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Diterpenes from the Hainan Soft Coral *Lobophytum cristatum* Tixier-Durivault

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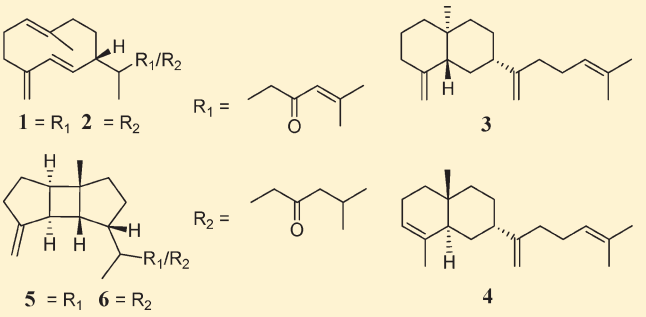
Supporting Information

ABSTRACT: Two new prenylgermacrane-type diterpenoids, lobophytumins A and B (**1** and **2**), two new prenyleudesmane-type diterpenoids, lobophytumins C and D (**3** and **4**), and two new spatane-type diterpenoids, lobophytumins E and F (**5** and **6**), were isolated from the Hainan soft coral *Lobophytum cristatum* Tixier-Durivault. Their structures, including relative configuration, were elucidated by detailed analysis of spectroscopic data and by comparison with related known compounds. In addition, the absolute configuration of lobophytumin C (**3**) was tentatively assigned by comparing its specific rotation with that of the closely related model compound (−)-β-selinene (**8**). On the basis of biogenetic considerations, the absolute configurations of lobophytumins A, B, and D−F were also tentatively suggested. This is the first report of spatane-type diterpenoids from a soft coral source. The present work supports Faulkner's proposal of prenylgermacrene as the precursor of many diterpenes. In a bioassay, lobophytumins C and D (**3** and **4**) showed weak *in vitro* cytotoxicities against the tumor cell lines A-549 and HCT-116.



Soft corals from the South China Sea have been extensively studied by Chinese marine natural product chemists and have yielded a plethora of steroids and terpenoids, the latter mainly including diterpenoids.[1] It has been suggested that such secondary metabolites are probably involved in the defensive mechanisms of the animals, which appear to be relatively free from predation.[2]

The soft corals of the genus *Lobophytum* are prolific in the South China Sea. Recently, as part of our ongoing research project with the purpose of discovering bioactive substances from Chinese marine invertebrates,[3−9] we collected the soft coral *Lobophytum cristatum*, off Lingshui Bay, Hainan Province, China. Chemical investigation of the Et_2O-soluble fraction from the acetone extract of this animal led to the isolation of six new diterpenes, lobophytumins A−F (**1**−**6**), belonging to three different structural classes. The present work deals with the isolation and structure elucidation of these new compounds.

RESULTS AND DISCUSSION

Freshly collected specimens of *L. cristatum* were immediately chilled to −20 °C and kept frozen until they were extracted exhaustively with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble portion was repeatedly subjected to silica gel and Sephadex LH-20 column chromatography followed by reversed-phase HPLC purification, yielding six new diterpenes (**1**−**6**).

Compound **1**, named lobophytumin A, was isolated as an optically active, colorless oil. The molecular formula was determined to be $C_{20}H_{30}O$ by HREIMS (*m*/*z* 286.2281, $C_{20}H_{30}O$), which corresponded to six degrees of unsaturation. Compound **1** exhibited IR absorptions indicative of the presence of a ketone carbonyl (ν_{max} 1760 cm^{-1}). The strong UV absorptions at λ_{max} 238 and 244 nm suggested the presence of two conjugated systems. The ^{1}H NMR spectrum showed two downfield diagnostic broad singlets at δ 4.74 (1H, H-16b) and 4.76 (1H, H-16a) assignable to an exomethylene. In addition, four olefinic protons (δ 02, s, 1H, H-14; 5.78, d, *J* = 15.9 Hz, 1H, H-5; 5.18, dd, *J* = 15.9, 9.9 Hz, 1H, H-6; and 5.10, m, 1H, H-1) attributable to one disubstituted olefin and two trisubstituted carbon−carbon double bonds, respectively, were also observed. Analysis of ^{13}C NMR and DEPT spectra of **1** (Table 1) confirmed the presence of four carbon−carbon

