JOURNAL OF NATURAL PRODUCTS

Volume 70, Number 6 June 2007

Full Papers

Lemnalosides A−D, Decalin-Type Bicyclic Diterpene Glycosides from the Marine Soft Coral *Lemnalia* sp.

Guangmin Yao,[†,‡] Nicholas B. Vidor,[†] Adam P. Foss,[†] and Leng Chee Chang*[,†]

Department of Chemistry and Biochemistry, College of Science and Engineering, University of Minnesota Duluth, 1039 University Drive, Duluth, Minnesota 55812

Received July 15, 2006

Four new decalin-type bicyclic diterpenoids, lemnalosides A−D (**1**−**4**), along with one known decalin-type bicyclic diterpenoid, lemnabourside (**5**), were isolated from an extract of the marine soft coral *Lemnalia* sp. The structures of the new compounds were elucidated on the basis of spectroscopic analysis including ^{1}H−^{1}H COSY, HMQC, HMBC, NOESY, HRESIMS, UV, IR, and chemical means. Compound **1** showed moderate activity in the hyphae formation inhibition (HFI) assay against *Streptomyces* 85E at a concentration of 20 μg/disk, while compound **2** displayed inhibitory activity in the HFI assay at a concentration of 2.5 μg/disk.

Protein phosphorylation is one of the major regulatory mechanisms involved in signal transduction pathways including apoptosis, cell proliferation, and metabolism. Abnormalities in protein phosphorylation are often associated with human diseases. Thus, the inhibitors of both protein kinases and phosphatases appear to be attractive drug targets in the chemotherapeutic treatment of cancer and have received wide attention.[1,2] Marine natural products have proven to be a rich source of untapped natural resources, and many diverse bioactive chemical structures remain to be discovered.[3] To achieve this aim, we have adopted a prokaryotic whole cell assay using the hyphae formation inhibition (HFI) in the search for protein kinase inhibitors.[4]

The eukaryotic signal transduction pathways are regulated by protein kinases and phosphatases that are phosphorylated at specific serine/threonine/tyrosine residues. Recently, these serine/threonine-phosphorylated enzymes were found in *Streptomyces coelicolor* A3-(2)[5,6] and *S. granaticolor*.[7] Aerial hyphae formation in *Streptomyces* species requires protein kinase activity. It has been shown that a variety of kinase inhibitors block this process.[4,8] For example, staurosporine, a eukaryotic protein kinase inhibitor, exhibited a strong inhibitory effect on several phosphorylations of cellular proteins and the aerial hyphae formation in *S. griseus*.[8] In addition, recent studies have found that *Streptomyces* sp. have a variety of proteins phosphorylated at tyrosine residues, and several strains sensitive to the formation of aerial hyphae inhibition by protein kinase inhibitors were identified.[4]

An organic extract of *Lemnalia* sp. was found to show inhibitory activity in the HFI assay, giving a 16 mm bald and clear phenotype at a concentration of 80 μg/disk. This preliminary result encouraged us to study *Lemnalia* sp. for novel protein kinase inhibitors. The family Nephtheidae comprises many genera, of which *Lemnalia*, *Paralemnalia*, *Capnella*, *Litophyton*, and *Nephthea* have received considerable attention from organic chemists.[3,9] Previous chemical investigation on the soft coral of the genus *Lemnalia* resulted in the isolation of sesquiterpenoids,[10−23] norsesquiterpenoids,[10,12,16,21] diterpenoids,[22−26] and steroids. Herein we report the isolation, structure elucidation, and biological activities of compounds **1**−**5**.

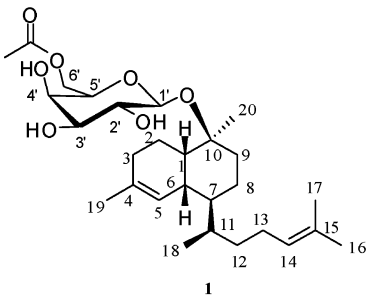
Results and Discussion

The crude CH_2Cl_2−MeOH (1:1) extracts of *Lemnalia* sp. exhibited inhibitory activity in the HFI assay at a concentration of 80 μg/disk. This extract was subjected to medium-pressure liquid chromatography (MPLC) eluting with a gradient of $CHCl_3$−MeOH, affording a series of diterpenoid-containing fractions, which were further purified by Sephadex LH-20 and semipreparative reversed-phase HPLC, yielding five compounds (**1**−**5**). The structure of

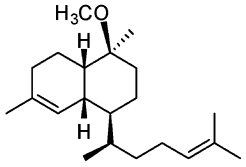
* To whom correspondence should be addressed. Tel: 218-726-7346. Fax: 218-726-7394. E-mail: lcchang@d.umn.edu.
† University of Minnesota Duluth.
‡ Shijiazhuang Pharmaceutical Group Zhongqi (sjz) Pharmaceutical Technology Co., Ltd.

