Ten-Membered Substituted Cyclic 2-Oxecanone (Decalactone) Derivatives from *Latrunculia corticata*, a Red Sea Sponge

Tomáš Řezanka*[a] and Valery M. Dembitsky[a]

Keywords: Glycosides / Lactones / Natural products / Marine sponges

Two novel glycosides, latrunculinosides A and B, containing substituted 2-oxecanones (decalactones) were isolated from *Latrunculia corticata* collected in the Gulf of Aqaba, Israel. They were characterized by spectroscopic methods, predominantly ^{1}H NMR, ^{13}C NMR, MS, IR and UV, and by chemical degradation. Both glycosides contain unusual saccharides such as β-D-olivose, β-L-digitoxose, α-L-amicetose, and β-D-oliose. The compounds gave positive results in antifeeding activity assays.

Introduction

Marine sponges belonging to the class Demospongiae have been the subject of extensive chemical and biological investigations. Numerous bioactive peptides have been isolated and identified in sponges,[1] and the continued interest of chemists in these marine organisms has yielded a large number of non-nitrogen-containing metabolites.[2] Sponges have also proven to be a rich source of a broad spectrum of natural products containing macrolactones, with fascinating structures and different biological activities.[3−5] The first compounds in this group were halicholactone and neohalicholactone, fatty acid metabolites found in the sponge *Halichondria okadai*, collected off the coast of Japan.[6,7] Similar compounds, didemnilactones A and B or neodidemnilactone,[8,9] were isolated from the colonial *Didemnum moseleyi* near the Japanese Islands and asciditrienolide A[10] from a marine tunicate (ascidian) *Didemnum candidum*. The correct structure of asciditrienolide was reported a few years later.[11]

Here we report the isolation of the ten-membered ring lactonic glycosides latrunculinosides A and B from the sponge *Latrunculia corticata*. These compounds were assigned the structures **1** and **10** on the basis of chemical and spectroscopic methods.

Results and Discussion

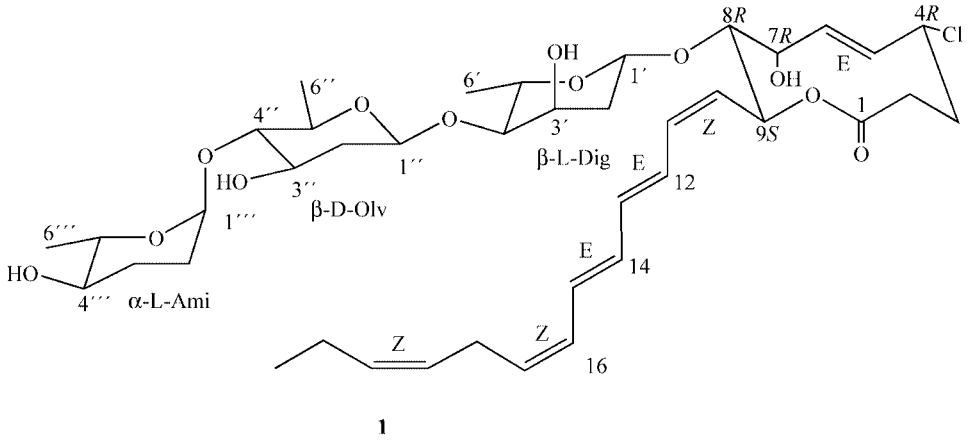
In the course of our program devoted to the search for new compounds in marine animals[12,13] we found that extracts from *Latrunculia corticata* collected in the Gulf of Aqaba, Israel, exhibited considerable biological activity. The sponges were extracted with butanol and the extract was separated on Sephadex LH-20. The fractions were further purified by RP-HPLC to give two glycosides (**1** and **10** − see Figure 1), which were identified by their IR, UV, MS, and ^{1}H and ^{13}C NMR spectroscopic data and by chemical degradation.

Compound **1** (latrunculinoside A) had the molecular formula $C_{40}H_{59}ClO_{12}$, established by HRFABMS (*m*/*z* = 767.3776 $[M + H]^+$ Δ 3.0 mmu). Negative FABMS gave four prominent ions at *m*/*z* = 765 $[M - H]^-$, *m*/*z* = 651 $[M - H - 114]^-$, *m*/*z* = 521 $[M - H - 114 - 130]^-$, and *m*/*z* = 391 $[M - H - 114 - 2 \times 130]^-$, the last three corresponding to the loss of one trideoxyhexosyl and two dideoxyhexosyl units, respectively. Infrared absorptions at 3550 and 1725 cm^{-1} revealed the presence of hydroxy and carbonyl functionalities, respectively. The UV spectrum indicated the presence of a conjugated tetrane (λ_{max} = 307 nm, log ε = 4.82),[14] as was also supported by the NMR spectroscopic data. Fortunately, the ^{1}H NMR spectra measured in a CD_3OD/[D_6]DMSO mixture displayed well separated signals, which enabled us to determine the gross structure of **1**. Interpretation of the COSY and HMBC, together with the HOHAHA and HMQC spectra, is described below.

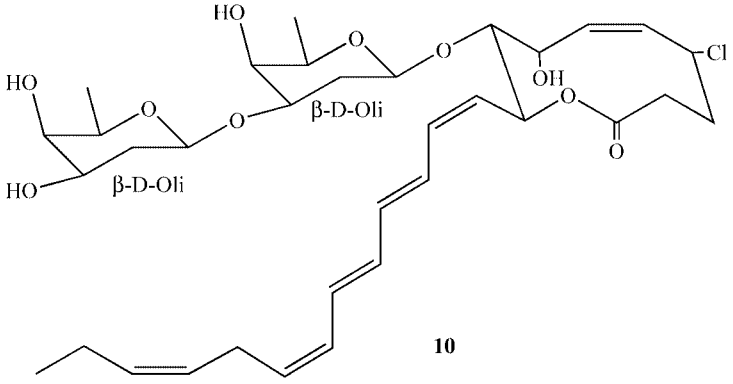
The glycoside was enzymatically hydrolyzed (Figure 2), and the aglycon latrunculin A (**2**) was isolated from the reaction mixture. The molecular formula of **2** was determined by HRFABMS as $C_{22}H_{29}ClO_4$ ($[M + H]^+$ at *m*/*z* = 393.1830). The LREIMS of **2** showed molecular ions at *m*/*z* = 392 and 394 in a ratio of about 3:1, indicating the presence of one chlorine atom in **2**. The side chain showed a UV spectrum suggesting the presence of a conjugated tetraene system (as α-parinaric acid; λ_{max} = 313, log ε = 4.94), as was also implied by the proton and carbon signals

[a] Institute of Microbiology,
Vídeňská 1083, Prague, 14220, Czech Republic
Fax: (internat.) +420-241-062347
E-mail: rezanka@biomed.cas.cz
[b] Department of Pharmaceutical Chemistry and Natural Products, School of Pharmacy, Hebrew University of Jerusalem,
P.O. Box 12065, Jerusalem 91190, Israel

  DOI: 10.1002/ejoc.200300012



1



10

Figure 1. Structure of new glucosides (latrunculinosides A and B; **1** and **10**) from *Latrunculia corticata*, a Red Sea sponge

