Gulf of Eilat (mainly) and the Gulf of Suez. It was soon realized that all the collections from the Gulf of Eilat contained only latrunculin B (2), while the sponges collected near Sharm El-Sheikh and in the Gulf of Suez contained only latrunculin A (1) (except for two collections where both compounds exist). This phenomenon is presently being investigated.

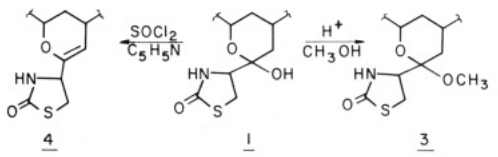
Latrunculin A [$C_{22}H_{31}NO_5S$ (MS and elemental analysis), λ_{max} 218 nm (ϵ 23 000),[5] eight unsaturations] possesses according to the ^{13}C and ^1H NMR spectra (Tables I and II and Figure 1) the following moieties:



(Y and Z electron-withdrawing groups) and three CH_2 groups. These assignments accounted for five out of the eight unsaturations for the molecule, so it was concluded that the molecule must contain three rings.

As will be seen below, the first two fragments are parts of a 16-membered macrolide found in the X-ray study, while the third moiety is consistent with a THP ring. Finally, the last two fragments above will be seen to conform with a 2-thiazolidinone system found in the crystallographic analysis.

Scheme I. Reactions of Latrunculin A



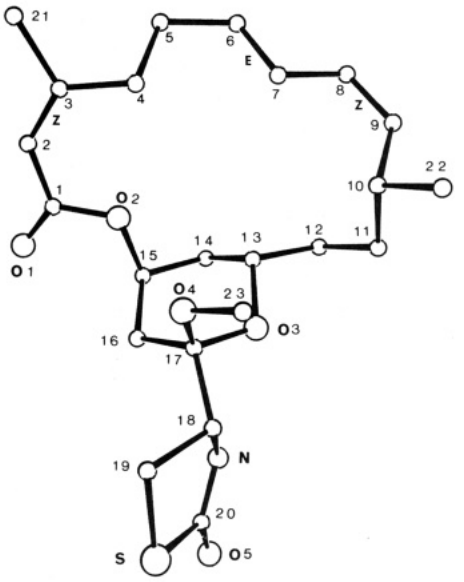


Figure 2. Computer-generated ORTEP drawing of compound 3.

Structure 1 was determined for latrunculin A by a single-crystal X-ray study of one of its derivatives. Latrunculin A itself was obtained as a foam and latrunculin B gave very poor crystals that were not amenable to X-ray analysis. Fortunately, we succeeded in preparing medium-quality crystals of another derivative, 3, which was obtained after treatment of compound 1 with 2% H_2SO_4 in CH_3OH. In this reaction the hemiketal function was converted into a ketal, with a methoxy group (see Scheme I and Table I).

The structure of 3 was solved by direct methods and could be refined to a moderate accuracy (R = 0.084). The location of all the hydrogen atoms was found to be rather difficult, and since the intensities of the monitoring reflections decreased to about 80% of their initial values and an appropriate correction factor was applied to all the data, no attempts were made at refining the structure any further. However, the bond distances and angles within the heavy atoms skeleton show a fair agreement with those reported in the literature for similar structural fragments, and, therefore, the structure of compound 3 is correct as far as its conformation and relative configuration are concerned. An ORTEP drawing of the molecule is shown in Figure 2, and all its conventional structural parameters have been deposited as supplementary material (see below). As could be seen from the ORTEP drawing of 3 and from bond distances and dihedral angles, and as anticipated also from the NMR spectra, the geometry of the three double bonds is 2, 3*Z*; 6, 7*E*; and 8, 9*Z*. The methyl attached to the Δ^2 double bond appears at δ 24.7 in the ^{13}C NMR, which is indicative of a *Z* configuration, in contrast to δ 15–18 (due to a γ-effect) measured in the case of a methyl attached to an *E* double bond. Concerning the diene configuration, the $^3J_{H,H}$ coupling constants were most

(2) I. Spector, N. R. Shochet, Y. Kashman, and A. Groweiss, *Science (Washington, D.C.)*, **4584**, 493 (1983).
(3) Y. Kashman, A. Groweiss, and U. Shmueli, *Tetrahedron Lett.* **21**, 3629 (1980).
(4) J. A. Maclaren, *Aust. J. Chem.* **21**, 1891 (1968).
(5) The large ϵ value points to overlapping of a diene with another chromophore which is most likely responsible for the shoulder at λ_{max} 268 nm; compare also with the UV spectrum of compound 2.