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Chart 1

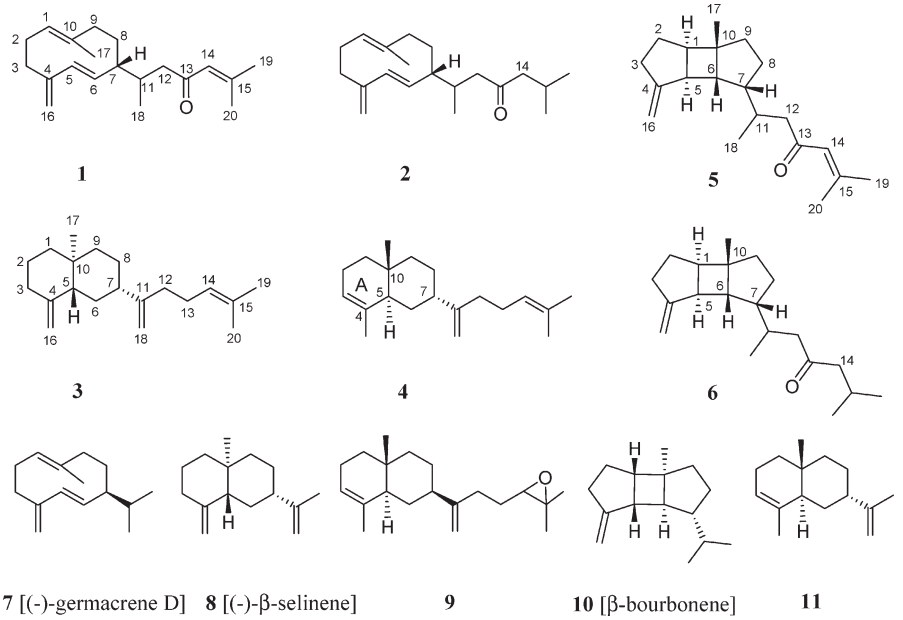

Table 1. ^{13}C NMR (100 MHz, $CDCl_3$) Data for Compounds 1−6[a]

	1	2	3	4	5	6
1	129.8, CH	129.9, CH	42.0, CH_2	40.4, CH_2	45.7, CH	45.7, CH
2	29.2, CH_2	29.2, CH_2	23.5, CH_2	23.0, CH_2	27.2, CH_2	27.2, CH_2
3	34.4, CH_2	34.4, CH_2	36.9, CH_2	120.9, CH	33.7, CH_2	33.7, CH_2
4	148.6, C	148.6, C	151.0, C	135.1, C	157.2, C	157.3, C
5	136.1, CH	136.2, CH	50.1, CH	47.0, CH	47.9, CH	48.0, CH
6	133.4, CH	133.3, CH	30.0, CH_2	29.4, CH_2	55.1, CH	55.2, CH
7	52.0, CH	51.9, CH	44.1, CH	45.7, CH	55.1, CH	55.2, CH
8	26.1, CH_2	26.2, CH_2	27.4, CH_2	27.4, CH_2	29.7, CH_2	29.8, CH_2
9	40.6, CH_2	40.6, CH_2	41.4, CH_2	38.0, CH_2	41.9, CH_2	42.0, CH_2
10	133.8, C	133.8, C	36.0, C	32.4, C	43.2, C	43.2, C
11	34.4, CH	33.7, CH	154.9, C	155.0, C	33.1, CH	32.5, CH
12	49.0, CH_2	48.1, CH_2	35.0, CH_2	34.8, CH_2	50.8, CH_2	49.8, CH_2
13	201.3, C	210.9, C	27.0, CH_2	26.9, CH_2	201.4, C	211.1, C
14	124.4, CH	52.5, CH_2	124.4, CH	124.4, CH	124.2, CH	52.4, CH_2
15	154.3, C	24.5, CH	131.5, C	131.5, C	154.6, C	24.5, CH
16	109.5, CH_2	109.6, CH_2	105.4, CH_2	21.1, CH_3	103.8, CH_2	103.8, CH_2
17	15.9, CH_3	15.8, CH_3	16.4, CH_3	15.7, CH_3	21.5, CH_3	21.4, CH_3
18	18.1, CH_3	18.2, CH_3	107.0, CH_2	107.0, CH_2	18.6, CH_3	18.7, CH_3
19	27.6, CH_3	22.5[b], CH_3	25.7, CH_3	25.7, CH_3	27.6, CH_3	22.5[b], CH_3
20	20.6, CH_3	22.6[b], CH_3	17.7, CH_3	17.7, CH_3	20.6, CH_3	22.6[b], CH_3

