Cembrane Diterpenes from the Gorgonian *Lophogorgia peruana*

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The chemical study of the gorgonian *Lophogorgia peruana* collected at the Gulf of California has led to the isolation of the new diterpenes lophodienone (**2**), lophodiol A (**3**), lophodiol B (**4**), 17-acetoxylophotoxin (**5**), 15,16-epoxylophotoxin (**6**), 17-acetoxy-15,16-epoxylophotoxin (**7**), and isoepoxylophodione (**8**), together with the known compounds lophotoxin (**1**), rubifolide (**9**), lopholide (**10**), deoxylophotoxin (**11**), and the lophotoxin analogues **12** and **13**. The structures of the new metabolites have been established by spectroscopic techniques and chemical correlation. The in vitro cytotoxicity has been tested against three tumor cell lines.

Gorgonian octocorals are a prolific source of natural products such as acetogenins, mono- and polyhydroxylated steroids, and terpenes. Cembrane-type diterpenes and their cyclized derivatives are the most abundant metabolites.[1] Although these compounds seem to play an important role in chemical defense against other corals, fishes, or microorganisms,[2] previous work demonstrates that some cembrane derivatives also possess significant biomedical activities, including anti-inflammatory,[3] Ca-antagonistic,[4] antiparasitic,[5] and, more frequently, cytotoxic properties.[6,7]

As a part of our research on marine organisms of the Gulf of California (Mexico), we have investigated the gorgonian *Lophogorgia peruana* (Verrill, 1868). Gorgonians from this genus have been the source of sesquiterpenes such as (+)-lepidozene,[8] hydroxylated steroids,[9] and diketone cembranolides.[10] The best known compound is the furanocembranolide lophotoxin (**1**), a neuromuscular toxin first isolated from *L. rigida*.[11] More recently, the study of *L. violacea* from Brazil has also led to the isolation of lophotoxin (**1**) together with four related compounds displaying feeding-deterrent activity.[12]

Results and Discussion

Freeze-dried specimens of *L. peruana* were extracted with acetone/MeOH (1:1), and the resulting residue was partitioned between H_2O and Et_2O. The organic layer was concentrated under reduced pressure to give an extract that showed growth inhibitory activity of the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). This extract was subjected to column chromatography eluting with hexane/Et_2O mixtures of increasing polarities, then $CHCl_3$/MeOH, and finally MeOH. Cytotoxicity-guided isolation of selected fractions afforded the new diterpenes lophodienone (**2**), lophodiol A (**3**), lophodiol B (**4**), 17-acetoxylophotoxin (**5**), 15,16-epoxylophotoxin (**6**), 17-acetoxy-15,16-epoxylophotoxin (**7**), and isoepoxylophodione (**8**), along with the known compounds lophotoxin (**1**),[5,11] rubifolide (**9**),[13] lopholide (**10**),[5,14] deoxylophotoxin (**11**),[12] and the lophotoxin analogues **12** and **13**.[12]

Compound **2** was isolated as a colorless oil. Its HREIMS exhibited a molecular ion peak at *m*/*z* 302.2254, consistent with the molecular formula $C_{20}H_{30}O_2$ and six degrees of unsaturation. The NMR resonances (Table 1) comprising three olefinic protons [δ_H 6.42 (1H, dd, J = 15.6, 11.0 Hz, H-6), 6.00 (1H, brd, J = 11.0 Hz, H-5), and 5.94 (1H, dd, J = 15.6, 7.9 Hz, H-7)], four olefinic carbons [δ_C 144.3 (d, C-7), 136.6 (s, C-4), 132.4 (d, C-5), and 125.3 (d, C-6)], and one ketone carbonyl [δ 208.0 (s, C-3)] suggested the presence of a conjugated dienone moiety. This assumption was supported by the IR absorptions at 1685 and 1643 cm^{-1} and the UV absorption λ_{max} 272 nm. The ^1H NMR spectrum of **2** further exhibited the resonances of an isopropenyl group [δ 4.77 (1H, m, H-16b), 4.76 (1H, brs, H-16a), and 1.66 (3H, d, J = 0.6 Hz, H-17)] and three methyl groups [δ 1.99 (3H, d, J = 1.2 Hz, H-18), 1.05 (3H, d, J = 6.6 Hz, H-19), and 1.14 (3H, s, H-20)]. Two signals in the ^{13}C NMR spectrum at δ 61.4 (s, C-12) and 61.8 (d, C-11), the latter coupled in the HSQC spectrum with a proton at δ 2.67 (1H, dd, J = 6.8, 4.7 Hz, H-11), established a trisubstituted epoxide function in the molecule. The remaining signals of the NMR spectra were due to five methylenes and two methines. These structural features accounted for five degrees of unsaturation and then compound **2** had to contain an additional ring. Analysis of the COSY and HMBC spectra were diagnostic in determining that compound **2** possessed a 14-membered cembrane-type diterpenoid skeleton and in locating the dienone and epoxide moieties. Thus, the carbonyl group at δ 208.0 (s, C-3) showed HMBC correlations (Table 1) with two methylene protons at δ 2.52 (1H, dd, J = 20.8, 11.9 Hz, H-2β) and 2.42 (1H, dd, J = 20.8, 11.9 Hz, H-2α), which, in turn, were correlated with the quaternary carbon of the isopropenyl group (δ 145.5). These data permitted location of the carbonyl group at C-3. The presence of the C_4-C_5 and C_6-C_7 double bonds was further supported by the HMBC correlations of the ketone carbonyl with the methyl group at δ 1.99 (Me-18) and the olefinic proton at δ 6.00 (H-5), which in turn was correlated with the olefinic carbon at δ 144.3 (C-7). In the NOESY spectrum the olefinic proton H-7 (δ 5.94) showed NOE interactions with H-5 (δ 6.00), which further interacted with Me-18 (δ 1.99), indicating *Z* and *E* geometry for the C_4-C_5 and C_6-C_7 double bonds, respectively. The southern part of the molecule was assembled starting from the epoxide proton at δ 2.67 (H-11), which was coupled in the COSY spectrum with two methylene protons at δ 1.59 (2H, m, H-10), which in turn were correlated in the HMBC spectrum with the C-8 allylic methine at δ 36.0.

Although NOE effects can be controversial in the assignment of the relative configuration of conformationally flexible molecules, the correlations observed for compound **2** provided reasonable evidence to support the relative configurations depicted in Figure 1. Thus, Me-19 exhibited NOE associations with H-7 and H-9β. In addition, the olefinic proton H-6 showed an NOE with H-1, indicating a β orientation for this proton and thus an α orientation for the isopropenyl group. The relative configuration around the oxirane ring was determined by the NOE interactions of H-11 with H-6, H-1, and H-13α together with those observed for Me-20 with H_2-10, H-13β, and H-14β.