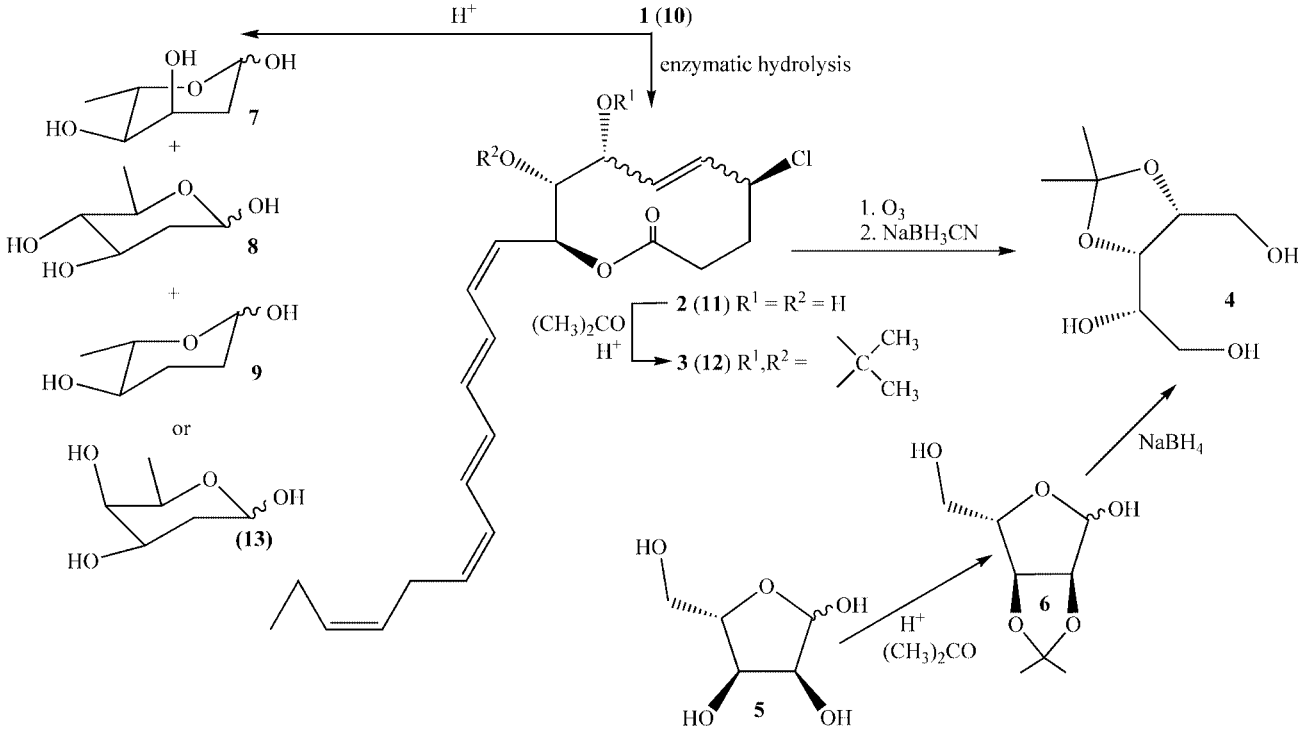


Figure 2. Reaction schemes for degradation compounds from glycoside **1** (**10**) and synthesis of standard 2,3-*O*-isopropylidene-L-ribitol (**4**)

(see Table 1, Table 2 and Table 3). The ^{13}C NMR spectrum contained the expected 22 signals with 27 attached protons, which required that there be two hydroxy groups in compound **2**. The ^{13}C NMR spectrum contained signals due to a lactone carbonyl at δ = 172.3 ppm and six olefinic bonds. Since the molecular formula requires eight unsaturation equivalents, compound **2** must be monocyclic.

Chemical shifts indicated that four methine carbons (C-4, C-7, C-8, and C-9) resonating between δ = 65.9 and δ = 79.3 ppm are attached to heteroatoms and the quaternary carbon C-1 belongs to an ester-type carbonyl group. Analysis of the corresponding ^1H NMR spectrum revealed that the remaining methine protons resonating between δ = 5.36 ppm and δ = 6.91 ppm are part of the (*Z*,*E*,*E*,*Z*) conjugated tetraene, while two isolated (*E*,*Z*) double bonds were shown to be present in the molecule of aglycon **2**.

The presence of two hydroxy groups attached at C-7 and C-8 in **2** followed from the formation of the acetonide **3**, with the characteristic downfield shifts experienced by the signals assigned to 7-H and 8-H (Δδ = 0.60 and 0.67 ppm, respectively).

Further analysis of the COSY spectrum allowed us to trace the ^1H-^1H couplings of a moiety from 2-H_2 to the 22-H_3 methyl group, and these assignments were confirmed by the HMQC and HMBC data .

The relative stereochemistry was inferred from the large couplings between α 9-H and 10-H (14.0 Hz), and between α 5-H and β 4-H (14.0 Hz) and 6-H (15.3 Hz). These coup-

Table 1. ^1H NMR data for latrunculinosides A and B (**1** and **10**)