[a] Chemical shifts (ppm) referred to $CHCl_3$ (δ 77.0). [b] Interchangeable values.

double bonds, two of which were disubstituted [δ 109.5, C-16; 148.6, C-4 and δ 136.1, C-5; 133.4, C-6] and the others trisubstituted [δ 129.8, C-1; 133.8, C-10 and δ 124.4, C-14; 154.3, C-15]. A ketone carbonyl [δ 201.3, C-13] was also observed in the ^{13}C NMR spectrum of **1**. The ketone carbonyl and four double bonds accounted for five degrees of unsaturation. The remaining degree of unsaturation was attributed to a ring in **1**.

Detailed analysis of the ^{1}H−^{1}H COSY spectrum in combination with HMQC and HMBC experiments allowed the assignment of all of the chemical shifts in the ^{1}H and ^{13}C NMR spectra and led to structure **1**, which has a prenylgermacrane-like skeleton. Comparison of ^{1}H and ^{13}C NMR data of **1** with those of germacrane D (**7**),[10] a common sesquiterpene in many higher plants and lower organisms, further confirmed the assignments for the 10-membered ring of **1**. Support for the rest of the molecule, namely, the eight-carbon side chain, was obtained from the HMBC correlations from H-11 (δ 2.07) to C-13 (δ 201.3) and C-7 (δ 52.0) and from H-14 (δ 6.02) to C-13 (δ 201.3) and C-19 (δ 27.6). The geometries of

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the double bonds at $\Delta^{1(10)}$ and Δ^{5} were presumed to be both *E* from the ^{13}C NMR chemical shift of C-17 (δ 15.9)[11] and the large coupling constant (J = 15.9 Hz) between H-5 and H-6.

There are two stereogenic centers (C-7 and C-11) in the molecule of **1**. The flexibility of the 10-membered ring and the free rotation of the single bond between C-7 and C-11 prevented the determination of the relative configuration between C-7 and C-11 by either a ROESY experiment or a simple chemical reaction. The configuration of C-7, as depicted in **1**, was tentatively assigned on the basis of a biogenetic argument (see below), while the configuration of C-11 remains undefined.

Lobophytumin B (**2**) has the molecular formula $C_{20}H_{32}O$, established by HREIMS (m/z 288.2447), representing two more mass units than that of **1**. Comparison of the ^{1}H and ^{13}C NMR data of **1** and **2** revealed that **2** shares the same germacrane D nucleus as **1**, differing from **1** only at the side chain where the double bond at Δ^{14} was reduced, in agreement with the mass data. The reduction of the double bond caused the ^{13}C NMR resonance of C-13 to be shifted significantly downfield (from δ 201.3 to δ 210.9). The assigned structure for **2** was confirmed by ^{1}H−^{1}H COSY and HMBC spectra. Compound **2** is therefore the 14,15-dihydroderivative of **1**.

Compound **3**, lobophytumin C, was obtained as an optically active, colorless oil {$[\alpha]_{25}^{D}$ −36.6 (*c* 0.45, $CHCl_3$)}. Its molecular formula was established as $C_{20}H_{30}$ on the basis of the observed molecular ion at m/z 272.2488 in its HREIMS spectrum. This formula indicates five degrees of unsaturation in **3**. Analysis of the ^{13}C NMR and DEPT spectra of **3** revealed the presence of three methyls, eight sp^3 methylenes, two sp^2 methylenes, two sp^3 methines, one sp^2 methine, and three sp^2 and two sp^3 quaternary carbons. The presence of two terminal methylenes and one trisubstituted olefin in **3** was confirmed by the diagnostic ^{1}H NMR data: δ 5.13 (1H, m, H-14), δ 4.80 (1H, brs, H-18*Z*), 4.74 (1H, brs, H-18*E*), and δ 4.71 (1H, brs, H-16*E*), 4.44 (1H, brs, H-16*Z*). Three double bonds accounted for three degrees of unsaturation. Thus, the remaining two degrees of unsaturation were attributed to a bicyclic ring system in **3**. With the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC experiments, a prenyleudesmane skeleton was established.