Lophodiol A (**3**) was isolated as an amorphous, white solid whose molecular formula $C_{22}H_{26}O_9$ was deduced from HREIMS data. The

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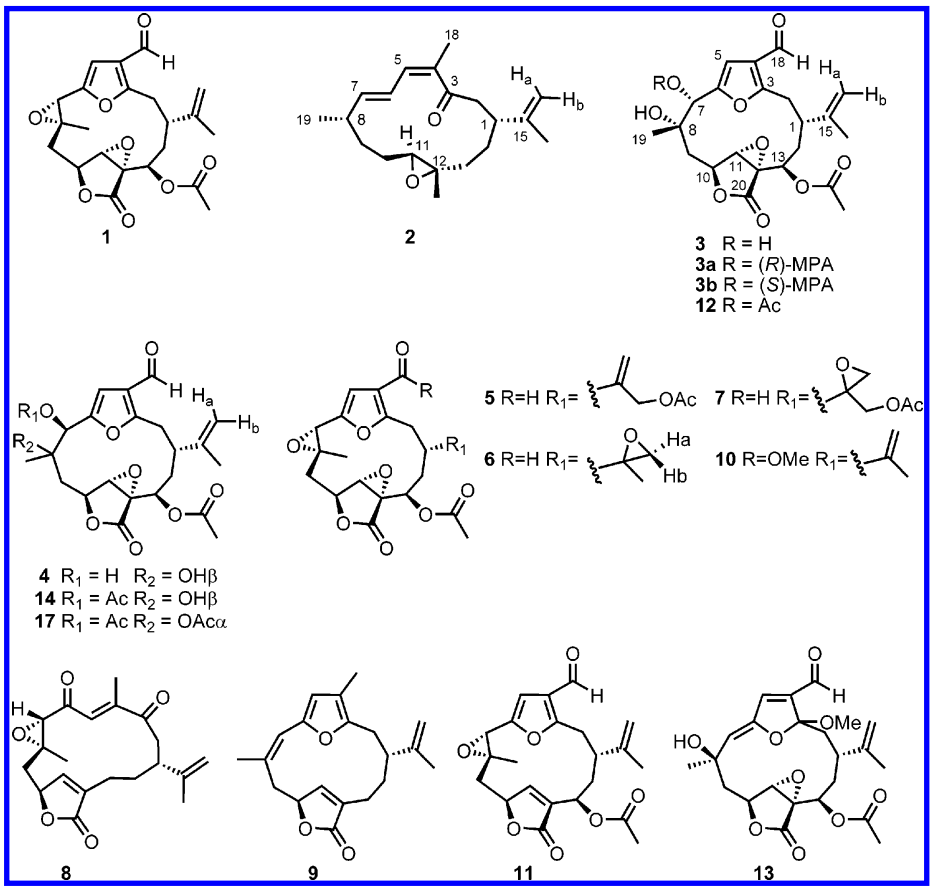
Chart 1



IR spectrum of compound **3** displayed absorptions assigned to hydroxyl (3500 cm^{-1}), γ-lactone (1785 cm^{-1}), ester (1735 cm^{-1}), and aldehyde (1684 cm^{-1}) functionalities. The presence of an acetate group was readily inferred from the NMR signals at δ_H 2.08 (s, 3H), δ_C 20.6 (q) and 170.4 (s) (Tables 2 and 3). The remaining resonances in the NMR spectra were attributable to a cembrane-based diterpene. In particular, the ^{1}H NMR spectrum showed the presence of an aldehyde [δ 9.88 (1H, s, H-18)] and an isopropenyl group [δ 4.88 (1H, brs, H-16b), 4.87 (1H, brs, H-16a), and 1.85 (3H, brs, H-17)], while the NMR resonances at δ_H 6.70 (1H, s) and δ_C 161.9 (s, C-3), 154.3 (s, C-6), 123.9 (s, C-4), and 106.6 (d, C-5) were attributable to an α,α',β-trisubstituted furane ring. In addition, the carbon signals at δ 168.6 (s, C-20), 74.4 (d, C-10), 63.3 (d, C-11), and 59.0 (s, C-12) were consistent with an α,β-epoxy-γ-lactone moiety. These data suggested a furanocembranolide structure for compound **3**, closely related to the known compound lophotoxin (**1**),[11] whose absolute configuration has recently been assigned.[5] The most significant differences were the absence in the NMR spectra of **3** of the resonances due to the 7,8-epoxy group and the presence of those attributable to a 7,8-dihydroxy moiety instead. Thus, a signal in the ^{13}C NMR spectrum at δ 73.4 (d) that was correlated in the HSQC spectrum with the signal at δ 5.24 (1H, brs) and in the HMBC spectrum with Me-19 [δ 1.40 (3H, s, H-19)] was assigned to C-7. The quaternary carbon at δ 74.0 that was correlated in the HMBC spectrum with H-7 (δ 5.24), H-9β (δ 1.76), H-9α (δ 1.55), and Me-19 (δ 1.40) was assigned to C-8 and confirmed the 7,8-dihydroxy substitution pattern.

The NOESY spectrum indicated that compound **3** possessed the same relative configuration as lophotoxin (**1**) at the C-1, C-10, C-11, C-12, and C-13 stereocenters. Finally, the α orientation of the hydroxyl groups at C-7 and C-8 was established from the correlations displayed by Me-19 with the protons H9-α, H-10, and H-7, and between H-7 and H-9α (Figure 2).

Treatment of **3** with Ac_2O/pyridine yielded compound **12**, also isolated as a metabolite of the gorgonian. However, a literature survey revealed that the spectroscopic data and optical rotation of **12** had been previously assigned to structure **14**,[12] a diastereomer of **12** that differs in configuration at C-7 and C-8. To solve this incongruence, we further investigated the configuration of **12** and its parent alcohol **3** through an alternative approach. In particular, derivatization of **3** with (*R*)- and (*S*)-α-methoxy-α-phenylacetic (MPA) acids followed by ^{1}H NMR analysis of the resulting diastereomeric esters **3a** and **3b** revealed negative $\Delta\delta$ ($\delta_R - \delta_S$) values for Me-19 and H-10 (-0.28 and -0.23, respectively) and positive $\Delta\delta$ values for H-5, H-18, and H_2-2 ($+0.22$, $+0.12$, and $+0.23/+0.09$, respectively). Following the MPA rules,[15] these data indicated an *S* configuration for C-7 in **3**. This result, taken together with the NOESY correlations for lophodiol A (Figure 2), supported the structure depicted in formula **3**, which also shows the absolute configuration of the molecule. Following this, the 7-*O*-acetyl derivative of lophodiol A (**3**) possesses a 7*S*,8*S* configuration, as depicted in formula **12**.

The molecular formula of lophodiol B (**4**) was identical to that of **3**, as indicated by HREIMS. The spectroscopic data of **4** were similar to those of **3**, including several broad ^{1}H NMR signals of low intensity due to intramolecular mobility within the 14-membered cycle. The main difference was the downfield shift of H-7 in **4** by $\Delta\delta$ 0.96 ppm [δ 6.20 (s, 1H)], suggesting a different configuration at C-7. Furthermore, upon irradiation of Me-19, NOE effects were observed on H9-β, H-7, and H-5, while the irradiation of H-7 caused NOE effects on Me-19 and H-5, indicating a β orientation for the C-7 and C-8 hydroxyl groups (Figure 2). This data led to the conclusion that lophodiol B (**4**) was 7,8-di-*epi*-lophodiol A.