Table I. ^{13}C NMR of Latrunculins and Derivatives[a]

carbon no.		1 δ (J, Hz)	1 [T_1, s]	δ 3	δ 4	δ 2[c]
1	s	166.0[f]		166.4	166.4	165.6
2	d	117.6 (160)	[0.52]	118.3	118.0	118.0
3	s	158.3		157.7	157.5	154.7
4	t	32.7	[0.40]	32.2[d]	32.9[d]	35.8[d]
5	t	30.6	[0.48]	30.7	31.1[d]	26.9[d]
6	d	131.8 (151)	[0.51]	132.2	132.4	
7	d	126.3 (149)	[0.52]	125.0	126.0	
8	d	127.3 (154)	[0.53]	127.7	127.8	127.6
9	d	136.5 (153)	[0.50]	135.7	136.2	135.9
10	d	29.2	[0.52]	29.6	29.4	28.9
11	t	31.8[d]	[0.29][e]	31.4[d]	31.4[d]	31.2[d]
12	t	31.2[d]	[0.32][e]	31.1[d]	31.2[d]	31.2[d]
13	d	62.3 (143)	[0.43]	63.1	64.2	62.6
14	t	35.1[d]	[0.24][e]	35.2[d]	34.6[d]	35.4[d]
15	d	68.1 (153)	[0.48]	66.7	70.8	68.7
16	t	32.1[d]	[0.27][e]	29.1[d]	96.7 d	31.8[d]
17	s	96.9		99.9	156.2	97.7
18	d	62.1 (145)	[0.53]	56.7	56.2	61.8
19	t	28.7	[0.38]	28.0	32.9[d]	28.7[d]
20	s	175.5[f]		174.6	174.8	175.3
21	q	24.7 (126)	[>1]	25.0	24.9	24.1
22	q	21.8 (126)	[0.57]	21.6	21.7	22.3
23	q			47.9		

[a] Compound 1 was measured at 75.46 MHz in $CDCl_3$ solution and 2-4 at 22.63 MHz. Chemical shifts are given in δ values downfield from internal Me_4Si; J values are quoted in hertz; $\Delta T_1 = \pm 0.02$ s; ^{13}C NMR gated decoupling experiments and extensive SFORD studies enabled us to intercorrelate the chemical shift of the carbons of 1 with those of the protons. [b] s = singlet, d = doublet, t = triplet, q = quartet. [c] Numbers are given according to the structure of 1. [d] These methylene signals may be interchanged. [e] These signals may be interchanged. [f] These signals were assigned after a suitable 2-thiazolidinone model was prepared.[4]

significant. Thus, the protons attached to Δ^6 exhibited a mutual coupling constant of 15 Hz while in Δ^8 it was found to be 10.5 Hz, confirming the *E* and *Z* configuration, respectively.

Proton chemical shifts and the correlations deduced from extensive double-resonance irradiations (difference-decoupling experiments) enabled us to assign most signals in the ^1H NMR spectrum (see Figure 1 and Table II). As mentioned above, the ^1H and the ^{13}C NMR spectra (Tables I and II) are in full agreement with the structure determined by the X-ray analysis and in addition give information on the conformation and internal motions of the molecule.

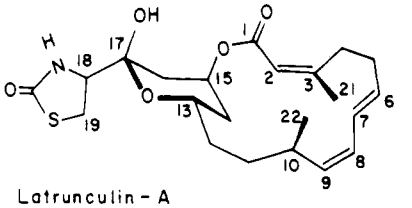
H-13 and H-15 were found, in the solid state, to be axial and equatorial respectively. This finding is consistent with the results of the ^1H NMR study (Table II), thus pointing to the same conformation of the tetrahydropyrane ring in both solution and solid states (Chart I). This observation is of particular importance for the C-17 configuration assignment in compound 1. Ketalization of the hemiketal is expected to go through the oxonium ion, and thus C-17 loses its original configuration. However, as the 2-thiazolidinone group (the largest substituent of the tetrahydropyrane ring) of compound 3 is equatorial in both liquid and solid states and as the H-13 and H-15 multiplicities in 1 are essentially the same as in 3 (Table II and Experimental Section), the configuration of C-17 is also expected to be the same in both compounds.