No.	**1**	**10**
2α	2.25 (ddd, *J* = 11.9, 3.5, 3.1 Hz, 1 H)	2.27 (m, 1 H)
2β	2.07 (ddd, *J* = 11.9, 14.8, 4.8 Hz, 1 H)	2.09 (m, 1 H)
3α	2.20 (dddd, *J* = 10.8, 4.8, 3.5, 2.5, 1 H)	2.08 (m, 2 H)
3β	1.96 (dddd, *J* = 14.8, 13.5, 10.8, 3.1, 1 H)	
4	3.95 (dddd, *J* = 14.5, 13.5, 2.5, 1.1, 1 H)	4.15 (ddd, *J* = 14.6, 13.1, 2.4 Hz, 1 H)
5	5.73 (dd, *J* = 14.5, 15.8 Hz, 1 H)	5.93 (dd, *J* = 14.5, 10.6 Hz, 1 H)
6	5.82 (ddd, *J* = 15.8, 4.4, 1.1 Hz, 1 H)	5.87 (dd, *J* = 10.6, 4.5 Hz, 1 H)
7	4.07 (dd, *J* = 4.4, 3.2 Hz, 1 H)	4.27 (dd, *J* = 4.5, 3.1 Hz, 1 H)
8	4.38 (dd, *J* = 3.2, 14.6 Hz, 1 H)	3.88 (dd, *J* = 3.1, 14.6 Hz, 1 H)
9	5.58 (dd, *J* = 14.6, 14.1 Hz, 1 H)	5.08 (dd, *J* = 14.6, 14.2 Hz, 1 H)
10	5.44 (dd, *J* = 14.1, 12.3 Hz, 1 H)	5.46 (dd, *J* = 14.2, 12.2 Hz, 1 H)
11	6.37 (dd, *J* = 12.3, 11.5 Hz, 1 H)	6.06 (dd, *J* = 12.2, 11.6 Hz, 1 H)
12	6.93 (dd, *J* = 11.5, 14.7 Hz, 1 H)	6.44 (dd, *J* = 11.6, 14.8 Hz, 1 H)
13	6.38 (dd, *J* = 14.7, 11.2 Hz, 1 H)	6.40 (dd, *J* = 14.8, 11.4 Hz, 1 H)
14	6.49 (dd, *J* = 11.2, 13.8 Hz, 1 H)	6.51 (dd, *J* = 11.4, 13.9 Hz, 1 H)
15	6.51 (dd, *J* = 13.8, 11.3 Hz, 1 H)	6.57 (dd, *J* = 13.9, 11.6 Hz, 1 H)
16	6.16 (dd, *J* = 11.3, 10.8 Hz, 1 H)	6.10 (dd, *J* = 11.6, 10.4 Hz, 1 H)
17	5.66 (ddd, *J* = 10.8, 6.4, 6.4 Hz, 1 H)	5.68 (ddd, *J* = 10.4, 6.3, 6.3 Hz, 1 H)
18	2.63 (t, *J* = 6.4 Hz, 2 H)	2.64 (t, *J* = 6.3 Hz, 2 H)
19	5.43 (m, 1 H)	5.45 (m, 1 H)
20	5.37 (m, 1 H)	5.39 (1H. *m*)
21	2.00 (m, 2 H)	2.01 (m, 2 H)
22	1.06 (t, *J* = 7.6 Hz, 3 H)	1.04 (t, *J* = 7.6 Hz, 3 H)
1′	4.93 (dd, *J* = 1.7, 9.8 Hz, 1 H)	4.85 (dd, *J* = 10.0, 1.5 Hz, 1 H)
2a′	1.67 (ddd, *J* = 10.7, 9.8, 3.3 Hz, 1 H)	2.40 (ddd, 1 H, *J* = 12.5, 10.5, 10.0)
2e′	2.01 (ddd, *J* = 10.7, 2.8, 1.7 Hz, 1 H)	2.24 (ddd, *J* = 10.5, 4.1, 1.5 Hz, 1 H)
3′	3.28 (dt, *J* = 3.3, 2.8 Hz, 1 H)	3.23 (ddd, *J* = 12.5, 4.6, 4.1 Hz, 1 H)
4′	3.55 (dd, *J* = 9.4, 2.8 Hz, 1 H)	3.34 (dd, *J* = 4.8, 4.6 Hz, 1 H)
5′	3.94 (dq, *J* = 9.4, 6.4 Hz, 1 H)	3.85 (dd, *J* = 6.4, 4.8 Hz, 1 H)
6′	1.31 (d, *J* = 6.4 Hz, 3 H)	1.29 (d, *J* = 6.4 Hz, 3 H)
1′′	4.74 (dd, *J* = 11.0, 1.3 Hz, 1 H)	5.05 (dd, *J* = 10.5, 2.1 Hz, 1 H)
2a′′	1.55 (ddd, 1 H, *J* = 11.8, 11.0, 10.3)	2.65 (ddd, *J* = 10.8, 10.5, 9.2 Hz, 1 H)
2e′′	2.19 (ddd, *J* = 10.3, 4.7, 1.3 Hz, 1 H)	2.15 (ddd, *J* = 10.8, 5.2, 2.1 Hz, 1 H)
3′′	3.68 (ddd, *J* = 11.8, 8.2, 4.7 Hz, 1 H)	3.14 (ddd, *J* = 9.2, 5.2, 5.0 Hz, 1 H)
4′′	3.28 (dd, *J* = 9.7, 8.2 Hz, 1 H)	3.08 (dd, *J* = 5.0, 4.5 Hz, 1 H)
5′′	3.56 (dd, *J* = 9.7, 6.4 Hz, 1 H)	3.74 (dd, *J* = 4.5, 6.4 Hz, 1 H)
6′′	1.32 (d, *J* = 6.4 Hz, 3 H)	1.39 (d, *J* = 6.4 Hz, 3 H)
1′′′	5.02 (d, *J* = 2.4 Hz, 1 H)	–
2a′′′	2.03 (m, 1 H)	–
2e′′′	1.75 (m, 1 H)	–
3a′′′	1.92 (m, 1 H)	–
3e′′′	1.73 (m, 1 H)	–
4′′′	3.08 (ddd, *J* = 9.9, 9.5, 4.3 Hz, 1 H)	–
5′′′	3.31 (dq, *J* = 9.9, 6.4 Hz, 1 H)	–
6′′′	1.19 (d, *J* = 6.4 Hz, 3 H)	–

Table 2. ^{1}H NMR data for latrunculins A and B (**2** and **11**) and their derivatives (**3** and **12**)