We tried to establish chemical correlations of the diols **3** and **4** with their co-metabolite lophotoxin (**1**) through opening of the C-7,C-8 epoxide. However, treatment of **1** with different aqueous mixtures (CH_3CN/H_2O/cat. *p*-TsOH, CH_3CN/H_2O/cat. HOAc) led to no reaction or to complex mixtures formed by product degradation. Surprisingly, treatment of **1** with acetone/HOAc/$CrCl_2$ yielded

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Lophodienone (**2**)[a]

carbon	δ_H (mult, *J* in Hz)[b]	δ_C[b]	HMBC
1	2.42 (m)	46.4	C-16, C-17
2	2.42 (dd, 20.8, 11.9)α 2.52 (dd, 20.8, 11.9)*β*	46.4	C-3, C-15, C-14
3		208.0	
4		136.6	
5	6.00 (brd, 11.0)	132.4	C-3, C-7, C-18
6	6.42 (dd, 15.6, 11.0)	125.3	C-4, C-5, C-8
7	5.94 (dd, 15.6, 7.9)	144.3	C-5, C-8
8	2.42 (m)	36.0	
9	1.49 (m)α, 1.77 (dddd, 13.9, 10.0, 3.4, 3.4)*β*	32.9	
10	1.59 (m)	27.3	C-8, C-11
11	2.67 (dd, 6.8, 4.7)	61.8	C-10, C-13
12		61.4	
13	0.64 (td, 12.6, 2.4)α, 1.89 (td, 12.6, 6.2)*β*	38.6	C-1, C-12, C-14, C-20 C-11, C-14, C-20
14	1.46 (m)α, 1.06 (m)*β*	29.6	C-1
15		145.5	
16	4.76 (brs)a, 4.77 (m)b	111.8	C-1, C-15, C-17
17	1.66 (d, 0.6)	18.9	C-1, C-15, C-16
18	1.99 (d, 1.2)	19.8	C-3, C-4, C-5
19	1.05 (d, 6.6)	21.2	C-7, C-8, C-9
20	1.14 (s)	16.3	C-11, C-13

[a] ^{1}H and ^{13}C NMR were recorded in $CDCl_3$, at 600 and 150 MHz, respectively. [b] Assignments aided by COSY, HSQC, HMBC, and NOESY experiments.

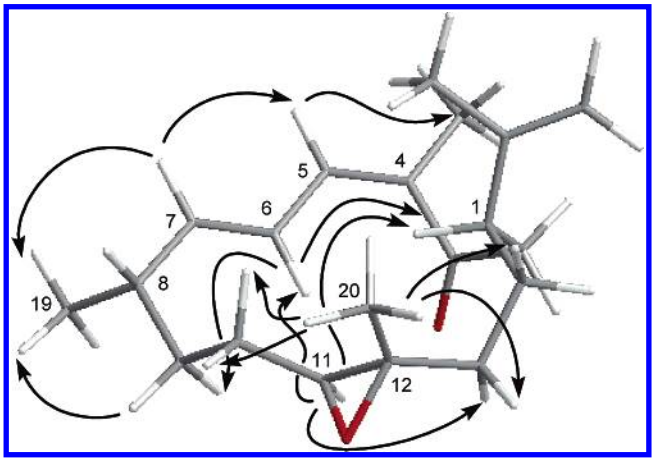


Figure 1. Selected NOESY-1D correlations for compound **2**.

the diol **15** (Scheme 1), a diastereomer of lophodiols A (**3**) and B (**4**). The main differences observed in the ^{1}H NMR spectrum of **15** with respect to those of **3** and **4** were the chemical shifts of Me-19 and H-7 at δ_H 1.49 and 5.72, respectively, indicating that **15** displayed a 7,8-dihydroxy functionality with a different configuration from those of **3** and **4**. The NOESY correlations were especially diagnostic to unravel the configuration at the reaction centers. Thus, Me-19 showed correlations with H-5, H-7, H-9α, and H-9*β*, while H-7 showed additional cross-peaks with H-5 and H-9α. These data were consistent with the 7*R*,8*S* configuration for compound **15** and thus with the structure 7-*epi*-lophodiol A. Taking into account that compound **15** has the opposite configuration at both C-7 and C-8 relative to lophotoxin (**1**), the formation of compound **15** could be explained through the acid-promoted isomerization of the 7*S*,8*R*-epoxide function of lophotoxin (**1**) into the less strained 7*S*,8*S* epoxide **16** and subsequent regioselective opening through S_N2 attack at C-7 (Scheme 1). To confirm this hypothesis, we investigated if the 7*S*,8*S* epoxide could be obtained from diol **15**. Interestingly, when **15** was treated under conventional acetylation conditions, no reaction occurred, but when the reaction was heated to 80 °C, it cleanly gave the epoxide **16** and minor amounts of the peracetylated derivative **17**.

The HREIMS of compound **16** established the molecular formula $C_{22}H_{24}O_8$, indicating that **16** was an isomer of lophotoxin (**1**). Comparison of the ^{1}H NMR spectra of compounds **15** and **16** showed a significant upfield shift of H-7 [δ 3.81 (s)] in **16**, consistent with an epoxide function at C-7,C-8. Furthermore, this proton was correlated in the HSQC spectrum with the carbon at δ 57.2 (d, C-7) and in the HMBC spectrum with C-8 (δ 59.5), C-6 (δ 148.9), C-5 (δ 110.6), and C-19 (δ 21.6). The NOESY correlation observed between H-7 and the Me-19 allowed definition of the configuration 7*S*,8*S* in the epoxide function. All these spectroscopic data and chemical correlations defined the structure 8-*epi*-lophotoxin for compound **16**.

On the other hand, the ^{1}H NMR spectrum of compound **17** differed from that of **15** by the downfield shifts of H-7 [δ 5.82 (s)] and Me-19 [δ 1.72 (s)] and the presence of two additional acetyl groups, inferred from the signals at δ 2.14 (3H, s, 7-OCO*CH*$_3$) and 2.09 (3H, s, 8-OCO*CH*$_3$). These and the remaining spectroscopic features confirmed that compound **17** was the peracetylated derivative of **15**.

17-Acetoxylophotoxin (**5**), isolated from *L. peruana* as a minor constituent, shared many spectroscopic features with lophotoxin (**1**). However, high-resolution mass spectrometry defined the molecular formula $C_{24}H_{27}O_{10}$ for **5**, suggesting the presence of an additional *O*-acetyl group. This assumption was confirmed by the NMR signals at δ_H 2.13 (3H, s) and δ_C 170.6 (s, O*C*OCH$_3$) and 20.9 (q, OCO*C*H$_3$). The additional *O*-acetyl group could be readily accommodated at C-17 upon comparison of the ^{1}H NMR spectra of compounds **1** and **5**. The main difference was the replacement of the Me-17 at δ 1.90 (3H, s) in **1** by an oxygenated methylene in **5**, evidenced by the signals at δ_H 4.79 (1H, d, J = 14.7 Hz, H-17) and 4.82 (1H, d, J = 14.7 Hz, H-17). In addition, the oxygenated methylene carbon at δ_C 65.9 (t, C-17) was correlated in the HMBC spectrum with the olefinic protons H-16 [δ 5.2 (1H, t, J = 1.4 Hz) and 5.1 (1H, s)] defining the structure of compound **5** as 17-acetoxylophotoxin. The correlations observed in the NOESY spectrum indicated that compound **5** possessed the same relative configuration as lophotoxin (**1**).[5,11]