Comparison of ^{13}C NMR data of **3** with those of model compound **8** ((−)-β-selinene)[12] confirmed the bicyclic partial structure, while the downfield ^{1}H NMR chemical shifts of Me-19 (δ 1.69) and Me-20 (δ 1.62) located the double bond at Δ^{14} of the eight-membered side chain. Furthermore, the almost identical ^{13}C NMR data for C-1−C-11, C-16, C-17, and C-18 of **3** and **8** (Table S1) strongly suggested that the relative configurations at C-5, C-7, and C-10 of **3** are the same as those in **8**. The assigned relative configurations at C-5, C-7, and C-10 were further supported by ROESY experiments (Figure 1). Thus, in the ROESY spectrum of **3** (Figure S19), H-5 (δ 1.81) exhibited clear NOE correlations with H-1*ax* (δ 1.26), H-3*ax* (δ 2.01), H-7 (δ 1.95), and H-9*ax* (δ 1.53), suggesting that all of these protons are oriented on the same side (β) of the molecule. Furthermore, the NOE cross-peak observed between H_3-17 (δ 0.76) and H-6*ax* (δ 1.27) and the absence of NOE correlations between H_3-17 and both H-5 and H-7 implied that CH_3-17 and the side chain at C-7 are both α oriented.

As described above, the structure of **3** is closely related to (−)-β-selinene (**8**), the only exception being the side chain at C-7. Moreover, the sign and magnitude of the $[\alpha]_D$ value for compound **3** are similar to those of **8** {$[\alpha]_D$ −46 (*c* 10, $CHCl_3$)}. This fact allowed us to tentatively suggest that the absolute configurations at C-5, C-7, and C-10 of **3** should be the same (5*R*, 7*S*, 10*S*) as those of (−)-β-selinene. The minor structural variation due to the extra "prenyl" group at C-12 of the side chain of **3** is not expected to have a large effect on the specific rotation.

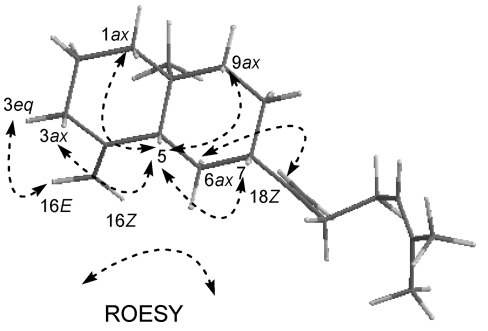


Figure 1. ROESY spectrum of compound **3**.

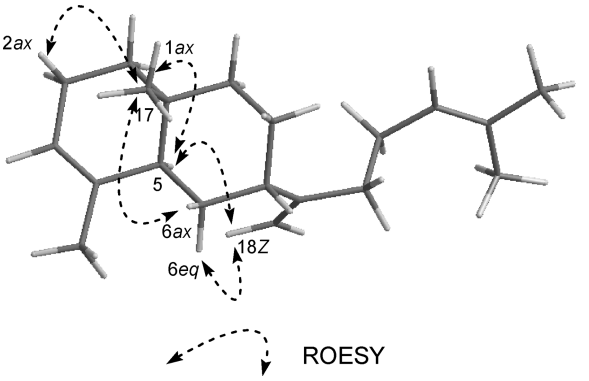


Figure 2. ROESY spectrum of compound **4**.

The establishment of the absolute configuration of **3** gave support for the configurations (*R*) at C-7 of **1** and **2**, which were only arbitrarily assigned above, since compounds **1**−**3** are co-occurring and biogenetically related to each other.

Lobophytumin D (**4**) has the molecular formula $C_{20}H_{30}$, which is identical to that of **3**, as indicated by HREIMS. Careful comparison of the ^{1}H and ^{13}C NMR data of **4** and **3** revealed similarities between them indicating that they share the same prenyleudesmane framework possessing three stereogenic centers at C-5, C-7, and C-10, respectively. In fact, the planar structure of **4** differs from **3** only by the location of the double bond in ring A, where the exomethylene group in **3** was replaced by a vinyl methyl (H_3-16) accompanying the isomerization of the olefin from the $\Delta^{4(16)}$ to Δ^{3}.