No.	**2**	**3**	**11**	**12**
2α	2.25 (ddd, *J* = 11.7, 3.2, 3.0 Hz, 1 H)	2.24 (ddd, *J* = 11.9, 3.5, 3.1 Hz, 1 H)	2.27 (ddd, *J* = 11.6, 3.5, 3.2 Hz, 1 H)	2.26 (ddd, *J* = 11.5, 3.4, 3.1 Hz, 1 H)
2β	2.07 (ddd, *J* = 11.7, 14.4, 4.7 Hz, 1 H)	2.08 (ddd, *J* = 11.9, 14.8, 4.8 Hz, 1 H)	2.09 (ddd, *J* = 11.6, 14.3, 4.9 Hz, 1 H)	2.06 (ddd, *J* = 11.5, 14.7, 4.8 Hz, 1 H)
	2.20 (dddd, *J* = 10.8, 4.7, 3.2, 2.6, 1 H)	2.21 (dddd, , *J* = 10.8, 4.8, 3.5, 2.5 1 H)	2.19 (dddd, 1 H, , *J* = 10.8, 4.9, 3.5, 2.4)	2.22 (dddd, *J* = 10.8, 4.8, 3.4, 2.5, 1 H)
3β	1.96 (dddd, *J* = 14.4, 13.4, 10.8, 3.0,1 H)	1.95 (dddd, *J* = 14.8, 13.5, 10.8, 3.1, 1 H)	1.97 (dddd, 1 H, *J* = 14.3, 13.3, 10.8, 3.2)	1.98 (dddd, *J* = 14.7, 13.5, 10.8, 3.1, 1 H)
4	4.05 (ddd, *J* = 14.0, 13.4, 2.6 Hz, 1 H)	3.95 (ddd, *J* = 14.5, 13.5, 2.5 Hz, 1 H)	4.15 (ddd, *J* = 14.5, 13.3, 2.4 Hz, 1 H)	3.95 (ddd, *J* = 14.4, 13.5, 2.5 Hz, 1 H)
5	5.77 (dd, *J* = 14.0, 15.3 Hz, 1 H)	5.73 (dd, *J* = 14.5, 15.8 Hz, 1 H)	5.91 (dd, *J* = 14.5, 10.5 Hz, 1 H)	5.73 (dd, *J* = 14.4, 10.8 Hz, 1 H)
6	5.89 (dd, *J* = 15.3, 4.7 Hz, 1 H)	5.82 (dd, *J* = 15.8, 4.3 Hz, 1 H)	5.81 (dd, *J* = 10.5, 4.5 Hz, 1 H)	5.82 (dd, *J* = 10.8, 4.4 Hz, 1 H)
7	3.97 (dd, *J* = 4.7, 3.3 Hz, 1 H)	4.57 (dd, *J* = 4.3, 3.1 Hz, 1 H)	3.98 (dd, *J* = 4.5, 3.3 Hz, 1 H)	4.59 (dd, *J* = 4.4, 3.1 Hz, 1 H)
8	3.94 (dd, *J* = 3.3, 14.8 Hz, 1 H)	4.61 (dd, *J* = 3.1, 14.5 Hz, 1 H)	3.96 (dd, *J* = 3.3, 14.6 Hz, 1 H)	4.55 (dd, *J* = 3.1, 14.7 Hz, 1 H)
9	4.88 (dd, *J* = 14.8, 14.0 Hz, 1 H)	4.98 (dd, *J* = 14.5, 14.1 Hz, 1 H)	4.72 (dd, *J* = 14.6, 14.0 Hz, 1 H)	4.96 (dd, *J* = 14.7, 14.3 Hz, 1 H)
10	5.54 (dd, *J* = 14.0, 12.4 Hz, 1 H)	5.51 (dd, *J* = 14.1, 12.5 Hz, 1 H)	5.54 (dd, *J* = 14.0, 12.6 Hz, 1 H)	5.51 (dd, *J* = 14.3, 12.4 Hz, 1 H)
11	6.35 (dd, *J* = 12.4, 11.7 Hz, 1 H)	6.38 (dd, *J* = 12.5, 11.6 Hz, 1 H)	6.36 (dd, *J* = 12.6, 11.5 Hz, 1 H)	6.37 (dd, *J* = 12.4, 11.2 Hz, 1 H)
12	6.91 (dd, *J* = 11.7, 14.6 Hz, 1 H)	6.93 (dd, *J* = 11.6, 14.7 Hz, 1 H)	6.92 (dd, *J* = 11.5, 14.8 Hz, 1 H)	6.90 (dd, *J* = 11.2, 14.6 Hz, 1 H)
13	6.39 (dd, *J* = 14.6, 11.1 Hz, 1 H)	6.40 (dd, *J* = 14.7, 11.2 Hz, 1 H)	6.36 (dd, *J* = 14.8, 11.0 Hz, 1 H)	6.41 (dd, *J* = 14.6, 11.5 Hz, 1 H)
14	6.46 (dd, *J* = 11.1, 13.6 Hz, 1 H)	6.48 (dd, *J* = 11.2, 13.8 Hz, 1 H)	6.49 (dd, *J* = 11.0, 13.7 Hz, 1 H)	6.50 (dd, *J* = 11.5, 13.7 Hz, 1 H)
15	6.51 (dd, *J* = 13.6, 11.5 Hz, 1 H)	6.50 (dd, *J* = 13.8, 11.3 Hz, 1 H)	6.52 (dd, *J* = 13.7, 11.4 Hz, 1 H)	6.53 (dd, *J* = 13.7, 11.0 Hz, 1 H)
16	6.14 (dd, *J* = 11.5, 10.6 Hz, 1 H)	6.17 (dd, *J* = 11.3, 10.7 Hz, 1 H)	6.15 (dd, *J* = 11.4, 10.8 Hz, 1 H)	6.18 (dd, *J* = 11.0, 10.6 Hz, 1 H)
17	5.68 (ddd, *J* = 10.6, 6.3, 6.3 Hz, 1 H)	5.66 (ddd, *J* = 10.7, 6.2, 6.2 Hz, 1 H)	5.69 (ddd, *J* = 10.8, 6.4, 6.4 Hz, 1 H)	5.64 (ddd, *J* = 10.6, 6.5, 6.5 Hz, 1 H)
18	2.64 (t, *J* = 6.3 Hz, 2 H)	2.66 (t, *J* = 6.2 Hz, 2 H)	2.63 (t, *J* = 6.4 Hz, 2 H)	2.65 (t, *J* = 6.5 Hz, 2 H)
19	5.45 (m, 1 H)	5.43 (m, 1 H)	5.47 (m, 1 H)	5.44 (m, 1 H)
20	5.36 (m, 1 H)	5.38 (m, 1 H)	5.37 (m, 1 H)	5.39 (1H. *m*)
21	2.00 (m, 2 H)	2.01 (m, 2 H)	2.03 (m, 2 H)	2.05 (m, 2 H)
22	1.03 (t, *J* = 7.4 Hz, 3 H)	1.05 (t, *J* = 7.5 Hz, 3 H)	1.06 (t, *J* = 7.3 Hz, 3 H)	1.04 (t, *J* = 7.4 Hz, 3 H)
CH_3	–	1.40 (s, 1 H)	–	1.41 (s, 1 H)
CH_3	–	1.40 (s, 1 H)	–	1.41 (s, 1 H)

lings point to the *anti* orientations shown in Figure 3, while the small couplings exhibited by 6-H and 7-H (4.7 Hz), and also by 7-H and 8-H (3.3 Hz), are compatible with *syn* interactions. In the NOE difference spectrum of **2**, the 8-H and 4-H signals showed peak enhancements when 6-H was irradiated. When 5-H was irradiated, the 3-H and 9-H signals showed peak enhancements. Furthermore, the NOEs observed for β 4-H and 8-H upon irradiation of 6-H, as well as the NOEs observed between 9-H, 5-H, and α 3-H and 10-H, 8-H, 6-H, 4-H, and α H-2, are in agreement with the above evidence (see Figure 3).

The results from MM2 calculations and molecular modeling, together with those described above, indicated that the most stable conformation of **2** has all three hydroxy groups at C-7, C-8, and C-9 located with the same orientation (*ribo*). These findings suggest that **2**, as depicted in Figure 4, has the (4*R**,7*R**,8*R**,9*S**; i.e., relative) configuration.

The absolute stereochemistry of **2** was determined as follows. When the isopropylidene derivative **3**, which retained the asymmetric carbons (C-4, C-7, C-8, C-9) in **2**, was subjected successively to ozonolysis and $NaBH_3CN$ reduction, it afforded the isopropylidene of ribitol (**4**), $[\alpha]_D^{21} = +4.0$ ($c = 0.35$). Our ribitol (**4**) was directly compared with both its L and D epimers, prepared in our lab by the reported method,[15] and our synthesized D epimer was found to be identical in all respects (^{1}H NMR, ^{13}C NMR, and optical rotation) with the D isomer[16] derived from D-ribose. Therefore, **4** has the (7*R*,8*R*,9*S*) absolute configuration, and so **2** has the (4*R*,7*R*,8*R*,9*S*) absolute configuration, and latrun-

Table 3. ^{13}C NMR data for latrunculinosides A and B (**1** and **10**), latrunculins A and B (**2** and **11**), and their derivatives (**3** and **12**)