The molecular formula $C_{22}H_{24}O_9$ of compound **6**, established by HRCIMS, contained one oxygen atom more than the molecular formula of lophotoxin (**1**). The comparison of the IR and NMR spectra of **6** with those of **1** confirmed that the α,*β*-epoxy-*γ*-lactone, the trisubstituted furane, and the C-7,C-8-epoxy functionalities of **1** were also present in **6**. However, compound **6** had one oxygen-bearing methylene, as indicated by the ^{13}C NMR signal at δ 55.4 (t, C-16) that was correlated in the HSQC with the protons at δ 3.05 (1H, d, J = 4.6 Hz, H-16a) and 2.83 (1H, d, J = 4.6 Hz, H-16b). These latter signals showed HMBC correlations with a fully substituted carbon at δ 58.2 (s, C-15) and the methine at δ 36.4 ascribed to the C-1 position of the lophotoxin skeleton. Moreover, the Me-17 signal was upfield shifted from δ 1.89 ppm in **1** to δ 1.34 ppm in the new metabolite **6**. These features permitted accommodation of an epoxy group at C-15,C-16 and the establishment of the structure of compound **6** as 15,16-epoxylophotoxin. The correlations observed in the NOESY spectrum were consistent with the relative configuration around the cembrane ring as depicted in the structure **6**, while the configuration at C-15 remains undetermined.

The HRCIMS of compound **7** established the molecular formula $C_{24}H_{26}O_{10}$ and thus 12 degrees of unsaturation. The NMR data of **7** indicated that it was also a furane diterpene related to 17-acetoxylophotoxin (**5**) and 15,16-epoxylophotoxin (**6**). In particular, the most distinctive signals in the NMR spectra of **7** were those corresponding to two oxygen-bearing methylenes [δ_C 50.3 (t, C-16) and δ_H 3.05 (1H, d, J = 4.6 Hz, H-16a)/2.83 (1H, d, J = 4.6 Hz, H-16b); δ_C 64.9 (t, C-17) and δ_H 4.43 (1H, d, J = 12.3 Hz, H-17)/

Table 2. ^{1}H NMR Spectroscopic Data for Compounds **3**, **4**, **15**, **16**, and **8**[a]

carbon	**3** δ (mult, *J* in Hz)	**4** δ (mult, *J* in Hz)	**15** δ (mult, *J* in Hz)	**16** δ (mult, *J* in Hz)	**8** δ (mult, *J* in Hz)
1	3.37 (brm)	3.40 (brm)	3.70 (brt, 11.4)	2.55 (ddd, 13.0, 7.5, 3.7)	2.45 (m)
2	3.02 (dd, 17.4, 5.0) 3.05 (dd, 17.4, 9.1)	3.03 (d, 7.5)	2.94 (dd, 17.7, 3.2)*β*, 3.07 (dd, 17.7, 11.4)α	3.17 (dd, 15.0, 3.7)*β*, 3.23 (dd, 15.0, 13.0)α	3.12 (dd, 12.7, 3.1)*β*, 2.49 (d, 12.7)α
3					
4					
5	6.70 (s)	6.66 (s)	6.79 (s)	6.86 (s)	6.66 (q, 1.3)
6					
7	5.24 (s)	6.20 (s)	5.72 (s)	3.81 (s)	3.61 (s)
8					
9	1.55 (m)α, 1.76 (dd, 14.8, 6.4)*β*	1.52 (m)α, 1.77 (dd, 14.9, 6.4)*β*	1.39 (dd, 15.0, 11.9)α, 1.93 (dd, 15.0, 4.2)*β*	1.67 (m)α, 2.65 (dd, 14.0, 4.7)*β*	1.74 (dd, 15.4, 4.3)*β*, 3.04 (dd, 15.4, 3.7)α
10	4.84 (dd, 10.0, 6.4)	4.82 (dd, 9.6, 6.5)	4.92 (dd, 11.9, 4.2)	4.59 (dd, 12.6, 4.7)	5.24 (m)
11	4.15 (brs)	4.07 (brs)	3.76 (brs)	3.72 (brs)	7.29 (dd, 2.7, 1.4)
12					
13	4.97 (dd, 7.8, 2.4)	4.95 (dd, 7.5, 2.0)	4.87 (d, 7.2)	4.93 (t, 7.0)	2.55 (m)
14	1.72 (brd, 14.7)α 2.50 (ddd, 14.7, 8.0, 7.8)*β*	1.66 (m) α 2.45 (m) *β*	1.47 (d, 15.6)α, 2.63 (dd, 15.6, 11.4)*β*	1.72 (m)α, 2.24 (ddd, 15.0, 7.5, 7.0)*β*	1.66 (m)α, 2.27 (dddd, 14.2, 9.8, 5.7 4.2)*β*
15					
16	4.87 (brs)a, 4.88 (brs)b	4.87 (brs)a, 4.89 (brs)b	4.95 (brs)a, 4.92 (brs)b	4.76 (brs)a, 4.85 (brs)b	4.40 (brs)a, 4.72 (brs)b
17	1.85 (brs)	1.85 (brs)	1.87 (brs)	1.83 (brs)	1.60 (brs)
18	9.88 (s)	9.87 (s)	9.89 (s)	9.94 (s)	2.18 (d, 1.3)
19	1.40 (s)	1.41 (s)	1.49 (s)	1.56 (s)	1.14 (s)
20					
AcO−	2.08 (s)	2.08 (s)	2.07 (s)	2.04 (s)	

[a] Spectra recorded in $CDCl_3$ at 600 MHz. Assignments aided by COSY, HSQC, HMBC, and NOESY experiments.

Table 3. ^{13}C NMR Spectroscopic Data for Compounds **3**, **4**, **15**, **16**, and **8**[a]

carbon	**3**	**4**	**15**	**16**	**8**
1	37.6	37.4	35.8	41.8	44.8
2	32.3	31.9	33.2	30.4	43.0
3	161.9	162.2	161.4	163.3	202.6
4	123.9	124.3	124.5	123.6	150.7
5	106.6	107.1	108.6	110.6	125.7
6	154.3	151.0	152.3	148.9	192.6
7	73.4	74.3	62.2	57.2	67.5
8	74.0	73.7	74.3	59.5	60.0
9	40.9	41.1	40.2	35.9	40.2
10	74.4	74.1	73.6	74.9	78.6
11	63.3	63.1	62.9	62.9	146.1
12	59.0	59.0	59.2	58.4	136.1
13	69.3	69.4	70.0	65.7	23.4
14	32.9	32.6	32.4	34.1	29.4
15	147.0	148.0	147.5	145.7	144.5
16	111.5	111.7	111.7	111.0	113.8
17	20.6	20.6	20.5	21.7	17.5
18	184.4	184.1	184.2	183.8	14.2
19	22.7	23.3	24.1	21.6	17.6
20	168.6	167.9	168.3	167.4	171.9
13-O*CO*CH_3	170.4	170.4	170.5	169.9	
13-OCO*C*H_3	20.6	20.6	20.5	20.7	

[a] Spectra recorded in $CDCl_3$ at 150 MHz. Assignments aided by HSQC and HMBC experiments.

4.18 (1H, d, J = 12.3 Hz, H-17)]. In the HMBC spectrum both methylenes were correlated with an oxygenated carbon at δ 58.6 (s, C-15) and the methine carbon at δ 32.7 (d, C-1), indicating an epoxy group at C-15,C-16 and an *O*-acetyl group at C-17. Therefore, the structure 17-acetoxy-15,16-epoxylophotoxin was determined for compound **7**. The relative configuration of **7** was deduced from the NOESY spectrum, which indicated a relative configuration identical to that of **1** at comparable centers, although the configuration at C-15 remains undetermined.