Interestingly, although the planar bicyclic structure of **4** is the same as the corresponding part of model compound **9**,[13] their NMR data (Table S1) are somewhat different. In particular, the ^{13}C NMR chemical shifts of C-3, C-5, C-7, and C-10 of **4** were found to be significantly upfield shifted (−3.5, −5.4, −2.7, and −3.4 ppm, respectively) with respect to those of **9**. These facts suggest that the differences in the bicyclic structures of **4** and **9** could be attributed only to the different relative configurations at C-5, C-7, and C-10. This assumption was supported by a ROESY experiment (Figure 2). In the ROESY spectrum of **4** (Figure S26), H-5 (δ 1.91) correlated with H-18*Z* (δ 4.80) and H-1*ax* (δ 1.45); H_3-17 (δ 0.90) exhibited NOE correlationss with H-2*ax* (δ 2.09) and H-6*ax* (δ 1.15), whereas no NOE cross-peaks between H-5 and H-7 (δ 1.95) and H-5 and H_3-17 were

observed, indicating that H-5 and the side chain at C-7 are cofacial (α) and the orientation of CH_3-17 is β. It is worth noting that the NOE correlation between H-5 (δ 1.81) and H-7 (δ 1.95) in the ROESY spectrum of **3** (Figure S19) is very clear.

Finally, on the basis of biogenetic considerations, the absolute configuration at C-7 of **4** was tentatively suggested as *S*. As a consequence, the absolute configurations at C-5 and C-10 were assigned *R* and *R*, respectively.

A literature search revealed that the assigned bicyclic structure of **4** is the same as the corresponding known sesquiterpene **11** (7β*H*-eudesma-3,11-diene).[14] Surprisingly, their ^{13}C NMR data for the bicyclic system are significantly different (Table S1). Unfortunately, the ^{13}C NMR data for **11**, which was prepared as a synthetic intermediate, are incomplete.[14] Additionally, only carbon shifts were reported, without assignments supported by 2D NMR experiments. Therefore, the basis for the NMR differences between **4** and **11** remains to be resolved.

Lobophytumin E (**5**), an optically active, colorless oil, has the molecular formula $C_{20}H_{30}O$ by HREIMS (*m*/*z* 286.2296), indicating six degrees of unsaturation. The IR absorption at 1760 cm^{-1} was indicative of the presence of a ketone carbonyl. A strong UV absorption at $\lambda_{244\ nm}$ (log ε 4.3) suggested the presence of an α,β-unsaturated ketone moiety. The ^{1}H NMR spectrum showed two broad 3H singlets at δ 2.13 (s, H_3-20) and 1.86 (s, H_3-19), attributable to two vinyl methyls. A 3H singlet at δ 1.00 (s, H_3-17) was assigned to a methyl linked to a quaternary carbon, whereas a 3H doublet at δ 0.85 (d, J = 6.2 Hz, H_3-18) was assigned to a methyl linked to an sp^3 methine. In addition, two diagnostic broad singlets at δ 4.69 (1H, H-16b) and 4.72 (1H, H-16a) and a singlet at δ 6.03 (1H, H-14) assignable to an exomethylene and an olefinic proton, respectively, were also observed. The ^{13}C NMR and DEPT spectra of **5** confirmed the presence of two double bonds [δ 103.8 (CH_2) and 157.2 (C); δ 124.2 (CH) and 154.6 (C)], a ketone carbonyl [δ 201.4], and four methyls. The remaining resonances between δ 55.1 and 27.2 were attributed to five methylenes, five methines, and one quaternary carbon. The carbonyl and two carbon−carbon double bonds left three sites of unsaturation, which was attributed to a tricyclic skeleton. The foregoing spectroscopic data indicated that compound **5** was a spatane-type diterpenoid having a conjugated enone moiety in the side chain. Extensive interpretation of the 2D NMR spectra (^{1}H−^{1}H COSY, HMQC, and HMBC) led to complete assignments for the ^{1}H and ^{13}C NMR (Table 1) data of the tricyclic nucleus and the eight-membered side chain. The proposed structure was unambiguously confirmed by comparing ^{1}H and ^{13}C NMR data of **5** with those of model compound **10** (β-bourbonene)[10] and **1**.