No.	1	2	3	10	11	12
1	172.0	172.3	172.1	172.0	172.2	172.1
2	34.7	33.0	32.9	33.4	33.0	32.8
3	25.4	28.8	29.4	26.0	29.1	29.7
4	70.3	65.9	65.6	69.0	66.8	67.1
5	129.4	129.3	129.2	129.7	129.0	129.4
6	135.9	133.4	132.3	134.1	133.2	131.7
7	73.6	75.8	79.4	72.5	74.2	77.8
8	74.8	79.3	84.7	73.5	78.7	82.3
9	71.9	71.8	72.0	72.1	70.9	71.5
10	127.0	128.7	131.0	128.2	127.9	130.9
11	134.8	132.1	128.1	133.1	132.4	129.9
12	127.5	129.9	129.7	127.5	128.7	129.0
13	134.6	134.5	134.3	134.8	133.1	133.7
14	131.2	131.4	131.5	131.7	132.6	132.4
15	134.2	134.2	134.4	133.5	133.8	133.1
16	122.5	122.4	122.2	123.1	123.0	122.7
17	125.7	125.8	125.9	126.1	125.3	125.7
18	24.4	24.3	24.0	26.2	25.1	24.6
19	124.5	124.2	124.6	124.9	124.8	124.7
20	129.9	129.4	128.5	130.4	128.7	128.5
21	20.2	20.1	20.3	20.1	20.0	20.3
22	14.0	14.2	14.1	14.2	14.2	14.1
1′	100.4	–	–	98.1	–	–
2′	39.1	–	–	32.2	–	–
3′	63.8	–	–	62.0	–	–
4′	76.5	–	–	77.6	–	–
5′	69.7	–	–	68.7	–	–
6′	19.1	–	–	14.9	–	–
1′′	99.5	–	–	97.6	–	–
2′′	37.6	–	–	34.1	–	–
3′′	70.6	–	–	64.4	–	–
4′′	80.7	–	–	79.5	–	–
5′′	71.3	–	–	68.4	–	–
6′′	17.7	–	–	14.9	–	–
1′′′	97.1	–	–	–	–	–
2′′′	25.2	–	–	–	–	–
3′′′	32.0	–	–	–	–	–
4′′′	71.4	–	–	–	–	–
5′′′	71.2	–	–	–	–	–
6′′′	18.5	–	–	–	–	–
C	–	–	101.8	–	–	101.8
CH_3	–	–	28.7	–	–	28.7
CH_3	–	–	28.7	–	–	28.7

culin A is therefore (4*R*,5*E*,7*R*,8*R*,9*S*,10*Z*,12*E*,14*E*,16*Z*,19*Z*)-4-chloro-7,8-dihydroxy-5,10,12,14,16,19-docosahexaeno-9-lactone.

The ^1H NMR and ^{13}C NMR spectroscopic data of the saccharide unit of **1** are also shown in Table 1 and Table 3. ^1H and ^{13}C NMR spectroscopic data, as well as decoupling experiments on **1**, were used to assign the signals of the monosaccharide moieties. For a dideoxyhexose, strong *J* coupling were observed between 1′-H/2a′-H and 4′-H/5′-H and weak coupling between 1′-H/2′-H_e, and 2′-H_e/3′-H and 3′-H/4′-H, while the NOE patterns were strong only between 2′-H and 4′-H. The coupling constant of the anomeric proton of sugar was $J_{1'\text{-H}-2a'\text{-H}}$ = 9.8 Hz and $J_{1'\text{-H}-2e'\text{-H}}$ = 1.7 Hz. The glycoside linkage of the sugar was thus determined to be β. These observations indicated that this monosaccharide is β-digitoxose (2,6-dideoxy-β-*ribo*-hexose).

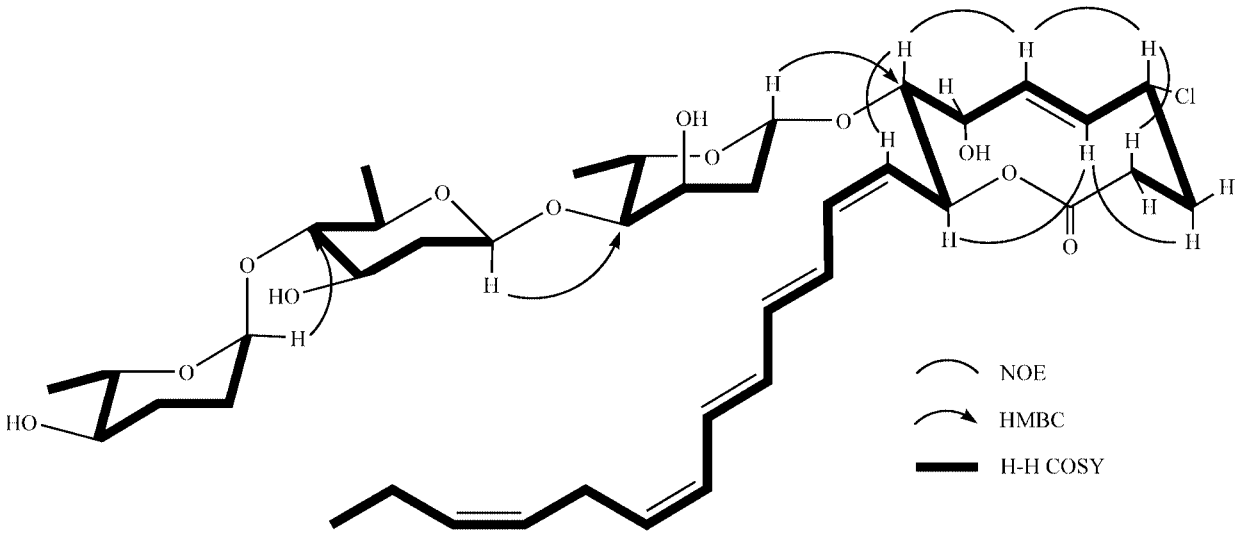
As to the second monosaccharide, strong NOEs were observed between H-l′′/3′-H′, H-l′′/5′′-H, and 3′′-H/5′′-H, while strong *J* couplings appeared between H-l′′/2a′-H′, 2a′-H′/3′′-H, 3′′-H/4′-H′, and 4′′-H/5′′-H. These observations indicate that this monosaccharide is β-olivose (2,6-dideoxy-β-*arabino*-hexopyranose).

The coupling constants (*J* = 9.9 Hz) between 4′′′-H and 5′′′-H of the monosaccharide indicate diaxial orientations. The anomeric proton 1′-H′′ appears as doublet (*J* = 2.4 Hz). On the basis of data from 2D NMR (i.e., COSY and NOE), the monosaccharide was identified as α-amicetose (2,3,6-trideoxy-α-*erythro*-hexopyranose).

The monosaccharides from the hydrolysate of **1** were purified by NH_2-HPLC. After evaporation of eluent, the three saccharides were obtained as colorless syrups. The first peak (amicetose) had $[\alpha]_D^{21} = -39.4$. Catelani[17] gave $[\alpha]_D = -39.0$ for an α,β-anomeric mixture of L-amicetose. In addition, the ^1H NMR spectrum is in good agreement with published data.[18] The optical rotation value of our olivose was +24, which is practically identical with the reported[19] value for D-olivose ($[\alpha]_D = +22$). The optical rotation of the compound corresponding to the third peak (digitoxose) in water ($[\alpha]_D^{21} = -37$) was also in good agreement with the literature data[20] ($[\alpha]_D = -38$ for the L and $[\alpha]_D = 36.5$ for the D configuration). This result indicates that one monosaccharide of compound **1** is in the D-form and two in the L-form.