10.1021/np0603424 CCC: $37.00
Published on Web 05/17/2007

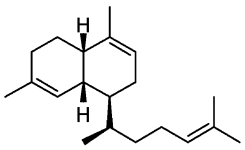
Chart 1



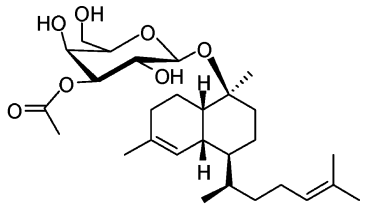
1



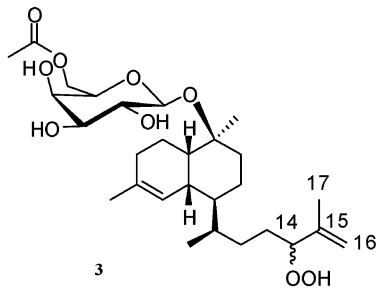
Dictyotin D methyl ether



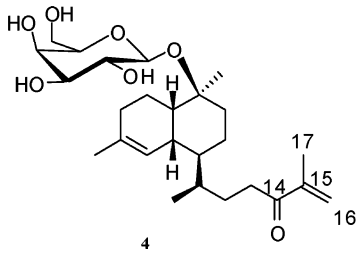
Biflora-4,9,15-triene



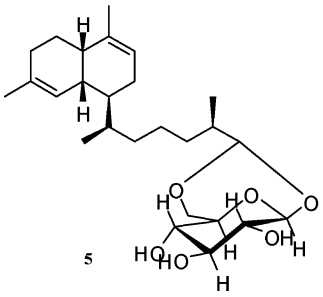
2



3



4



5

Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

no.	**1**[b]	**2**[b]	**3**[c]	**4**[b]
1	1.68 br d (13.5)	1.68 br d (13.5)	1.74 dd (3.3, 13.5)	1.68 br d (13.0)
2	1.05 qd (3.5, 13.5)	1.05 qd (3.5, 13.0)	1.10 qd (3.5, 13.5)	1.07 qd (3.5, 13.0)
	1.40 m	1.40 m	1.41 m	1.44 m
3	1.94 m	1.94 m	1.93 m	1.93 m
5	5.47 d (4.5)	5.47 d (4.5)	5.54 br. s	5.46 d (4.5)
6	2.01 dd (1.5, 10.5)	2.04 m	2.08 m	2.03 m
7	1.42 m	1.41 m	1.49 m	1.41 m
8	2.08 br d (11.5)	2.08 m	2.29 m	2.08 br d (10.5)
	1.49 m	1.47 m	1.40 m	1.47 m
9	1.73 m	1.73 m	1.78 m	1.75 m
	1.55 m	1.53 m	1.53 m	1.57 m
11	1.74 m	1.76 dd (3.0, 10.5)	1.44 m, overlap	1.73 dd (3.0, 13.5)
12	1.20 m	1.20 m	1.20 m	1.51 q (7.2)
13	1.85 m	1.87 m	1.40 m	2.59 td (7.2, 16.0)
	1.99 m	1.96 m		2.68 dt (7.2, 16.0)
14	5.05 t (7.0)	5.05 t (7.0)	4.21 dt (3.0, 6.6)	
16	1.65 s	1.65 s	4.89 d (1.5); 4.88 d (1.5)	5.92 s; 5.73 s
17	1.57 s	1.57 s	1.68 s	1.84 s
18	0.78 d (7.0)	0.78 d (7.0)	0.83 (6.9)	0.81 d (7.0)
19	1.63 s	1.63 s	1.63 s	1.63 s
20	1.30 s	1.32 s	1.34 s	1.32 s
1′	4.41 d (6.5)	4.55 d (7.5)	4.48 d (6.9)	4.46 d (7.0)
2′	3.60 m (overlap)	3.80 dd (7.5, 10.0)	3.48 t (8.2)	3.60 m, overlap
3′	3.58 m (overlap)	4.86 dd (3.5, 10.0)	3.52 dd (3.0, 8.2)	3.61 m, overlap
4′	3.88 br s	4.07 br s	3.81 br s	3.96 br s
5′	3.62 m (overlap)	3.54 dd (5.0, 6.0)	3.72 m	3.51 dd (4.5, 6.5)
6′	4.26 dd (5.5, 11.5)	3.78 dd (5.0, 11.5)	4.13 dd (4.5, 11.1)	3.78 dd (4.3, 12.0)
	4.29 dd (7.5, 11.5)	3.89 dd (6.5, 11.5)	4.30 dd (7.8, 11.1)	3.91 dd (6.5, 12.0)
−OOH			10.53 s	
CH$_3$CO	2.04 s	2.15 s	1.98 s	

[a] *J* values (Hz) are showed in parentheses. [b] Spectra obtained in $CDCl_3$. [c] Spectra obtained in acetone-d_6.

compound **5** was assessed by comparison of its spectroscopic data with those reported in the literature,[18] whereas compounds **1**−**4** are new.