From the X-ray study it could also be concluded that the rare 2-thiazolidinone ring, according to solid-state best plane calculations, is in an envelope-like conformation, with the CH_2 group (C-19) bent substantially out of a plane passed through the other atoms.

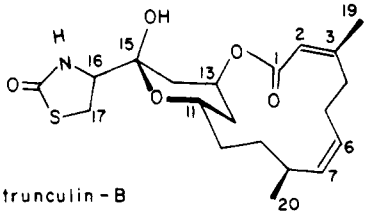
For the ^{13}C line assignment and the study of the molecule's flexibility, we undertook the ^{13}C relaxation time measurements.

The T_1 values of the various methyls, methylenes, and methines were measured by the inversion–recovery method. Our main interest centered on the differences in relaxation times between the macrolide carbons and those belonging to the smaller rings. Thus, for example, the relaxation times (T_1) of the vinylic carbons C-2, C-6, to C-9 and the saturated methine C-10, all of which belong to the macrolide ring, were ca. 0.50–0.53 s while those of C-13 and C-15 (0.43 and 0.48 s, respectively) were somehow shorter. This and other measurements concerning the CH_2 carbons (Table I) are consistent with a moderately faster internal motion of the macrolide, as compared to the motion of the other ring systems present.

Chart I. Relative Configuration of 1 and 2



1 Latrunculin - A



2 Latrunculin - B

Latrunculin A and its derivatives showed several simple fragmentation patterns in their mass spectra. Thus, for example, the 14-eV spectrum of compound 1 showed only four significant peaks: *m/e* 421 (the parent peak, 20% in intensity), 403 ($M^+ - H_2O$, 47), 385 ($M^+ - 2H_2O$, 46, the origin of the second molecule of water is not well under-

Table II. ^{1}H NMR Data of Latrunculin A and B[a]

H	δ		coupled bands
		1	
7	6.41	dd (15, 10.5)	5.98, 5.74
8	5.98	t (10.5)	6.41, 5.02
NH	5.80	br s (ex with D_2O)	
6	5.74	dt (15, 4.5)[b]	6.41, 2.26
2	5.69	d (1.3)	1.92
15	5.43	br t (3)	1.35-1.50, 1.80
9	5.02	t (10.5)	5.98, 2.83
13	4.29	mu	1.35-1.50
OH	3.93	s (ex with D_2O)	
18	3.87	dd (8, 7)	3.51, 3.48
19	3.51	dd (11.5, 7)	3.87, 3.48
19′	3.48	dd (11.5, 8)	3.87, 3.51
4	3.00	dt (13, 8)	2.60, 2.26
10	2.83	mu	5.02, 0.98
4′	2.60	dt (13, 8)[c]	3.00, 2.26
5, 5′	2.26	mu	5.02, 3.00, 2.60
21	1.92	d (1.3)	5.69
22	0.98	d (6.7)	2.83
		2	
NH	6.10	br s (ex with D_2O)	1.90
2	5.68	d (1.5)	1.5-1.8
13	5.43	br t (3)	5.04, 2.38
6	5.25	br t (3.3, 11.2)	5.25, 2.71
7	5.04	t (11.2)	
11	4.24	br t (10)	
OH	3.90	br s	3.47, 3.39
16	3.83	dd (8.6, 6.3)	3.83, 3.39
17	3.47	dd (11.5, 8.6)	3.83, 3.47
17′	3.39	dd (11.5, 6.3)	5.04, 2.38, 0.95
4, 8	2.71	mu	5.25, 2.71
5, 5′	2.38	mu	5.68
19	1.90	d (1.5)	2.71
20	0.95	d (6.6)	

[a] Measured at 270 MHz in $CDCl_3$. Chemical shifts are reported in δ values downfield from internal Me_4Si. Coupling constants are given in hertz. All abbreviations are the same as those in Table I. mu = multiplet unresolved, br = broad, ex = exchangeable. [b] This signal, partially obscured by the NH, could be seen after D_2O addition. [c] Analyzed in a difference double resonance irradiation experiment.

stood), and 301 ($M^+ - H_2O - C_3H_4NOS$, 100). This base peak originated from the strong cleavage of the 2-thiazolidinone moiety (analyzed by HRMS). Very intense peaks due to loss of *m/e* 102 mass units (C_3H_4NOS) were also observed in the spectra of latrunculin B (**2**) and several derivatives of **1** (e.g., **3** and **4**).