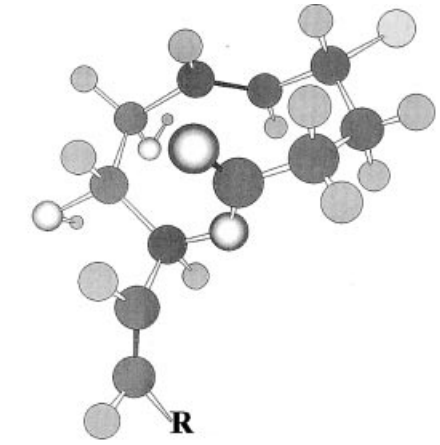
In compound **1**, the glycosidation shift at C-8 (ca. +4.5 ppm) and the chemical shifts of 1′-H (δ = 4.93 ppm) and C-1′ (δ = 100.4 ppm) of digitoxose indicated that this monosaccharide was glycosidated at C-8 of the aglycon. The signal due to the anomeric proton (1′′-H) of olivose (δ = 4.74 ppm), correlating to the C-1′′ resonance at δ = 99.5 ppm by HETCOR, indicated that the olivose unit was linked to a secondary alcoholic carbon (C-4′ of the digitoxose). The amicetose was assigned as terminal by the absence of any glycosylation shift at 4′′′-H and/or C-4′′′. These deductions were confirmed by a COLOC spectrum, which showed some diagnostic long-range correlations between 1′-H of digitoxose (δ 4.93) and C-8 (δ 74.8) of the aglycon, between 1′′-H (δ = 4.74 ppm) of the olivose unit and C-4′ (δ 76.5) of digitoxose, and between 1′′′-H (δ = 5.02 ppm) of the amicetose unit and C-4′′ (δ = 80.7 ppm) of olivose (see Table 1). The structure of glycoside **1** was thus (8*R*)-8-*O*-α-L-amicetosyl-(1→4)-β-D-olivosyl-(1→4)-β-L-digitoxosyl latrunculin A (i.e., latrunculinoside A).

High-resolution FABMS analysis of **10** suggested a molecular formula of $C_{34}H_{49}ClO_{10}$. The negative FABMS gave an $[M - H]^-$ ion at *m/z* = 650, with prominent fragments at *m/z* = 520 $[M - H - 130]^-$ and 390 $[M - H - 2 \times 130]^-$ (cleavage of one and two dideoxyhexose units, respectively). The molecular formula was supported by the presence of 34 signals in the ^{13}C NMR spectrum (3 × CH_3, 6 × CH_2, 24 × CH, and 1 × quaternary carbon). The high level of oxygenation in the molecular formula hinted at the presence of sugars in the molecule. The glycoside **10** was



1

Figure 3. The NOE, HMBC, and H-H COSY correlations of latrunculinoside A (**1**)



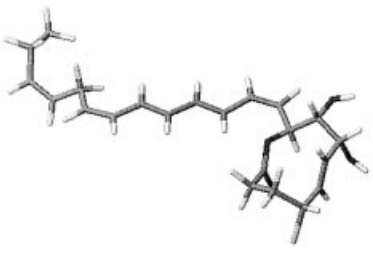


Figure 4. 3D-MM2 models (with and without side chain) of compound **1** are included

enzymatically hydrolyzed analogously to compound **1**, and the spectra of the aglycon (**11**; i.e., latrunculin B), were practically identical − with one exception − to those of aglycon **11**. Only the ^3J value corresponding to the dihedral angle between 5-H and 6-H was shifted ($^3J_{5\text{-H}-6\text{-H}}$ = 10.5 Hz). The value of the dihedral angle conformed to a *Z* double bond between C-5 and C-6. The two anomeric protons (δ = 4.85 and 5.05 ppm) in the ^{1}H NMR spectrum and the corresponding ^{13}C signals (δ = 98.1 ppm and 97.6 ppm) in the ^{13}C NMR spectra revealed the presence of two sugars (Table 3). The ^{1}H-^{1}H connectivities of the sugars were determined by COSY and TOCSY experiments, which indicated that they were both 2,6-dideoxy sugars. The sugars revealed large couplings ($J \geq 10$ Hz) between 1-H and 2a-H, and 2a-H and 3-H, and small couplings ($J \approx 4.5$ Hz) between 3-H/4-H and 5-H, indicating it to be a β-oliose. This was further verified by NOESY correlations of the respective 1,3-diaxial protons 1-H and 2a-H (Table 2). The proton and the carbon spectral assignments of the sugar moieties were made possible by a combination of COSY, TOCSY, HMQC, and HMBC experiments. The linkages between the sugars were deduced from the HMBC correlation from the anomeric proton of one sugar to the carbon of the neighboring sugar at the position of linkage and vice versa (see Figure 3). This allowed the two sugars to be linked as disaccharide moiety. The HMBC correlation from 1′-H (δ = 4.85 ppm) of the first sugar to the aglycon carbon at δ = 73.5 ppm allowed the placement of the disaccharide at C-8 of the aglycon. The stereochemistry of our oliose was determined by comparison with authentic D-oliose (obtained after hydrolysis of mithramycin A) by chiral gas chromatography (see Exp. Sect.).

The relative and absolute stereochemistry of aglycon **11** was demonstrated analogously to the case of compound **2**, and the structure was identified as (4*R*,5*Z*,7*R*,8*R*,9*S*,

Table 4. Bioactivities of latrunculinosides A and B (**1** and **10**)

Compound[a]	10 μg/mL[b]	50 μg/mL[b]	100 μg/mL[b]	Nat. conc.[c] μg/mL
1	4.3±0.6	1.7±0.6	0	29.2
10	6.0±1.0	3.3±0.6	0.7±0.6	9.0

[a] Aquarium assay results from feeding by goldfish on pellets treated with glycoside (**1** and **10**). All control pellets were eaten in all assays. Three replicate assays were performed at each concentration (mean ± SD is indicated in columns 2 to 4). [b] Concentration of the pure compound (**1** or **10**) in the pellet. [c] Natural concentration of **1** and **10** in *L. corticata*. Sponge volume was determined by displacement of water with frozen material according to Pawlik.[24]

10*Z*,12*E*,14*E*,16*Z*,19*Z*)-4-chloro-7,8-dihydroxy-5,10,12,14,16,19-docosahexaeno-9-lactone (i.e., latrunculin B). The full structure of glycoside **10** was therefore (8*R*) 8-*O*-β-D-oliosyl-(1→4)-β-D-oliosyl latrunculin B (i.e., latrunculinoside B).

A search for related compounds identified[21,22] pinolidoxin and epipinolidoxin from the fungus *Aschochyta pinodes*, and the more closely related didemnilactones A−B, neodidemnilactone, and ascitrienolides A−C from the marine ascidian *Didemnum*. These compounds exhibit weak binding activity to leukotriene B_4 receptors in human polymorphonuclear leukocyte membrane fractions. They share the same fatty acid structure element in their lactone ring and a similar unsaturated side chain with the latrunculinosides A and B.

Neither glycoside displayed antibacterial or antifungal activity. The compounds also did not give positive results in a brine shrimp toxicity assay, a sea urchin eggs test (*Paracentrotus lividus*), or a crown gall tumors on potato disks test (*Agrobacterium tumefaciens*).

Sponges are common inhabitants of the benthos and excellent candidates for chemical defenses, inasmuch as they have a sessile lifestyle and soft unprotected body tissues. Some macrolactones are known to be the principal defensive strategy of Caribbean sponges against predatory reef fishes.[23] To investigate the antifeeding activity of our two metabolites, aquarium assays were performed by previously described methods.[24]

As shown in Table 4, both compounds (**1** and **10**) deter feeding by goldfish in aquarium bioassays at concentrations of 10−100 μg/mL, equal to their natural concentrations; this strong bioactivity suggests a role in the chemical defense of *L. corticata*. The *trans* compound (**1**) had a higher activity than its *cis* counterpart (**10**). As shown in the literature[25] and confirmed by our results, halogenation increases the antifeeding activity.