Compound **1** was obtained as a colorless oil, with the molecular formula $C_{28}H_{46}O_7$ determined from HRESIMS (*m*/*z* 517.3119 [M + Na]$^+$, calc 517.3136) indicating six degrees of unsaturation. The IR spectrum of **1** showed the presence of one or more hydroxyl groups (3415 cm^{-1}) and an ester carbonyl (1730 cm^{-1}). Its ^{1}H NMR spectrum (Table 1) showed the signals for two olefinic protons (δ_H 5.47, d, J = 4.5 Hz, 1H; 5.05, t, J = 7.0 Hz, 1H), seven sugar protons (δ_H 4.41, d, J = 6.5 Hz, 1H; 4.29, dd, J = 7.5, 11.5 Hz, 1H; 4.26 dd, J = 5.5, 11.5 Hz, 1H; 3.88, br s, 1H; 3.62, m, 1H; 3.60, m, 1H; 3.58, m, 1H), one acetyl (δ_H 2.04, s, 3H), four tertiary methyl groups (δ_H 1.65, s, 3H; 1.63 s, 3H; 1.57, s, 3H; 1.30, s, 3H), and a secondary methyl group (δ_H 0.78, d, J = 7.0 Hz, 3H). The ^{13}C NMR spectrum indicated 28 signals, including one ester carbonyl (δ_C 171.1), one sugar unit (δ_C 63.1, 68.7, 72.2, 72.3, 73.8, 97.1), six methyls (δ_C 13.6, 17.9, 21.1, 23.8, 24.4, 25.9), six methylenes (δ_C 18.3, 21.6, 26.5, 31.4, 32.8, 36.1), six methines (δ_C 31.4, 36.5, 42.4, 44.5, 124.5, 125.0), and three quaternary carbons (δ_C 80.5, 131.3, 134.7). An ester carbonyl, two double bonds, and one sugar moiety from the ^{13}C NMR spectra accounted for four degrees of unsaturation, and the remaining two degrees of unsaturation required the presence of a bicyclic system in **1**. Thus, compound **1** must be a bicyclic diterpene glycoside. The configuration of the sugar unit was assigned after hydrolysis of **1** with 1

Table 2. ^{13}C NMR Data of Compounds **1**−**4**

no.	**1**[a]	biflora-4,9,15-triene[a]	dictyontin D methyl ether[a]	**2**[a]	**3**[b]	**4**[a]
1	44.5	39.8	42.2	44.8	45.3	44.7
2	21.6	24.8	20.8	21.6	22.0	21.7
3	31.4	31.0	31.6	31.3	32.0	31.3
4	134.7	134.6	133.1	134.7	135.1	135.1
5	124.5	124.1	125.4	124.5	125.5	124.2
6	36.5	36.6	34.0	36.5	37.1	36.5
7	42.4	39.1	42.2	42.4	43.3	42.9
8	18.3	24.8	19.6	18.6	18.9	18.5
9	32.8	121.7	31.8	32.9	33.5	32.9
10	80.5	136.8	76.1	80.5	79.4	80.2
11	31.5	31.6	31.7	31.4	32.3	32.0
12	36.1	35.9	36.0	36.1	32.6	30.9
13	26.5	26.4	26.4	26.5	30.0	36.3
14	125.0	125.2	125.2	125.1	89.4	202.8
15	131.3	131.2	130.9	131.3	146.0	144.8
16	25.9	25.9	25.7	25.9	113.6	124.5
17	17.9	17.8	17.7	17.9	17.2	17.9
18	13.6	13.5	13.6	13.5	13.8	13.4
19	23.8	24.1	23.6	23.8	23.9	23.8
20	24.4	21.9	22.5	24.5	24.5	24.6
1′	97.1			97.6	98.1	97.1
2′	72.2			69.8	72.5	72.4
3′	73.8			75.3	74.8	73.9
4′	68.7			68.6	69.8	69.5
5′	72.3			74.3	73.0	74.5
6′	63.1			62.8	64.3	62.9
CH$_3$CO	21.1			21.3	20.9	
CH$_3$*C*O	171.1			170.8	170.9	
MeO			47.9			

[a] Spectra obtained in $CDCl_3$. [b] Spectra obtained in acetone-d_6.