The molecular formula of isoepoxylophodione (**8**), $C_{20}H_{24}O_5$, was determined by HRCIMS. ^{1}H NMR data (Table 2), including correlations from COSY, illustrated diagnostic differences with respect to the compounds above-described. Particularly, the ^{1}H NMR signal at δ 7.29 (1H, dd, J = 2.7, 1.4 Hz, H-11) suggested the replacement of the α,*β*-epoxy-*γ*-lactone unit by an α,*β*-unsaturated-*γ*-lactone moiety. This feature was also evidenced by the ^{13}C NMR signals observed at δ 146.1 (d, C-11), 136.1 (s, C-12), and 171.9 (s, C-20). Furthermore, the aldehyde group at C-18, present in the lophotoxin analogues, was absent in **8**, being replaced by a signal at δ 2.18 (3H, d, J = 1.3 Hz, H-18) attributed to a methyl group at C-18. In the NMR spectra, the signals corresponding to the furane system were also absent, being replaced by the signals of two ketone carbonyls [δ 202.6 (s, C-3) and 192.6 (s, C-6)] and a trisubstituted double bond [δ_C 150.7 (s, C-4), 125.7 (d, C-5), and δ_H 6.66 (s, H-5)]. This olefinic proton showed long-range correlations in the HMBC with both ketone carbonyls and with Me-18, indicating the presence of an α-methyl-α,*β*-unsaturated 1,4-diketone moiety. The *E* geometry of the C-4,C-5 double bond was supported by the chemical shift of C-18 at δ 14.2 and the absence of a NOESY cross-peak between Me-18 and the olefinic proton H-5.

The relative configuration of **8** was deduced by combination of the NOESY data with the ^{1}H−^{1}H coupling constants. In particular, the NOESY cross-peaks for H-16a/H-2α, H-5/H_2-2, H-5/H-7, H-7/H-9*β*, Me-19/H-9α, H-10/H-9α, H-10/H-11, H-11/H-13, and H-11/H-14α supported the configuration depicted in structure **8**.

Lophotoxin (**1**) and the new compounds (**2**−**8**) isolated from *L. peruana* together with the semisynthetic derivatives **15**−**17** were tested in assays directed to detect in vitro cytotoxic activity against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma) (Tables 4, 5, and 6). Compounds **1**, **2**, and **3** were inactive against the three tumor cell lines at concentrations lower than 25 μM, while compounds **4** and **5** were only mildly active. Compounds **6**−**8** and **15**−**17** showed a significant activity against the three tumor cell lines, with most of the values lower than 5.0 μM. Compound **8** was the most active, with GI_{50} values of 2.7, 2.9, and 4.1 μM against MDA-MB-231, A-549, and HT-29, respectively. In addition, compounds **6**−**8** and **15**−**17** showed total growth inhibition (TGI) values lower than 10 μM, as shown in Table 5. Finally, LC_{50} values (Table 6) lower than 10 μM were registered for compounds **6**, **8**, and **15**−**17**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX spectrophotometer. UV

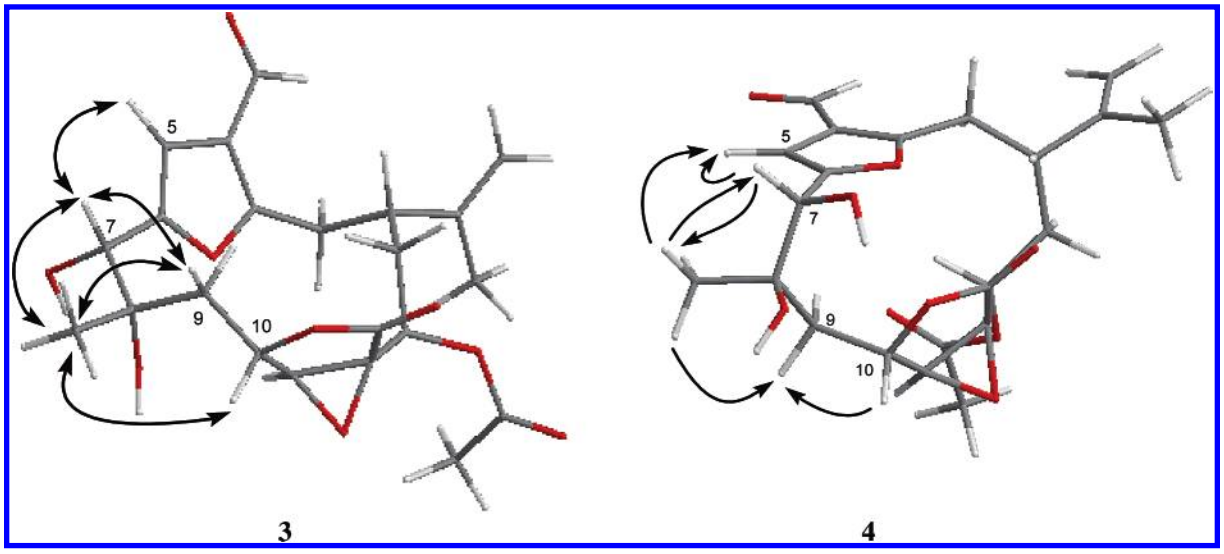


Figure 2. Selected NOE effects for compounds **3** (from NOESY, ↔) and **4** (from NOESY-1D, →).

spectra were obtained on a Philips PU 8710 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 600 spectrometer using $CDCl_3$ as solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at δ 7.26, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ 77.0. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Low-resolution mass spectra were recorded on a Finnigan Voyager GC8000^{top} spectrometer. High-resolution mass spectra (HRMS) were obtained on a Autospec-Q spectrometer. Column chromatography was carried out on Merck silica gel 60 (70−230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal phase and LiChrosorb RP-18 columns in reversed phase, using a differential refractometer RI-71 or an UV detector. All solvents were spectral grade or were distilled prior to use.

Collection and Identification. Specimens of *L. peruana* (family Gorgoniidae, order Alcyonacea, subclass Octocorallia, class Anthozoa) were collected by hand using scuba at the Gulf of California and liophilized. A voucher specimen is stored at the collection of the Laboratorio de Ecología del Bentos, Instituto de Ciencias del Mar y Limnología (UNAM), with the code M149.