Experimental Section

General Experimental Procedures: UV spectra were measured with a Cary 118 (Varian) apparatus in EtOH in the 200−350 nm range. A Perkin−Elmer Model 1310 (Perkin−Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of compounds as neat films. NMR spectra were recorded with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H), 125.7 MHz (^{13}C). High- and low-resolution MS were with a VG 7070E − HF spectrometer (70 eV). GC-MS analyses of the sugar derivatives were performed with a Finnigan 1020 B single-state quadrupole GC-MS instrument in the EI mode. A C_{18} reversed-phase column (5 μm, 7.8 × 250 mm, Supelco, USA), was employed. A linear gradient from 40% H_2O and 60% acetonitrile to pure acetonitrile over 36 min, flow rate 1.7 mL/min with detection at 256 nm, was used to separate the two compounds in the crude extract.

Animal Materials: The sponge *Latrunculia corticata* (Carter 1879), family Styelidae, phylum Porifera, class Demospongiae, subclass Monaxonida, family Latrunculiidae, was collected by hand (scuba) from the Coral Reef (from 5 to 10 m deep), on 27 August 2001 in the Red Sea, Gulf of Aqaba (Eilat, Israel). The voucher specimens are deposited in the collection of the second author (V.M. Dembitsky). *Latrunculia corticata*, also known as Magnificant Fire Sponge, had a brown-red color.

Extraction and Isolation: Fresh sponges (850 g wet weight) were frozen and stored at −18 °C. Sample of sponge were extracted with 90% butanol. Chromatography of the extract on a Sephadex LH-20 column (100 × 5 cm) with elution with MeOH gave organic fractions (8 mL) checked by two-dimensional TLC (silica gel plates, *n*BuOH/AcOH/H_2O, 12:3:5 and $CHCl_3$/MeOH/H_2O, 40:9:1). Fraction 7 was further fractionated by RP-HPLC on a C18-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH/H_2O (4:1) to yield two compounds: **1** (24.8 mg, 0.0029%) and **10** (7.7 mg, 0.0009%).

Enzymatic Hydrolysis of Glycosides (Autolysis): An aliquot of crude extract (after extraction of BuOH) was added to 50 g of defrost sponge and the solution was then incubated at 37 °C for 48 h. The resulting mixture was extracted with CH_2Cl_2, the solution was evaporated to dryness, and the residue was chromatographed on a silica gel column (10 g) with CH_2Cl_2/MeOH/H_2O (90:10:1), to afford **2** or **11.**

Preparation of Isopropylidene Aglycon: The aglycon **2** (3.6 mg) was dissolved in dry acetone (200 μL) and 2,2-dimethoxypropane (2 mL). A catalytic amount of *p*-toluenesulfonic acid was added to this solution. The reaction mixture was stirred at room temperature for 4 h under nitrogen. After that, the mixture was neutralized with saturated $NaHCO_3$ solution and extracted with 3 × 3 mL of $CHCl_3$. The combined organic layers were concentrated to dryness in vacuo to afford the corresponding isopropylidene derivative **3** (2.9 mg).

Ozonolysis: The isopropylidene derivative **3** was dissolved in methanol (10 mL), cooled in a dry ice/acetone bath (−78 °C). Ozone was bubbled through the solution for 5−10 min. Excess ozone was then removed with a stream of nitrogen for 2 min. Sodium cyanoborohydride (4 mg) was added at −78 °C and the mixture was stirred for 1 h, after which acetic acid (20 μL) was added. After stirring had continued for an additional 30 min at room tempera-

ture, the solution was evaporated to dryness. The residue was dissolved in methanol and was then chromatographed on a silica gel column with methanol (flow rate 1 ml/ minute) to yield the main product **4**. The compound **4** was dissolved in the methanol and 10% BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) in hexane were added. After 3 min, 2 μL of the reaction solution was analyzed by chiral GC (conditions see above).

Hydrolysis of Glycosides: The glycoside (≈ 15 mg) was dissolved in hydrogen chloride (5%, 8 mL) and the mixture was heated at reflux for 4 h. The reaction mixture was neutralized with silver carbonate and then filtered, and the filtrate was extracted with diethyl ether. The water layer was evaporated to dryness and the mixture was chromatographed on an NH_2-HPLC column with water/acetonitrile (8:2) to yield α,β-anomeric mixtures of L-digitoxose (**7**) $[\alpha]_D^{21} = -37$, D-olivose (**8**) $[\alpha]_D^{21} = +24$, and L-amicetose (**9**) $[\alpha]_D^{21} = -39.4$, obtained as colorless syrups.

Chiral Chromatography: A Macherey−Nagel GmbH & Co. KG (Düren, Germany) Permabond L-Chirasil-Val capillary column − 25 m × 0.25 mm ID − was used. Oven temperature: 80 °C (5 min iso; 4 °C/min to) 190 °C (10 min), carrier gas helium, 20 cm/s, detector FID, 300 °C, injection of 1 μL mixture in dichloromethane (for standards: containing 0.5 mg/ml of each analyte), split (100:1), 250 °C.

Preparation of Standards

2,3-*O*-Isopropylidene-α/β-L-ribofuranose (6): A solution of H_2SO_4 in dry acetone (0.1%) was added to L-ribofuranose (75 mg, 0.5 mmol) and the mixture was kept in a refrigerator at 4 °C for 3 d, followed by stirring of the reaction mixture at room temperature for 24 h. Solid Na_2CO_3 was added to the solution for 2 h, after which the residual solid material was filtered away. The acetone was evaporated, and the crude mixture was subjected to silica gel chromatography to obtain 2,3-*O*-isopropylidene-α/β-L-ribofuranose (54 mg, 72%). The ^1H and ^{13}C NMR were in agreement with the literature data.[15]

2,3-*O*-Isopropylidene-L-ribitol (4): The isopropylidene derivative (**6**) was dissolved in a mixture of water (5 mL) and ethanol (1 mL) and, after the mixture had been cooled in an ice bath, a cold solution of sodium borohydride (0.3 mmol) in water (2 mL) was added dropwise. After the mixture had been stirred at room temperature for 15 h, the pH was adjusted to 5−6 with acetic acid (1.7 N). The mixture was extracted with ethyl acetate, and the combined organic layer was washed with water, dried, and concentrated to give **4** as a pale yellow syrup. The yield was 80%. $[\alpha]_D^{22} = +4.1$ ($c = 0.11$, EtOH).

D-Oliose: After hydrolysis of mithramycin A, the sugars were methylated[26] by use of a mixture of MeI and Ag_2O and the mixture was chromatographed (see above). The retention times of oliose from mithramycin A were $t_R = 7.43$, 7.53, 8.34, and 8.44 min, respectively. These values are in good accordance with data for oliose isolated after hydrolysis of latrunculinoside B (the retention times were $t_R = 7.42$, 7.52, 8.33, and 8.42 min, respectively).