N HCl. The hydrolysate was trimethylsilated by 1-(trimethylsilyl)-imidazole, and the GC retention time of the sugar was compared with that of the authentic sample prepared in the same manner.[27,28] In this way, the sugar unit of **1** was determined to be D-galactose. So, compound **1** was a galactoside. A detailed analysis of the 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC) of **1** is given in Tables 1 and 2. Comparison of its ^{13}C NMR data (Table 2) with values for the known compound, dictyotin D methyl ether,[29] indicated that **1** was a dictyotin D methyl ether analogue, except the *O*-methyl group in dictyotin D methyl ether was replaced by an acetyl galactoside unit in **1**. In the HMBC spectrum of **1**, the glycosidation position was determined by a three-bond correlation between the galactosyl anomeric proton H-1′ (δ_H 4.41, d, J = 6.5 Hz) and C-10 (δ_C 80.5). The β-galactosyl linkage was deduced from the coupling constant (J = 6.5 Hz) of H-1′. Moreover, the HMBC correlation of H-6′ (δ_H 4.29, dd, J = 7.5, 11.5 Hz; 4.26, dd, J = 5.5, 11.5 Hz) with acetate carbonyl (δ_C 171.1), and an acetyl group (δ_H 2.04, s, 3H) with acetate carbonyl (δ_C 171.1), suggested that the C-6′ hydroxyl group of the galactose was acetylated. Unambiguous confirmation of the planar structure of **1** came from the ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

A *cis*-decalin configuration in the diterpene portion was deduced from the ^{13}C chemical shifts in C-1 (δ_C 44.5) and C-6 (δ_C 36.5), which were very similar to those of lemnaflavoside,[23] lemnabourside,[24,25] and dictyotin D methyl ether.[29] These chemical shifts differed from the δ_C values for the bridge C atoms in the *trans*-decalin of γ-cadinene (δ_C 49.2, 41.6).[30] Acid hydrolysis of **1** afforded a dehydration product of the aglycone, biflora-4,9,15-triene,[31] which further supported the *cis*-decalin configuration of **1**. In addition, the *cis*-fusion of the decalin skeleton was also indicated by the NOE correlation between H-1 (δ_H 1.68) and H-6 (δ_H 2.01) as well as the small coupling constant (~1 Hz) between H-1 and H-6. The large coupling constant (J = 10.5 Hz) between H-6 and H-7 established the equatorial orientation of the side chain at C-7.[27] NOESY correlations of H-5 (δ_H 5.47) with H-6(δ_H 2.01), H-11(δ_H 1.74), and H-18 (δ_H 0.78), and H-6 (δ_H 2.01) with H-18 (δ_H 0.78), revealed the *cis* relationship of H-6 and H-18. The NOE

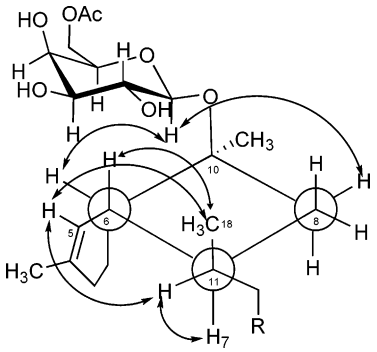


Figure 1. Key NOEs of lemnaloside A (**1**).

from the anomeric proton H-1′ (δ_H 4.41, d, J = 6.5 Hz, 1H) to H-1(δ_H 1.68, br d, J = 13.5 Hz, 1H) indicated their *syn* relation. The key NOESY correlations of compound **1** are shown in Figure 1. On the basis of the above evidence, **1** was determined to be the 6′-*O*-acetate of dictyotin D, 10-*O*-β-D-galactopyranoside, named lemnaloside A.

Compound **2**, a colorless oil, was shown to have a molecular formula of $C_{28}H_{46}O_7$ by HRESIMS (*m*/*z* 517.3112, calcd for $[M + Na]^+$ 517.3136). Acid hydrolysis of **2** with 1 N HCl also yielded D-galactose, as determined by GC methods after treating with 1-(trimethylsilyl)imidazole.[27,28] The same molecular formula of compounds **2** and **1** indicated they were isomer. The ^{1}H NMR and ^{13}C NMR data of **2** were very similar to those of **1**; the major difference was that H-3′ (δ_H 4.86, dd, J = 3.5, 10.0 Hz) and C-3′ (δ_C 75.3) of the galactose in **2** were shifted to a lower field than those in **1** [H-3′ (δ_H 3.58, m) and C-3′ (δ_C 73.8)], which suggested the C-3′ hydroxyl group of the galactose was acetylated. The HMBC correlation of H-3′ of the galactose and the carbonyl (δ_C 170.8) of the acetate further supported the above deduction. ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments allowed the complete assignment for structure **2**.