Extraction and Isolation. Freeze-dried specimens of the octocoral *L. peruana* (178 g) were extracted for 2 h with 3.5 L of acetone/MeOH (1:1) at room temperature. After filtration, the solution was evaporated under reduced pressure (bath T_a = 30 °C) to obtain a residue that was partitioned between H_2O and Et_2O. The organic layer was evaporated to dryness to give an extract (4.5 g), which was chromatographed on a SiO_2 column using solvents of increasing polarities from hexane to Et_2O, then $CHCl_3$/MeOH (8:2), and finally MeOH. The fraction eluted with hexane/Et_2O (8:2) was repeatedly purified by normal-phase HPLC using mixtures of hexane/EtOAc (9:1, 85:15, and 8:2) to yield compounds **2** (13.3 mg, 0.007% dry wt) and **9** (321.5 mg, 0.18% dry wt). Fractions of the general chromatography eluted with $CHCl_3$/MeOH (8:2) were subjected to column chromatography using solvents of increasing polarities from hexane to Et_2O and then $CHCl_3$/MeOH mixtures (9:1 and 8:2). The fraction eluted with hexane/Et_2O (1:9) was subjected to repeated normal-phase HPLC using hexane/AcOEt (6:4 and 1:1) to give **10** (7.8 mg, 0.0044% dry wt), **4** (3.0 mg, 0.0017% dry wt), and **11** (1.0 mg, 0.0006% dry wt). The fractions eluted with Et_2O and $CHCl_3$/MeOH (9:1) were subjected to normal- and reversed-phase HPLC using $CHCl_3$/MeOH (98:2) or MeOH/H_2O (9:1 and 8:2), respectively, to give compounds **5** (1.0 mg, 0.0006% dry wt), **6** (5.0 mg, 0.0028% dry wt), **7** (1.7 mg, 0.001% dry wt), **8** (1.2 mg, 0.0007% dry wt), **12** (4 mg, 0.0022% dry wt), and **13** (1.3 mg, 0.0007% dry wt). Finally, the fraction eluted with $CHCl_3$/MeOH (8:2) yielded, after purification on normal-phase HPLC using $CHCl_3$/MeOH (98:2), compounds **1** (130 mg, 0.073% dry wt) and **3** (5 mg, 0.0028% dry wt).

Lophodienone (2): colorless oil; $[\alpha]^{25}_D$ −51.0 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 2925, 1685, 1643, 1454, 1381, 892 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 272 (3.7); ^{1}H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; EIMS *m*/*z* 302 (3) $[M]^+$, 245 (4), 161 (59), 108 (100); HREIMS *m*/*z* 302.2254 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Lophodiol A (3): amorphous solid; $[\alpha]^{25}_D$ −6.8 (*c* 0.05, $CHCl_3$); IR (film) ν_{max} 3500, 2932, 1785, 1735, 1684, 1220 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 272 (3.0); ^{1}H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 3; EIMS *m*/*z* 434 (5) $[M]^+$, 416 (20), 374 (9), 356 (24), 237 (40), 137 (100); HREIMS *m*/*z* 434.1587 (calcd for $C_{22}H_{26}O_9$, 434.1577).

Lophodiol B (4): amorphous solid; $[\alpha]^{25}_D$ −10.0 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3500, 2926, 1785, 1735, 1684, 1238 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 271 (3.4); ^{1}H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 3; EIMS *m*/*z* 434 (10) $[M]^+$, 416 (18), 400 (7), 374 (7), 356 (26), 338 (26), 237 (44), 137 (100); HREIMS *m*/*z* 434.1595 (calcd for $C_{22}H_{26}O_9$, 434.1577).

17-Acetoxylophotoxin (5): colorless oil; $[\alpha]^{25}_D$ −12.7 (*c* 0.12, $CHCl_3$); IR (film) ν_{max} 2926, 1785, 1736, 1232 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.87 (1H, s, H-18), 6.57 (1H, d, *J* = 1.1 Hz, H-5), 5.2 (1H, t, *J* = 1.4, H-16b), 5.1 (1H, s, H-16a), 5.01 (1H, d, *J* = 7.0 Hz, H-13), 4.82 (1H, d, *J* = 14.7 Hz, H-17), 4.80 (1H, dd, *J* = 4.4, 2.9 Hz, H-10), 4.79 (1H, d, *J* = 14.7 Hz, H-17) 4.17 (1H, s, H-11), 4.09 (1H, s, H-7), 3.94 (1H, td, *J* = 10.5 and 2.6 Hz, H-1), 3.18 (1H, dd, *J* = 18.5 and 2.6 Hz, H-2α), 2.97 (1H, dd, *J* = 18.5 and 12.5 Hz, H-2*β*), 2.54 (1H, ddd, *J* = 15.7, 10.5 and 7.0 Hz, H-14*β*), 2.52 (1H, dd, *J* = 15.4 and 2.6 Hz, H-9α), 2.13 (3H, s, 17-OCOC*H*$_3$) 2.08 (1H, m, H-9*β*), 2.04 (3H, s, 13-OCOC*H*$_3$), 1.75 (1H, d, *J* = 15.7 Hz, H-14α), 1.13 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 184.4 (CH, C-18), 170.6 (C, 17-O*C*OCH$_3$), 170.0 (C, 13-O*C*OCH$_3$), 167.9 (C, C-20), 161.2 (C, C-3), 149.8 (C, C-6), 147.5 (C, C-15), 123.0 (C, C-4), 111.8 (CH_2, C-16), 105.7 (CH, C-5), 76.5 (CH, C-10), 69.9 (CH, C-13), 65.9 (CH_2, C-17), 64.2 (CH, C-11), 61.2 (C, C-12), 56.0 (C, C-8), 55.3 (CH, C-7), 39.1 (CH_2, C-9), 33.7 (CH_2, C-2), 32.5 (CH_2, C-14), 32.4 (CH, C-1), 20.9 (CH$_3$, 17-OCO*C*H$_3$), 20.4 (CH$_3$, 13-OCO*C*H$_3$), 20.3 (CH$_3$, C-19); CIMS *m*/*z* 475 (5) $[M + H]^+$, 415 (100); HRCIMS *m*/*z* 475.1636 (calcd for $C_{24}H_{27}O_{10}$, 475.1604).

15,16-Epoxylophotoxin (6): colorless oil; $[\alpha]^{25}_D$ +3.2 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 2933, 2856, 1784, 1750, 1684, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.90 (1H, s, H-18), 6.57 (1H, d, *J* = 1.1 Hz, H-5), 5.14 (1H, d, *J* = 7.0 Hz, H-13), 4.79 (1H, dd, *J* = 4.4 and 2.6 Hz, H-10) 4.19 (1H, s, H-11), 4.04 (1H, s, H-7), 3.39 (1H, dd, *J* = 18.2 and 2.4 Hz, H-2*β*), 3.21 (1H, td, *J* = 11.7 and 2.4 Hz, H-1), 3.18 (1H, d, *J* = 5.1 Hz, H-16a), 2.95 (1H, dd, *J* = 18.2 and 12.2 Hz, H-2α), 2.69 (1H, d, *J* = 5.1 Hz, H-16b), 2.51 (1H, dd, *J* = 15.4 and 2.6 Hz, H-9α), 2.22 (1H, ddd, *J* = 15.8, 11.7 and 7.0 Hz, H-14*β*), 2.06 (1H, dd, *J* = 15.4, 4.4 Hz, H-9*β*), 1.66 (1H, bd, *J* = 15.8 Hz, H-14α), 2.17 (3H, s, 13-OCOC*H*$_3$), 1.34 (3H, s, H-17), 1.13 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 184.5 (CH, C-18), 170.0 (C, 13-O*C*OCH$_3$), 167.9 (C, C-20), 161.1 (C, C-3), 149.6 (C, C-6), 122.9 (C, C-4), 105.9 (CH, C-5), 76.5 (CH, C-10), 69.6 (CH, C-13), 64.4 (CH, C-11), 61.1 (C, C-12), 58.2 (C, C-15), 55.9 (C, C-8), 55.4 (CH_2, C-16), 55.2 (CH, C-7), 39.1 (CH_2, C-9), 36.4 (CH, C-1), 30.8 (CH_2, C-14), 29.4 (CH_2, C-2),