Latrunculinoside A (1): Colorless powder (24.8 mg). $[\alpha]_D^{23} = +72$ ($c = 0.13$, MeOH). UV (EtOH): λ_{max} (log ε) = 307 (4.82) nm. IR (film): $\tilde{\nu}_{max}$ = 3550 (OH), 1725 (C=O) cm^{-1}. HRFABMS: m/z = 767.3779 $[M + H]^+$, calcd. for $[C_{40}H_{59}ClO_{12} + H]^+$ 767.3776; negative LRFABMS: m/z = 765 $[M - H]^-$, 651 $[M - H - 114]^-$, 521 $[M - H - 114 - 130]^-$, 391 $[M - H - 114 - 2 \times 130]^-$. NMR spectra see Table 1−3.

Latrunculin A (2): (4*R*,5*E*,7*R*,8*R*,9*S*,10*Z*,12*E*,14*E*,16*Z*,19*Z*)-4-Chloro-7,8-dihydroxy-5,10,12,14,16,19-docosahexaeno-9-lactone, colorless oil. $[\alpha]_D^{23} = +36$ ($c = 0.09$, MeOH). UV (EtOH): λ_{max} (log ε) = 313 (4.94) nm. IR (film): $\tilde{\nu}_{max}$ = 3550 (OH), 1725 (C=O) cm^{-1}. HRFABMS: m/z = 393.1830 $[M + H]^+$, calcd. for $[C_{22}H_{29}ClO_4 + H]^+$ 393.1832; negative LREIMS: m/z = 394 $[M]^-$, 392 $[M]^-$ in a ratio 3:1; NMR spectra see Table 1−3.

Latrunculinoside B (10): Colorless powder (7.7 mg). $[\alpha]_D^{22} = -18.5$ ($c = 0.09$, MeOH). HRFABMS: m/z = 652.3100 $[M + H]^+$, calcd. for $[C_{34}H_{49}ClO_{10} + H]^+$ 653.3092; negative LRFABMS: m/z = 650 $[M - H]^-$, 520 $[M - H - 130]^-$, 390 $[M - H - 2 \times 130]^-$. The UV, IR and ^1H and ^{13}C NMR spectra were practically identical to those of **1**, see also Table 1−3 for NMR spectroscopy.

Latrunculin B (11): (4*R*,5*Z*,7*R*,8*R*,9*S*,10*Z*,12*E*,14*E*,16*Z*,19*Z*)-4-Chloro-7,8-dihydroxy-5,10,12,14,16,19-docosahexaeno-9-lactone, colorless oil. $[\alpha]_D^{23} = -9$ ($c = 0.08$, MeOH). UV (EtOH): λ_{max} (log ε) = 312 (4.73) nm. IR (film): $\tilde{\nu}_{max}$ = 3550 (OH), 1722 (C=O) cm^{-1}. HRFABMS: m/z = 393.1828 $[M + H]^+$, calcd. for $[C_{22}H_{29}ClO_4 + H]^+$ 393.1832; negative LREIMS: m/z = 394 $[M]^-$ and m/z = 392 $[M]^-$ in a ratio 3:1; NMR spectra see Table 1−3.

Bioassay for Antifeeding Activity Assay: Purified compounds (**1** and **10**) were dissolved in a minimal volume of methanol and mixed with alginate-based food matrix[24] (100 μL) until all organic and water-soluble components were distributed uniformly throughout the paste. The alginate food matrix was then dispensed by use of a 0.1 mL syringe into a $CaCl_2$ solution (0.25 M), forming a strand that was allowed to harden for 2 min. The hardened strand was rinsed with filtered water and cut into 3 mm pellets with a scalpel. Control pellets were prepared identically but without the addition of natural or synthetic compounds. Feeding assays were performed with goldfish (*Carassius auratus*).

[1] N. Fusetani, S. Matsunaga, *Chem. Rev.* **1993**, *93*, 1793−1806.
[2] D. J. Faulkner, *Nat. Prod. Rep.* **2002**, *19*, 1−48 and the earlier references in this series cited therein.
[3] G. Rousseau, *Tetrahedron* **1995**, *51*, 2777−2849.
[4] G. Drager, A. Kirschning, R. Thiericke, M. Zerlin, *Nat. Prod. Rep.* **1996**, *13*, 365−375.
[5] M. Ishibashi, in *Macrolide Antibiotics, Chemistry, Biology and Practice*, Second Edition, (Ed.: S. Omura), pp. 57−98, Elsevier **2002**.
[6] H. Niwa, K. Wakamatsu, K. Yamada, *Tetrahedron Lett.* **1989**, *30*, 4543−4546.
[7] H. Kigoshi, H. Niwa, K. Yamada, T. J. Stout, J. Clardy, *Tetrahedron Lett.* **1991**, *32*, 2427−2428.
[8] H. Niwa, M. Watanabe, H. Inagaki, K. Yamada, *Tetrahedron* **1994**, *50*, 7385−7400.
[9] H. Niwa, H. Inagaki, K. Yamada, *Tetrahedron Lett.* **1991**, *32*, 5127−5128.
[10] N. Lindquist, W. Fenical, *Tetrahedron Lett.* **1989**, *30*, 2735−2738.
[11] M. S. Congreve, A. B. Holme, A. B. Hughes, M. G. Looney, *J. Am. Chem. Soc.,* **199**3, *115*, 5816−5818.
[12] T. Řezanka, V. M. Dembitsky, *Eur. J. Org. Chem.* **2002**, 2400−2404.
[13] T. Řezanka, V. M. Dembitsky, *Eur. J. Org. Chem.* **2003**, 309−316.
[14] G. A. J. Pitt, R. A. Morton, *Prog Chem. Fats Lipids* **1957**, *4*, 228−274.
[15] B. Kaskar, G. L. Heise, R. S. Michalak, B. R. Vishnuvajjala, *Synthesis-Stuttgart* **1990**, 1031−1032.
[16] L. Hough, J. K. N. Jones, D. L. Mitchell, *Can. J. Chem.* **1958**, *36*, 1720−1728.

[17] G. Catelani, F. Colonna, P. Rollin, *Gazz Chim. Ital.* **1989**, *119*, 389−393.
[18] G. Berti, P. Caroti, G. Catelani, L. Monti, *Carbohydrate Research* **1983**, *124*, 35−42.
[19] M. Miyamoto, Y. Kawamatsu, M. Shinohara, *Tetrahedron Lett.* **1964**, 2371−2377.
[20] F. Tomita, T. Tamaoki, K. Shirahata, M. Kasai, M. Morimoto, S. Ohkubo, K. Mineura, S. Ishii, *J. Antibiotics* **1980**, *33*, 668−670.
[21] A. Evidente, R. Capasso, M. A. Abouzeid, R. Lanzetta, M. Vurro, A. Bottalico, *J. Nat. Prod.* **1993**, *56*, 1937−1943.
[22] A. Evidente, R. Lanzetta, R. Capasso, M. Vurro, A. Bottalico, *Phytochemistry* **1993**, *34*, 999−1003.
[23] J. R. Pawlik, *Chem. Rev.* **1993**, *93*, 1911−1922.
[24] J. R. Pawlik, B. Chanas, R. J. Toonen, W. Fenical, *Marine Ecol.-Prog. Ser.* **1995**, *127*, 183−194.
[25] M. Assmann, E. Lichte, J. R. Pawlik, M. Kock, *Marine Ecol-Prog. Ser.* **2000**, *207*, 255−262.
[26] C. J. Biermann, G. D. McGinnis, *Analysis of carbohydrates by GLC and MS*, CRC Press, Boca Raton, FL, USA, **1989**.

Received January 13, 2003