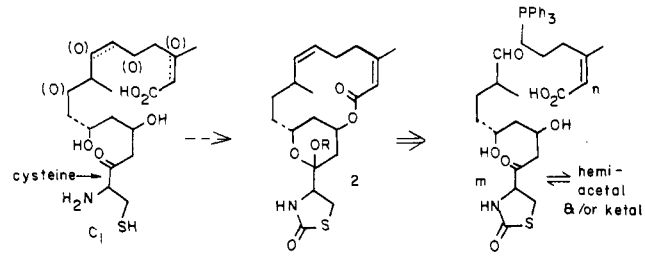
Water elimination from compound **1** ($SOCl_2$/pyridine, room temperature) afforded compound **4** (Scheme I). In the ^{13}C NMR spectrum of **4**, the original hemiketal carbon and one of the saturated methylenes vanished, while two new resonance lines (δ 156.2 s and 96.7 d) were observed. The latter two lines are in good agreement with a vinyl ether (the ^{1}H NMR exhibited a new vinyl proton at δ 5.22 (doublet) which is coupled to the methine at δ 5.19, formerly at 5.43 in compound **1**).

Microozonolysis of latrunculin A (**1**), performed in order to degrade the molecule for the purpose of determining its absolute configuration (in progress), afforded levulinaldehyde. The same aldehyde was also obtained from latrunculin B (see below).

The second toxin that was isolated from *L. magnifica* is close in structure as well as biological activities to compound **1**.

Latrunculin B [$C_{20}H_{29}NO_5S$, (from MS and elemental analysis)] revealed only 20 carbon atoms in its ^{13}C NMR spectrum, among which only 4 had chemical shifts between δ 160–118, in comparison to 6 such atoms in compound **1**.

Scheme II. Retrosynthesis and Biogenesis of Latrunculin B (**2**)



Furthermore, the ^{1}H NMR disclosed the absence of olefinic signals downfield from δ 5.7, suggesting that the diene fragment of **1** was replaced in **2** by a disubstituted monoene. The chemical shifts of the two hydrogen atoms (H-6 and -7) were found to be δ 5.25 and 5.04, respectively, with a mutual coupling constant of 11.2 Hz, which points to a *Z* configuration. Microozonolysis of **2**, which gave levulinaldehyde, confirmed the 6,7 position of the unconjugated double bond. All the rest of the spectral properties, including the ^{1}H NMR (Table II) and ^{13}C NMR (Table I), are essentially the same as those found for latrunculin A. Substituting the diene of **1** by a single double bond reduces the macrocycle of **2** to a 14-membered ring.

To the best of our knowledge, the latrunculins are the first marine macrolides and the first natural products possessing the 2-thiazolidinone heterocycle.

The latrunculins most likely belong to the polyketides, and it is suggested here that their biosynthesis starts from cysteine (Scheme II). Cysteine is also proposed by us as a starting material for compound m, one of the two major pieces that, by coupling, can construct latrunculin B (Scheme II).

Latrunculin A and B have been evaluated with regard to their effects on cultured mouse neuroblastoma and fibroblast cells. In both types of cells, submicromolar toxin concentrations (as low as 50 ng/mL) rapidly induce striking changes in cell morphology that are reversible upon removal of the toxin. The toxins cause major alterations in specific cytoskeletal proteins but lacked neurotoxic properties.[2] The latrunculins represent a new class of highly potent compounds that disrupt microfilament organisation in cells.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Ultraviolet spectra were recorded on a Varian Cary 219 spectrophotometer in methanol solutions. Optical rotations were measured with a Bellingham and Stanley polarimeter in $CHCl_3$ solutions. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were taken with DuPont 21-491B instrument. Parent peaks of the compounds were analyzed on a HRMS Varian Mat 731 instrument. ^{13}C NMR were measured with Bruker WH-300 (75.46 MHz) and Bruker WH-90 (22.63 MHz) spectrometers in $CDCl_3$ or C_6D_6 solutions. ^{1}H NMR spectra were recorded, unless stated otherwise, on a Bruker WH-270 spectrometer. Chemical shifts are reported in δ values downfield from internal Me_4Si, and the coupling constants are quoted in hertz. All solvents used were either spectral grade or freshly distilled.