The relative configurations at C-1, C-5, C-6, C-7, and C-10 were established by extensive analysis of the ROESY spectrum of **5** (Figures 3 and S33), in which H-5 (δ 2.41) correlated with H-1 (δ 2.32) and H-11 (δ 1.72), suggesting that H-1, H-5, and the side chain at C-7 are oriented on the same side (α) of the molecule. Further, the cross-peaks of H-6 (δ 1.52)/H-7 (δ 1.63) and H_3-17 (δ 1.00) observed in the ROESY spectrum of **5**, as well as the absence of a NOE correlation between H-6 and H-5, implied that these protons are β oriented. The established relative configuration is in accordance with that reported for spatane diterpenoids. Due to the free rotation of the single bond between C-7 and C-11, the configuration of H_3-18 remains undefined.

Once again, by analogy to **4**, the absolute configuration at C-7 of **5** was tentatively assigned as *R*, and consequently, the absolute configurations at C-1, C-5, C-6, and C-10 were suggested as *S*, *S*, *S*, and *S*, respectively.

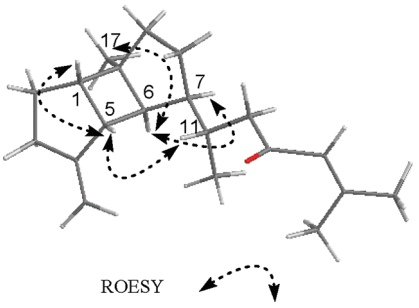
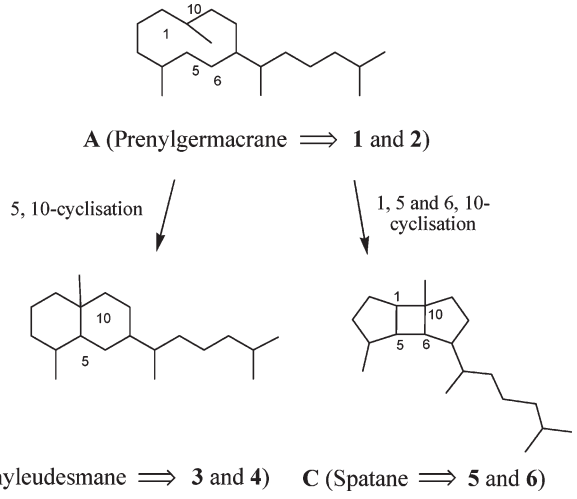


Figure 3. ROESY spectrum of compound **5**.

Scheme 1. Plausible Biogenetic Correlations between the Three Diterpenoid Skeletons



Lobophytumin F (**6**) has a molecular formula of $C_{20}H_{30}O$, established by HREIMS (*m*/*z* 288.2459), which indicated two additional hydrogens compared to **5**. Analysis of the ^{1}H and ^{13}C NMR spectra of **6** revealed a close relationship with both compounds **5** and **2**. In fact, **6** shares the same tricyclic system as **5** and possesses the same side chain as **2**. Detailed analysis of 2D NMR spectra allowed the unambiguous definition of the structure of **6**, which is the 14,15-dihydro derivative of **5**. Finally, the close similarity of the NMR data suggested that both compounds shared the same configurations at C-1, C-5, C-6, C-7, and C-10.

In conclusion, six new diterpenes that belong to three different diterpenoid structural classes were isolated from the Hainan soft coral *L. cristatum*. Although the structures of **1**−**6** are formally quite different, they are biogenetically related to each other. A prenylgermacrene is considered as the common precursor.[15] By analogy to Faulkner's proposal, a plausible biogenetic outline for compounds **1**−**6** is proposed as outlined in Scheme 1.

Prenylgermacrane-type[16,17] and prenyleudesmane-type[13,18−20] diterpenoids are quite rare in soft coral. While spatane-type diterpenes have been found only in brown algae previously,[21] this is the first reported isolation of spatane-type diterpenes from a soft coral source. The discovery of new compounds **1**−**6** has added to an extremely diverse and complex array of soft coral diterpenoids. Further studies should be conducted to unambiguously establish their absolute configurations by total synthesis as well as

to understand their biological/ecological roles in the life cycle of the animal.