Compound **3** was assigned a molecular formula of $C_{28}H_{46}O_9$ as deduced from its HRESIMS (*m*/*z* 549.3032, calcd for $[M + Na]^+$ 549.3034). Acid hydrolysis of **3** with 1 N HCl also yielded D-galactose, as determined by GC methods after treating with 1-(trimethylsilyl)imidazole.[27,28] Comparison of ^{1}H and ^{13}C NMR data of **3** and **1** (Tables 1 and 2) indicated that they were very closely related analogues, except for the presence of an exo-methylene (δ_H 4.89, 4.88, 2H; δ_C 113.6, 146.0) and an oxygenic group (δ_H 4.21, dt, J = 3.0, 6.6, 1H; δ_C 89.4) in **3** instead of a trisubstituted double bond (δ_H 5.05, t, J = 7.0, 1H; δ_C 125.0, 131.3) and a methyl (δ_H 1.65, 3H; δ_C 25.9) in **1**. In the HMBC spectrum of **3**, correlations from the H-17 (δ_H1.68, s, 3H) to the exo-methylene (δ_C 113.6, 146.0) and oxygenic methine (δ_C 89.4) and from the olefinic proton of the exo-methylene (δ_H 4.89, 4.88) to the oxygenic methine (δ_C 89.4) and C-17 (δ_C 17.2) revealed that the oxygenic group and the exo-methylene were located at C-14 and C-15, and C-16, respectively. Further consideration of the chemical shift of C-14 (δ_C 89.4) and the mass spectrum of **3** indicated that this oxygenic group was a hydroperoxy group.[32,33] An active proton signal appearing in δ_C 10.53 (s, 1H) in the ^{1}H NMR spectrum further supported the above deduction.

In the HMBC experiments, the δ 4.48 (H-1′) exhibited cross-peaks with signals at δ 79.4 (C-10). The H-6′ signal (δ 4.13 and 4.30) showed a connectivity with δ_C 170.9 (OAc), and δ_H 1.98 (acetyl) with δ_C 170.9 (OAc), indicating that the C-6′ hydroxyl group of the galactose was acetylated. The β-galactosyl linkage was deduced from the coupling constant value (J = 6.9 Hz) of anomeric proton H-1′. ^{1}H−^{1}H COSY, HMQC, and HMBC experiments allowed the complete assignment of the structure of **3**.

Compound **4** was obtained as a colorless oil, with the molecular formula $C_{26}H_{42}O_7$ determined from the HRESIMS (*m*/*z* 489.2799,

Table 3. Biological Activity of Tested Compounds on the Growth and Sporulation of *Streptomyces* 85 E[a]

compound	zone of inhibition observed (mm); 20 μg/disk	effect	comment
mitomycin C	30	clear	
U0126	15	bald	
1	11	bald	NA at 10 μg
2	18	clear	2.5 μg to 11B
3	NA[b]	na	NA
5	10	clear	NA at 10 μg

[a] Diameter of disk alone is 7 mm; Stock solutions were prepared in either DMSO or methanol. 1. No zones of inhibition were observed with MeOH or DMSO as negative controls. All compounds were tested at 20 μg/disk. Active compounds will be retested again at lower concentrations (20−1.25 μg/disk). [b] NA indicates not active.

calcd for $[M + Na]^+$, 489.2823). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** were very similar to those of compound **3**. The main difference was that the hydroperoxyl group in **3** (δ 79.4) was replaced with a ketone group (δ 202.8) in **4** at the C-14 position. In addition, the acetyl group in **3** disappeared. In the HMBC spectrum of **4**, correlations from CH_3-17 (δ_H 1.84) to C-14 (δ_C 202.8) and C-15 (δ_C 144.8) and from H-16 (δ_H 5.92; δ_H 5.73) to C-14 (δ_C 202.8) and C-15 (δ_C 144.8) also supported the carbonyl location at C-14. Moreover, the typical IR absorption at ν_{max} 1685 cm^{-1} and a strong UV absorption at λ_{max} 226 nm of **4** further confirmed the presence of an α,β-unsaturated ketone. The location of the galactose at C-10 was deduced from the cross-peak of the anomeric proton H-1′ (δ_H 4.46, d, J = 7.0 Hz) of galactose with C-10 (δ_C 80.2). The β-configuration of the galactose was deduced from the coupling constant value (J = 7.0 Hz) of the anomeric proton H-1′.

To date, the glycoside location in decalin-type bicyclic diterpenoids was either at C-16 or at C-18.[22−26] Thus, this is the first report of decalin-type bicyclic diterpenoids with glycosidation at C-10. The four new diterpene glycosides isolated from *Lemnalia* sp. were named lemnalosides A, B, C, and D, respectively.

Two phenotypes were observed in the HFI assay using *Streptomyces* 85E. Compounds that are cytotoxic to *Streptomyces* 85E result in a clear zone of inhibition that is distinctly different from aerial hyphae inhibition. For example, mitomycin C, which inhibited both growth and sporulation, gave a 30 mm clear zone of inhibition at 20 μg/disk, whereas, staurosporine, a protein kinase C inhibitor, exhibited a 13 mm bald zone at 20 μg/disk. A zone of "bald" cells indicated that the cells in this zone have only substrate hyphae as compared to the surrounding colonies. Furthermore, U0126, a highly selective inhibitor of mitogen-activated extracellular signal-regulated kinases, MEK 1 and MEK 2, exhibited a 15 mm bald phenotype at 5 μg/disk.