Collection, Extraction, and Isolation of the Lactrunculins. The sponge specimens were collected at depths of 6–30 m in several spots in the Gulf of Eilat and the Gulf of Suez. Each specimen was freeze-dried and then ground and extracted with petroleum ether in a Soxhlet apparatus for 24–48 h. The crude extract, after evaporation, was chromatographed in batches of 2–4 g on a LH-20 column (prepared and eluted with 2:1:1 petroleum ether–$CHCl_3$–CH_3OH). The latrunculin-containing fractions were combined and rechromatographed on a silica gel–H

Table III. Experimental Data for the X-ray Diffraction Studies

A. Crystal Parameters	
unit cell dimensions	a = 11.396 (1) Å
	c = 36.735 (3) Å
space group	tetragonal, $P4_122$ (z = 8)
d_{obsd}	1.19 g cm^{-3}
d_{calcd}	1.21 g cm^{-3}
M_r	435
B. Data Collection	
wavelength used	λ = 1.5418 Å
no. of observations (Inet > 0)	4137
no. of independent reflections	2464
R factor for symmetry related reflections	0.052
no. of independent reflections with $I > 3\sigma$ (obsd)	1421
angular range	$2° \leq \theta \leq 70°$
crystal scan range	1.2 + 0.15 tan θ (deg)
horizontal aperture	3.5 + 0.5 tan θ (mm)
C. Structure Determination	
phase problem	direct methods (MULTAN78)
refinement	least squares (ORFLS - local version)
final R factor	0.084

column. Elution with 40–50% ethyl acetate in petroleum ether afforded pure compounds.

Latrunculin A (1): $[\alpha]^{24}_D$ +152° (c 1.2, $CHCl_3$); UV (CH_3OH) 218 (23 500), 268 nm (sh); IR ($CHCl_3$) 3570, 3430, 3350, 2960, 2930, 1690 (strong), 1495, 1455, 1440, 1380, 1360, 1270, 1220, 1140, 1095, 1065, 990, 825 cm^{-1}; mass spectrum (14 eV), m/e (relative intensity) 421 (20), 403 (47), 385 (46), 301 (100), 149 (48), 135 (50), 102 (10), 85 (44); ^{1}H NMR, see Figure 1 and Table II; ^{13}C NMR, see Table I.

Latrunculin B (2): $[\alpha]^{24}_D$ +112° (c 0.48, $CHCl_3$); UV (CH_3OH) 212 (17 250), 269 nm (sh); IR ($CHCl_3$) 3520, 3400, 2910, 1675, 1205, 1115, 1085, 1045, 987, 975, 960 cm^{-1}; mass spectrum (14 eV), m/e (relative intensity) 377 (50), 359 (27), 326 (16), 275 (40), 256 (16), 149 (52), 128 (63), 109 (68), 95 (65), 81 (87), 71 (72), 69 (86), 57 (100); ^{1}H NMR, see Table II; ^{13}C NMR, see Table I.