All of the diterpenes were examined for growth-inhibition activities *in vitro* toward human lung adenocarcinoma A-549 cells and human colon carcinoma HCT-116 cells. Lobophytumins C (**3**) and D (**4**) showed weak cytotoxicity against the A-549 cell line with IC_{50} values of 35.66 ± 3.20 and 36.69 ± 5.11 μM, respectively. The IC_{50} values of **3** and **4** against the HCT-116 cell line were 37.10 ± 3.49 and 35.74 ± 4.63 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. UV spectra were recorded on a Varian Cary 300 Bio spectrometer. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer. NMR spectra were measured on either a Varian Mercury 400 spectrometer (100 MHz for ^{13}C) or a Varian Mercury 300 (300 MHz for ^{1}H) with residual $CHCl_3$ (δ_H 7.26 ppm; δ_C 77.0 ppm) as an internal standard. EIMS and HREIMS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. Reversed-phase HPLC {Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 10 mm (i.d.) × 250 mm] column} was also employed. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for CC, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Animal Material. The sample of *Lobophytum cristatum* (order Alcyonacea, family Alcyoniidae) was collected, by one of the authors (E.M.), off Lingshui Bay, Hainan Province, China, in July 2004, at a depth of 20 m, and was frozen immediately after collection. The specimen was subsequently identified by Associate Prof. H. Huang of South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (no. LS-335) for this collection is maintained for inspection at the Herbarium of the Shanghai Institute of Materia Medica, CAS. The colony has crestlike lobes and a distinct stalk. The polyps have small dentate rods, 0.05−0.09 mm long, and clublike sclerites of about 0.15 mm long. The surface layer consists of crests with clubs, 0.10−0.20 mm long, the smaller ones with a central wart. The interiors of the crests contain spindles up to 0.35 mm long. The surface layer consists of a base with clubs, 0.10−0.15 mm long, many with a central wart. The interior of the base contains capstans.

Extraction and Isolation. The frozen animals (210 g dry weight) were cut into small pieces and exhaustively extracted with acetone (3 × 3 L) at room temperature. The organic extract was evaporated to give a residue, which was partitioned between Et_2O and H_2O. The Et_2O solution was concentrated under reduced pressure to give a dark brown residue (5.4 g), which was fractionated by gradient Si gel column chromatography [0−100% Et_2O in light petroleum ether (PE)], yielding five fractions (A−E). Fraction B eluted by 95:5 PE/Et_2O was further purified on a second Si gel column eluting with 98:2 PE−Et_2O to afford **3** (9.0 mg) and **4** (8.8 mg). Fraction C, eluted by 9:1 PE/Et_2O, was further chromatographed on a Si gel column, eluting with 95:5 PE/Et_2O, and successively further purified by RP-HPLC to yield **1** (11.7 mg), **2** (10.6 mg), **5** (7.7 mg), and **6** (8.5 mg), respectively.

Lobophytumin A (1): colorless oil; $[\alpha]^{25}_D$ +116 (*c* 1.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (3.6), 244 (4.3) nm; IR (KBr) ν_{max} 2917, 1762, 1714, 1652, 1460, 1380, 957 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.02 (1H, s, H-14), 5.78 (1H, d, *J* = 15.9 Hz, H-5), 5.18 (1H, dd, *J* = 15.9, 9.9 Hz, H-6), 5.10 (1H, m, H-1), 4.76 (1H, brs, H-16a), 4.74 (1H, brs, H-16b), 2.51 (1H, dd, *J* = 15.4, 3.9 Hz, H-12a), 2.36 (1H, m, H-8a), 2.26 (1H, dd, *J* = 12.4, 2.5 Hz, H-9a), 2.20 (1H, m, H-9b), 2.11 (3H, s, H_3-20), 2.07 (3H, m, H-3a, H-3b, H-11), 2.05 (1H, m, H-12b), 2.04 (1H, m, H-7), 1.97 (1H, m, H-8b), 1.85 (3H, s, H_3-19), 1.55 (1H, m, H-2a), 1.49 (3H, s, H_3-17), 1.38 (1H, m, H-2b), 0.87 (3H, d, *J* = 6.7, H_3-18); ^{13}C NMR (see Table 1); EIMS *m*/*z* 286 $[M]^+$ (5), 239 (20), 188 (80), 173 (40), 159 (40), 83 (100), 55 (36); HREIMS *m*/*z* 286.2281 (calcd for $C_{20}H_{30}O$, 286.2297).