Compounds **1**−**3** and **5** were evaluated for their inhibitory activities against *Streptomyces* 85E in the hyphae formation inhibition assay (Table 3), according to an established protocol.[4] Compound **4** was not tested due to lack of material. Compound **1** showed moderate activity and gave a 11 mm bald zone of inhibition at 20 μg/disk. Compound **2** was the most active and gave a 18 mm clear zone of inhibition at 20 μg/disk. It also exhibited a clear zone at 2.5 μg/disk. Compounds **3** (with a hydroperoxy group at position C-14) and **5** (glycosidation at position C-16) were inactive.

Although the crude organic extract of *Lemnalia* sp. gave a 16 mm clear + bald phenotype at a concentration of 80 μg/disk, the purified isolates gave clear zones of inhibition. Diterpenes including dictyotins A, B, C, and D methyl ether were reported to show cytotoxicity against the murine B16 melanoma cells with an IC_{50} value in the range 3−19 μg/mL.[27] Dictyotin D methyl ether was reported to show cytotoxicity with an IC_{50} of 19 μg/mL. Lemnabboursides B and C, in the diterpene glycosides series in which the glycosidation unit occurs at position C-15, were reported to show weak cytotoxicity against hepatoma ascites cells with an IC_{50} in the range 33.5−41.4 μg/mL.[24]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 automatic polarimeter. UV spectra were recorded on a HP 8453 UV−visible spectrophotometer. The FT-IR spectra were recorded on a Perkin-Elmer BX FT-IR spectrometer. Mass spectra and high-resolution MS spectra were taken with a BioTOF II ESI mass spectrometer. 1D and 2D NMR spectra were recorded in acetone-d_6 and chloroform-*d* on Mercury Plus (300 MHz) and INOVA Unity (500 MHz) Varian spectrometers, the latter equipped with an *xyz*-shielded gradient triple-resonance probe. ^1H and ^{13}C chemical shifts were referenced to the central peak of acetone-d_6 (δ_H = 2.05 and δ_C = 29.92) and chloroform-*d* (δ_H = 7.24 and δ_C = 77.23), respectively. Reversed-phase HPLC was carried out on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech semipreparative Econosil C_{18} column (10 μm, 10 × 250 mm) run with a flow rate of 1.5 mL/min. Chromatographic fractions and pure compounds were monitored by TLC, detected by a color reaction by spraying with a solution of 10% sulfuric acid−ethanol solution followed by 5 min heating at 120 °C. Column chromatography (CC) was carried out on a column of Merck Si gel 60 (70−230 mesh). Gas chromotography was performed on a HP 5890-II plus chromatograph equipped with a HP 5971A mass selective detector using a DB-5 column (0.25 mm × 30 m).

Animal Material. The soft coral *Lemnalia* sp. (C025175) was collected at a depth of 10 m in the South China Sea, Malaysia, on April 11, 2003. The soft coral was frozen immediately upon collection and shipped to NCI (National Cancer Institute, Frederick, MD). After aqueous extraction of the frozen soft corals at 4 °C, the extracts were lyophilized and extracted subsequently with CH_2Cl_2−MeOH (1:1) and MeOH. The combined organic extracts were evaporated *in vacuo* and stored at −30 °C. A voucher specimen was deposited at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Extraction and Isolation. A crude organic extract (5.0 g) derived from 120 g wet weight of the initial collection (provided by the NCI Natural Products Open Repository) showed inhibitory activity in the HFI assay at 80 μg/disk. The residue (5.0 g) was chromatographed over Si gel (250 g) using $CHCl_3$ and then $CHCl_3$−MeOH mixtures of increasing polarity to afford six crude fractions. Of these, fractions 3−5 showed inhibitory activity in the HFI assay. Repeated Si gel CC on fraction 4 afforded fractions 4-1 and 4-2. Repeated application of fraction 4-2 to Si gel gave compound **5** (50 mg). Fraction 3 was subjected to a Si gel column eluting with $CHCl_3$−acetone (8:1) to afford fractions 3-1 and 3-2. Fraction 3-2 was subjected to RP HPLC eluting isocratically with MeOH−H_2O (85:15) to afford compound **1** (15 mg). Compound **2** (40 mg) was obtained from fraction 3-1 by a repeated Si gel column and then Sephadex LH-20 CC ($CHCl_3$−MeOH, 1:1). Fraction 5 was applied to a Si gel column to afford fractions 5-1 and 5-2. Fraction 5-2 was further purified by HPLC eluting isocratically with MeOH−H_2O (70:30) to give pure compound **3** (2.1 mg), and fraction 5-2 was purified by HPLC eluting isocratically with MeOH−H_2O (75:25) to afford compound **4** (1.3 mg).