Methylation of Hemiketal 1 To Give 3. A sample of 200 mg of 1 was dissolved in 10 mL of CH_3OH, cooled to 0 °C, and treated with 3 mL of 10% H_2SO_4 in CH_3OH during 30 min. The mixture was warmed to room temperature, and stirred for 2 h and was neutralized with $NaHCO_3$ solution; the solvent was removed, and the aqueous residue was extracted with CH_2Cl_2, dried ($MgSO_4$), and evaporated to give an oily material. Chromatography over a silica-H short column afforded 120 mg of pure 3, which was crystallized from benzene or CCl_4: mp 164–165 °C (C_6H_6); $[\alpha]^{24}_D$ +315° (c 0.33, $CHCl_3$); UV (CH_3OH) 218 (25 800), 269 nm (sh); IR (CCl_4) 3420, 3200, 2915, 1690, 1680, 1450, 1375, 1275, 1185, 1155, 1090, 1045, 977, 950, 930, 870 cm^{-1}; mass spectrum (70 eV), m/e (relative intensity) 403 (52), 385 (43), 377 (22), 333 (66), 180 ($C_{10}H_{14}NO_2$, 100), 121 (29), 110 (35), 107 (40), 93 (55), 81 (51); ^{1}H NMR δ 6.37 dd (J = 15, 11 Hz), 6.04 t (J = 11 Hz), 5.81 dt (J = 15, 5 Hz), 5.65 d (J = 1.2 Hz), 5.61 br s (NH), 5.17 m, 5.00 t (J = 11 Hz), 4.15 t (J = 7 Hz), 4.15 m, 3.42 m, 3.33 s (OCH_3), 3.34, 3.31 (unresolved multiplets, 2 H), 1.92 d (J = 1.2 Hz, 3 H), 1.01 d (J = 6.5 Hz, 3 H); ^{13}C NMR, see Table I.

Dehydration of Hemiketal 1 To Give Vinyl Ether 4. A solution of 90 mg of 1 in pyridine (2 mL) was cooled to 0 °C. A few drops of redistilled $SOCl_2$ were added, and the solution was stirred for 20 min. The organic material was extracted with ether, neutralized with dilute HCl solution, washed with $NaHCO_3$ then water, and dried ($MgSO_4$). The solvent was removed and the oily residue was chromatographed on a short silica-H column to give ca. 50 mg of pure 4: IR ($CHCl_3$) 3380, 2900, 1680, 1435, 1380, 1270, 990, 965, 910, 860 cm^{-1}: mass spectrum (12 eV) m/e (relative intensity) 403 (M^+, $C_{22}H_{29}NO_4S$); ^{1}H NMR δ 6.35 dd (J = 14.5, 10.6 Hz), 6.04 t (J = 10.6 Hz), 5.83 br s (NH), 5.76 dt (J = 14.5, 5.9 Hz), 5.63 d (J = 1.5 Hz), 5.22 br d (J = 5 Hz), 5.19 br t (J = 5 Hz), 5.01 t (J = 10.6 Hz), 4.35 dd (J = 7.9, 6.3 Hz), 4.06 br t (J = 12 Hz), 3.58 dd (J = 10.9, 7.9 Hz), 3.44 dd (J = 10.9, 6.3 Hz), 1.90 d (J = 1.5 Hz, 3 H), 1.04 d (J = 6.3 Hz, 3 H); ^{13}C NMR, see Table I.

X-ray Study of Compound 3. A crystal of compound 3 (from benzene) with approximate linear dimensions 0.2 × 0.2 × 0.4 mm was chosen for the structural study. The X-ray diffraction experiments were performed with the aid of a CAD4F diffractometer system using Ni-filtered copper radiation. Preliminary values of unit cell dimensions were obtained with the aid of an automatic search procedure, as well as from a Polaroid rotation photograph, and the final values to be used in intensity data collection were secured by carefully recentering 25 reflections that were selected from a fast run over the angular range $15° < \theta < 20°$. The crystal data, the data collection parameters, and a summary of the structure determination and refinement are presented in Table III. It must be pointed out that the intensitives of the monitoring reflections underwent a nearly linear decay to about 80% of their initial values and an appropriate correction factor was implied to all the data. The integrated intensities were not corrected for absorption or extinction.

After some false starts, an E map, based on phases that were obtained with the aid of the MULTAN 78 system,[6] revealed most of the non H atoms in compound 3, and the remaining ones were located in weighted Fourier syntheses computed with the SHELX program.[7]

The above trial structure was refined with the aid of a local modification of program ORFLS.[8] The least-squares refinement proceeded smoothly through the overall isotropic, individual isotropic, and anisotropic stages and was terminated with a few cycles of full-matrix anisotropic refinement of the non H part of the molecule. The maximum density in a final difference electron density synthesis was 0.35 e Å^{-3}.

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Supplementary Material Available: Additional X-ray crystallographic data for compound 3 and tables of positional and thermal parameters, bond distances and angles, and dihedral angles (5 pages). Ordering information is given on any current masthead page.

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(8) W. R. Busing, K. O. Martin, and H. A. Levy, ORFLS Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, TN, 1962.