Scheme 1. Chemical Transformation of Lophotoxin (**1**) into Diol **15**

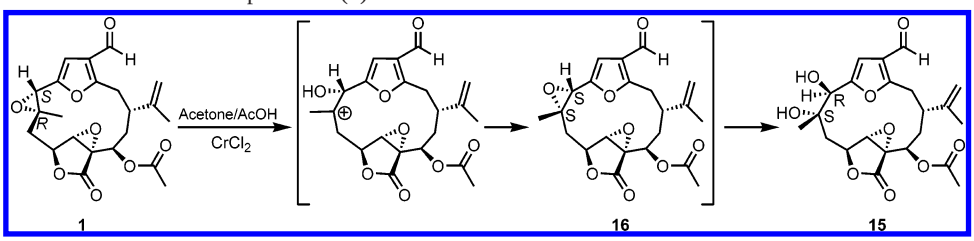


Table 4. GI_{50} Values (μM) in Cytotoxicity Assays for Cembrane Diterpenes from *L. peruana*

	4	**5**	**6**	**7**	**8**	**15**	**16**	**17**
MDA-MB-231		14.5	5.8	5.1	2.7	3.2	4.8	3.7
A-549		17.9	6.0	5.1	2.9	3.9	5.3	3.3
HT-29	14.7		4.6	8.2	4.1	2.8	5.0	6.2

Table 5. TGI Values (μM) in Cytotoxicity Assays for Cembrane Diterpenes from *L. peruana*

	6	**7**	**8**	**15**	**16**	**17**
MDA-MB-231			3.2	4.6	6.2	
A-549	9.7	6.9	5.5		8.4	3.5
HT-29	6.2		4.4	4.8	6.0	6.6

Table 6. LC_{50} Values (μM) in Cytotoxicity Assays for Cembrane Diterpenes from *L. peruana*

	6	**8**	**15**	**16**	**17**
MDA-MB-231		4.1	8.5		
A-549					3.9
HT-29	9.2	4.9		8.4	6.9

21.0 (CH_3, 13-OCO*C*H_3), 20.4 (CH_3, C-19), 17.6 (CH_3, C-17); CIMS *m*/*z* 373 (45) $[M + H - AcOH]^+$, 355 (50); HRCIMS *m*/*z* 433.1527 (calcd for $C_{22}H_{25}O_9$, 433.1499).

17-Acetoxy-15,16-epoxylophotoxin (7): colorless oil; $[\alpha]^{25}_D$ −6.3 (*c* 0.17, $CHCl_3$); IR (film) ν_{max} 2927, 2856, 1784, 1748, 1684, 1234 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.89 (1H, s, H-18), 6.59 (1H, d, J = 1.1 Hz, H-5), 5.2 (1H, d, J = 6.4 Hz, H-13), 4.43 (1H, d, J = 12.3 Hz, H-17), 4.80 (1H, dd, J = 4.4 and 2.6 Hz, H-10), 4.18 (1H, d, J = 12.3 Hz, H-17) 4.20 (1H, s, H-11), 4.05 (1H, s, H-7), 3.55 (1H, brt, J = 12.0 Hz, H-1), 3.43 (1H, dd, J = 18.3 and 2.9 Hz, H-2β), 3.05 (1H, d, J = 4.6 Hz, H-16a), 3.00 (1H, dd, J = 18.3 and 12.0 Hz, H-2α), 2.83 (1H, d, J = 4.6 Hz, H-16b), 2.50 (1H, dd, J = 15.4 and 2.6 Hz, H-9α), 2.18 (3H, s, 13-OCOC*H*$_3$), 2.13 (3H, s, 17-OCOC*H*$_3$), 2.11 (1H, m, H-14β), 2.07 (1H, dd, J = 15.4 and 4.4 Hz, H-9β), 1.63 (1H, d, J = 15.6 Hz, H-14α), 1.13 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 184.6 (CH, C-18), 170.6 (C, 17-O*C*OCH$_3$), 170.0 (C, 13-O*C*OCH$_3$), 168.0 (C, C-20), 160.2 (C, C-3), 149.8 (C, C-6), 123.0 (C, C-4), 106.3 (CH, C-5), 76.6 (CH, C-10), 68.8 (CH, C-13), 64.9 (CH_2, C-17), 64.5 (CH, C-11), 61.3 (C, C-12), 58.6 (C, C-15), 56.0 (C, C-8), 55.1 (CH, C-7), 50.3 (CH_2, C-16), 39.0 (CH_2, C-9), 32.7 (CH, C-1), 30.3 (CH_2, C-2), 29.4 (CH_2, C-14), 21.0 (CH_3, 13-OCO*C*H$_3$), 20.8 (CH_3, 17-OCO*C*H$_3$), 20.4 (CH_3, C-19); CIMS *m*/*z* 491 (5) $[M + H]^+$, 371 (30); HRCIMS *m*/*z* 491.1536 (calcd for $C_{24}H_{27}O_{11}$, 491.1553).

Isoepoxylophodione (8): colorless oil; $[\alpha]^{25}_D$ +58.7 (*c* 0.14, $CHCl_3$); IR (film) ν_{max} 2923, 2853, 1747, 1697, 1254 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 3; CIMS *m*/*z* 345 (55) $[M + H]^+$ 328 (25), 178 (100); HRCIMS *m*/*z* 345.1722 (calcd for $C_{20}H_{25}O_5$, 345.1702).

Acetylation of lophodiol A (3). A solution of compound **3** (1 mg, 2.3×10^{-3} mmol) in a mixture of Ac_2O (0.5 mL) and pyridine (0.7 mL) was stirred at rt for 3 h. Excess reagents were removed by evaporation under reduced pressure to yield a compound identical to the natural metabolite **12** (0.9 mg, 1.9×10^{-3} mmol, 82%): $[\alpha]^{25}_D$ −13.2 (*c* 0.09, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ 9.87 (1H, s, H-18), 6.67 (1H, s, H-5), 6.19 (brs, H-7), 4.95 (1H, dd, J = 7.4 and 2.2 Hz, H-13), 4.89 (1H, s, H-16b), 4.87 (1H, s, H-16a), 4.82 (1H, dd, J = 9.6 and 6.4 Hz, H-10), 4.07 (1H, brs, H-11), 3.40 (1H, brs, H-1), 3.03 (1H, d, J = 7.2 Hz, H$_2$-2), 2.42 (1H, ddd, J = 14.5, 7.8 and 7.8 Hz, H-14β), 2.17 (3H, s, 7-OCOC*H*$_3$), 2.08 (3H, s, 13-OCOC*H*$_3$), 1.77 (1H, dd, J = 14.8, 6.4 Hz, H-9β), 1.66 (1H, bd, J = 14.5 Hz, H-14α), 1.55 (1H, m, H-9α), 1.85 (3H, brs, H-17), 1.42 (3H, s, H-19).