Lemnaloside A (1): colorless oil; $[\alpha]_D^{20}$ +91.3 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (1.5) nm; IR (KBr) ν_{max} 3415 (br, OH), 2922, 1730 (ester carbonyl), 1377, 1249, 1079 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 517.3119 $[M + Na]^+$ (calcd for $C_{28}H_{46}NaO_7$, 517.3136).

Lemnaloside B (2): colorless oil; $[\alpha]_D^{20}$ +41.4 (*c* 0.41, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (1.5) nm; IR (KBr) ν_{max} 3411 (br, OH), 2928, 1729 (ester carbonyl), 1377, 1257, 1157, 1075, 808, 758 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 517.3112 $[M + Na]^+$ (calcd for $C_{28}H_{46}NaO_7$, 517.3136).

Lemnaloside C (3): colorless oil; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 549.3032 for $[M + Na]^+$ (calcd for $C_{28}H_{46}NaO_9$, 549.3034).

Lemnaloside D (4): colorless oil; $[\alpha]_D^{20}$ +39.2 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 226 (4.20) nm; IR (KBr) ν_{max} 3416 (br, OH), 2922, 1685 (chelated C=O), 1376, 1269, 1074 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 489.2799 $[M + Na]^+$ (calcd for $C_{26}H_{42}NaO_7$, 489.2823).

Acid Hydrolysis of Compounds 1 and 2. A solution of **1** (7.0 mg) dissolved in THF (0.5 mL) and in 1 N HCl (1 mL) was stirred at 80 °C for 4 h. After cooling to room temperature, the reaction mixture was separated with $CHCl_3$ and H_2O. The $CHCl_3$ layer was dried and then purified on Si gel chromatography [*n*-hexanes−acetone (40:1)] to give a dehydration byproduct, biflora-4,9,15-triene (2 mg). The H_2O layer was dried under vacuum and redissolved with a solution of 1-(trimethylsilyl)imidazole in pyridine (0.5 mL), and the solution was stirred at 60 °C for 30 min. After drying the solution, the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was analyzed by GC-MS using a DB-5 column (0.25 mm × 30 m) with a HP 5971A mass selective detector. Temperatures of the injector and detector were 250 and 280 °C, respectively. A temperature gradient system was used for the oven, starting at 90 °C for 3 min and increasing to 250 °C at a rate of 5 °C/min. Peaks of the hydrolysate were detected by comparison with retention time (t_R = 24.36 min) of an authentic sample of D-galactose (Acros) after treatment with 1-(trimethylsilyl)imidazole and pyridine (0.5 mL). Treated in the same way, standard D-glucose (Acros) gave a peak at t_R = 25.01 min. Analyses of **2**, **3**, and **4** were carried out following the same method as for **1**.

Hyphae Formation Inhibition Assay. The inhibition of hyphae formation assay in *Streptomyces* 85E was performed on purified isolates as described previously.[4] The mycelia fragments of *Streptomyces* were spread on minimal medium ISP 4 agar plates for the generation of a bacteria lawn. Compounds of known concentration dissolved in MeOH were dispensed onto disks in 20 μL aliquots. The air-dried disks were applied directly onto the plates and incubated at 30 °C. After 30 h of growth (during which the development of hyphae in *Streptomyces* species takes place), the results are identified by a clear zone of inhibition or bald phenotype around the disk. Surfactin, a sporulation inhibitor, and MeOH were employed as positive and negative controls, respectively. An inhibition zone > 9 mm is acceptable and considered active. Subfractions were tested at 80 μg/disk on 6 mm filter disks. Pure compounds will be retested again at lower concentrations (20, 10, 5, 2.5, 1.25 μg/disk). The assays were performed in duplicate.