Synthesis of the (*R*)-MPA Ester 3a. A solution of compound **3** (1.3 mg, 3.0×10^{-3} mmol) in 0.25 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (3.0 mg, 1.3×10^{-2} mmol in 0.25 mL), *N*,*N*-dimethylaminopyridine (1.4 mg, 1.1×10^{-2} mmol in 0.25 mL), and (*R*)-α-methoxy-α-phenylacetic acid (2.5 mg, 1.5×10^{-2} mmol in 0.25 mL) and stirred at room temperature for 1 h. The reaction mixture was purified on HPLC (hexane/EtOAc, 1:1) to obtain 1.0 mg (1.7×10^{-3} mmol, 57.3% yield) of (*R*)-MPA ester **3a**. ^1H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by a COSY experiment) δ 9.81 (1H, s, H-18), 6.61 (1H, s, H-5), 6.05 (brs, H-7), 5.03 (1H, dd, J = 7.2 and 3.9 Hz, H-13), 4.85 (1H, brs, H-16), 4.75 (1H, s, H-16), 4.45 (1H, brt, J = 7.3 Hz, H-10), 3.07 (1H, dd, J = 15.8 and 4.3 Hz, H-2β), 2.89 (1H, dd, J = 15.8 and 9.2 Hz, H-2α), 1.81 (3H, brs, H-17), 1.05 (3H, s, H-19).

Synthesis of the (*S*)-MPA Ester 3b. Compound **3** (1.9 mg, 4.4×10^{-3} mmol) was dissolved in 0.25 mL of CH_2Cl_2 and treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (3.0 mg, 1.3×10^{-2} mmol in 0.25 mL), *N*,*N*-dimethylaminopyridine (1.4 mg, 1.1×10^{-2} mmol in 0.25 mL), and (*S*)-α-methoxy-α-phenylacetic acid (2.5 mg, 1.5×10^{-2} mmol in 0.25 mL) as described above (rt., 1 h). The reaction mixture was purified on HPLC (hexane/EtOAc, 1:1) to obtain 0.7 mg (1.2×10^{-3} mmol, 40.1% yield) of (*S*)-MPA ester **3b**. ^1H NMR ($CDCl_3$, 600 MHz, selected data, assignments aided by a COSY experiment) δ 9.75 (1H, s, H-18), 6.39 (1H, s, H-5), 5.95 (brs, H-7), 4.88 (1H, dd, J = 7.4 and 4.2 Hz, H-13), 4.83 (1H, brs, H-16), 4.70 (1H, s, H-16), 4.68 (1H, brt, J = 7.9 Hz, H-10), 2.98 (1H, dd, J = 16.0 and 4.8 Hz, H-2β), 2.66 (1H, dd, J = 16.0 and 8.3 Hz, H-2α), 1.78 (3H, brs, H-17), 1.33 (3H, s, H-19).

Conversion of Lophotoxin (1) into 7-*epi*-Lophodiol A (15). Lophotoxin (**1**, 21 mg, 0.05 mmol) and anhydrous $CrCl_2$ (21 mg, 0.171 mmol) were dissolved in 2 mL of an acetone/HOAc (8:2) mixture and stirred at room temperature for 2 h. The reaction mixture was quenched with 5 mL of water and stirred for 5 min before adding 5 mL of EtOAc. The mixture was filtered through a cotton pad, and the organic layer was washed with $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated to dryness under vacuum to yield compound **15** (15 mg, 3.5×10^{-2}, 69% yield).

7-*epi*-Lophodiol A (15): amorphous solid; $[\alpha]^{25}_D$ −5.4 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3500, 2924, 1782, 1732, 1682, 1242 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 271 (3.4); ^1H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 3; EIMS *m*/*z* 434 (7) $[M]^+$, 416 (9), 398 (20), 374 (20), 356 (30), 338 (41), 156 (100); HREIMS *m*/*z* 434.1580 (calcd for $C_{22}H_{26}O_9$, 434.1577).

Acetylation of 15. A solution of **15** (14 mg, 3.2×10^{-2} mmol) dissolved in Py was treated with Ac_2O at 80 °C for 2 h. The reaction mixture was taken to dryness under reduced pressure and the residue purified by HPLC using hexane/EtOAc (6:4) to yield compounds **16** (8 mg, 1.9×10^{-2}, 60% yield) and **17** (1.2 mg, 2.3×10^{-3}, 6.7% yield).

8-*epi*-Lophotoxin (16): colorless oil; $[\alpha]^{25}_D$ +37.0 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 2928, 1789, 1739, 1682, 1232 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 3; EIMS *m*/*z* 416 (9) $[M]^+$, 356 (28), 136 (100), 122 (89); HREIMS *m*/*z* 416.1437 (calcd for $C_{22}H_{24}O_8$, 416.1472).

7-*epi*-7,8-Di-*O*-acetyllophodiol A (17): colorless oil; $[\alpha]^{25}_D$ +10.8 (*c* 0.08, $CHCl_3$); IR (film) ν_{max} 2924, 1789, 1738, 1682, 1228 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.89 (1H, s, H-18), 6.80 (1H, s, H-5), 5.82 (1H, s, H-7), 5.13 (1H, d, J = 6.4 Hz, H-13), 4.88 (1H, dd, J = 12.0 and 3.6 Hz, H-10), 4.85 (1H, brs, H-16b), 4.74 (1H, brs, H-16a), 4.20 (1H, s, H-11), 3.24 (1H, dd, J = 15.6 and 4.8 Hz, H-2β), 3.11 (1H, dd, J = 15.6 and 9.0 Hz, H-2α), 2.93 (1H, dd, J = 15.0 and 3.6 Hz, H-9α), 2.63 (1H, brm, H-1), 2.14 (3H, s, 7-OCOC*H*$_3$), 2.09 (3H,

s, 8-OCOCH_3)*, 2.08 (3H, s, 13-OCOCH_3)*, 2.24 (1H, m, H-14β), 1.91 (1H, m, H-9β), 1.81 (1H, m, H-14α), 1.81 (3H, s, H-17), 1.72 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 183.9 (CH, C-18), 170.0 (C, 13-O*C*OCH$_3$), 169.8 (C, 8-O*C*OCH$_3$), 169.6 (C, 7-O*C*OCH$_3$), 167.5 (C, C-20), 162.4 (C, C-3), 149.9 (C, C-6), 145.1 (C, C-15), 124.1 (C, C-4), 111.4 (CH_2, C-16), 109.6 (CH, C-5), 80.6 (C, C-8), 75.1 (CH, C-10), 73.4 (CH, C-7), 66.9 (CH, C-13), 63.1 (CH, C-11), 58.4 (C, C-12), 42.0 (CH, C-1), 37.5 (CH_2, C-9), 33.2 (CH_2, C-14), 29.2 (CH_2, C-2), 22.1 (CH_3, 8-OCO*C*H$_3$), 21.3 (CH_3, C-17), 20.8 (CH_3, 13-OCO*C*H$_3$), 20.7 (CH_3, 7-OCO*C*H$_3$), 20.4 (CH_3, C-19); signals with asterisk may be interchanged; EIMS *m*/*z* 518 (4) $[M]^+$, 458 (43), 416 (76), 398 (46), 356 (85), 138 (100); HREIMS *m*/*z* 518.1788 (calcd for $C_{26}H_{30}O_{11}$, 518.1788).

Cytotoxicity Assays. Compounds **2**−**8** and **15**−**17** were tested against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Cytotoxicity assays were performed by PharmaMar. A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability following the method described in the literature.[16]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **2**−**8** and **15**−**17**. This information is available free of charge via the Internet at http://pubs.acs.org.

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