Acknowledgment. We are grateful to J. Davies for providing the strain of *Streptomyces* 85E; G. Cragg, D. Newman, and E. Brown for providing extracts from the NCI Natural Products Open Repository; and I. Somasuntharam for performing the HFI assay. This work was supported, in part, by an American Society of Pharmacognosy Research Starter Grant and Tackling Interdisplinary Research Roadblocks (TIRR) Grants from University of Minnesota Chemical Biology Initiative (to L.C.C). We are particularly grateful to an anonymous reviewer for insightful advice on sugar stereochemistry.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for lemnalosides A−D (**1**−**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Persidis, A. *Nat. Biotech.* **1988**, *16*, 1082−1083.
(2) Cohen, P. *Nat. Rev. Drug Discovery* **2002**, *1*, 309−315.
(3) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and references cited in previous reviews in this series.
(4) Waters, B. D.; Saxena, G.; Wanggui, Y.; Kau, D.; Wrigley, S.; Stokes, R.; Davies, J. *J. Antibiot.* **2002**, *55*, 407−416.
(5) Matsumoto, A.; Hong, S. K.; Ishizuka, H.; Horinouchi, S.; Beppu, T. *Gene* **1994**, *146*, 47−56.
(6) Urabe, H.; Ogawara, H. *Gene* **1995**, *153*, 99−104.
(7) Vomastek, T.; Nádvorník, R.; Janecek, J.; Techniková, Z.; Weiser, J.; Branny, P. *Eur. J. Biochem.* **1998**, *257*, 55−61.
(8) Hong, S.-K.; Matsumoto, A.; Horinochi, S.; Beppu, T. *Mol. Gen. Genet.* **1993**, *236*, 347−354.
(9) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2006**, *23*, 26−78; **2005**, *22*, 15−61; **2004**, *21*, 1−49; **2003**, *20*, 1−48.
(10) El-Gamal, A. A. H.; Chiu, E.-P.; Li, C.-H.; Cheng, S.-Y.; Dai, C.-F.; Duh, C.-Y. *J. Nat. Prod.* **2005**, *68*, 1749−1753.
(11) Duh, C.-Y.; El-Gamal, A. A. H.; Song, P.-Y.; Wang, S.-K.; Dai, C.-F. *J. Nat. Prod.* **2004**, *67*, 1650−1653.
(12) Jurek, J.; Scheuer, P. J. *J. Nat. Prod.* **1993**, *56*, 508−513.
(13) Bowden, B. F.; Coll, J. C.; Engelhardt, L. M.; Tapiolas, D. M.; White, A. H. *Aust. J. Chem.* **1986**, *39*, 103−121.
(14) Kikuchi, H.; Tsukitani, Y.; Yamada, Y.; Iguchi, K.; Drexler, S. A.; Clardy, J. *Tetrahedron Lett.* **1982**, *23*, 1063−1066.
(15) Izac, R. R.; Fenical, W.; Tagle, B.; Clardy, J. *Tetrahedron* **1981**, *37*, 2569−2573.
(16) Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1980**, *33*, 2737−2747.
(17) Bowden, B. F.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 681−684.
(18) Huang, Z.-S.; Zhang, M. *Zhong Yao Cai* **1998**, *21*, 296−298.
(19) Daloze, D.; Braekman, J. C.; Georget, P.; Tursch, B. *Bull. Soc. Chim. Belg.* **1977**, *86*, 47−54.
(20) Kikuchi, H.; Manda, T.; Kobayashi, K.; Yamada, Y.; Iguchi, K. *Chem. Pharm. Bull.* **1983**, *31*, 1086−1088.
(21) Carney, J. R.; Pham, A. T.; Yoshida, W. Y.; Sheuer, P. J. *Tetrahedron Lett.* **1992**, *33*, 7115−7118.
(22) Zhang, B.-J.; Huang, Z.-S.; Li, H.-T.; Qiu, G.-G. *Tianran Canwu Yanjiu Yu Kaifa* **2003**, *15*, 487−489.
(23) Rudi, A.; Levi, S.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2002**, *65*, 1672−1674.
(24) Zhang, M.; Huang, Z.-S. *J. Nat. Prod.* **1998**, *61*, 1300−1301.
(25) Zhang, M.; Long, K.-H; Wu, H.-M; Ma, K. *J. Nat. Prod.* **1994**, *57*, 155−160.
(26) Long, K.-H.; Zhang, M. *Zhongshan Daxue Xuebao, Ziran Kexueban* **1991**, *30*, 58−61.
(27) Plaza, A.; Cinco, M.; Tubaro, A.; Pizza, C.; Piacente. *J. Nat. Prod.* **2003**, *66*, 1606−1610.
(28) Cioffi, G.; Piaz, F. D.; Capraríis, P. D.; Sanogo, R.; Marzocco, S.; Autore, G.; Tommasi, N. D. *J. Nat. Prod.* **2006**, *69*, 1323−1329.
(29) Ishitsuka, M. I.; Kusumi, T.; Ichikawa, A.; Kakisawa, H. *Phytochemistry* **1990**, *29*, 2605−2610.
(30) Sakurai, H.; Hosomi, A.; Saito, M.; Sasaki, K.; Ignchi, H.; Sasaki, J.; Araki, Y. *Tetrahedron* **1983**, *39*, 883−894.
(31) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359−2368.
(32) Duh, C. Y.; Chia, M. C.; Wang, S. K.; Chen, H. J.; Elgamal, A. A. H.; Dai, C. F. *J. Nat. Prod.* **2001**, *64*, 1028−1031.
(33) Iguchi, K.; Sawai, H.; Nishimura, H.; Fujita, M.; Yamori, T. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 131−136.

NP0603424