Lobophytumin B (2): colorless oil; $[\alpha]^{24}_D$ +46.6 (*c* 1.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (3.6) nm; IR (KBr) ν_{max} 1759, 1659, 1460, 1380, and 960 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (1H, d, *J* = 16.0 Hz, H-5), 5.16 (1H, dd, *J* = 15.9, 9.8 Hz, H-6), 5.10 (1H, m, H-1), 4.76 (1H, dd, *J* = 2.4, 0.9 Hz, H-16a), 4.74 (1H, brd, *J* = 2.4 Hz, H-16b), 2.51 (1H, m, H-12a), 2.36 (1H, m, H-8a), 2.26 (1H, m, H-9a), 2.26 (2H, d, *J* = 6.6 Hz, H_2-14), 2.20 (1H, m, H-9b), 2.11 (3H, s, H_3-20), 2.07 (3H, m, H-3a, H-3b, H-11), 2.05 (1H, m, H-12b), 2.04 (1H, m, H-7), 1.97 (1H, m, H-8b), 1.55 (1H, m, H-2a), 1.49 (3H, s, H_3-17), 1.38 (1H, m, H-2b), 0.89 (3H, d, *J* = 7.0, H_3-19), 0.88 (3H, d, *J* = 7.0, H_3-20), 0.87 (3H, d, *J* = 6.7, H_3-18); ^{13}C NMR (see Table 1); EIMS *m*/*z* 288 $[M]^+$ (16), 204 (16), 188 (100), 173 (38), 159 (42), 145 (20), 108 (34), 85 (36), 57 (40); HREIMS *m*/*z* 288.2447 (calcd for $C_{20}H_{32}O$, 288.2453).

Lobophytumin C (3): colorless oil; $[\alpha]^{25}_D$ −36.6 (*c* 0.45, $CHCl_3$); IR (KBr) ν_{max} 2917, 1460, 1380, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.13 (1H, m, H-14), 4.80 (1H, brs, H-18*Z*), 4.74 (1H, d, *J* = 1.4 Hz, H-18*E*), 4.71 (1H, d, *J* = 1.6 Hz, H-16*E*), 4.44 (1H, d, *J* = 1.6 Hz, H-16*Z*), 2.31 (1H, m, H-3*eq*), 2.13 (2H, m, H_2-13), 2.08 (2H, m, H_2-12), 2.01 (1H, m, H-3*ax*), 1.95 (1H, m, H-7), 1.81 (1H, brd, *J* = 10.6 Hz, H-5), 1.69 (3H, s, H_3-19), 1.62 (3H, s, H_3-20), 1.61 (2H, m, H_2-8), 1.59 (1H, m, H-6*eq*), 1.58 (2H, m, H_2-2), 1.53 (1H, m, H-9*ax*), 1.51 (1H, m, H-9*eq*), 1.45 (1H, m, H-1*eq*), 1.27 (1H, m, H-6*ax*), 1.26 (1H, m, H-1*ax*), 0.76 (3H, s, H_3-17); ^{13}C NMR (see Table 1); EIMS *m*/*z* 272 $[M]^+$ (92), 257 (24), 229 (35), 161 (95), 109 (70), 69 (100); HREIMS *m*/*z* 272.2488 (calcd for $C_{20}H_{32}$, 272.2504).

Lobophytumin D (4): colorless oil; $[\alpha]^{25}_D$ +7.2 (*c* 0.48, $CHCl_3$); IR (KBr) 2917, 1460, 1380, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.32 (1H, brs, H-3), 5.13 (1H, m, H-14), 4.80 (1H, brs, H-18*Z*), 4.74 (1H, d, *J* = 1.5 Hz, H-18*E*), 2.13 (2H, m, H_2-13), 2.09 (1H, m, H-2*ax*), 2.07 (2H, m, H_2-12), 1.96 (1H, m, H-2*eq*), 1.95 (1H, m, H-7), 1.91 (1H, m, H-5), 1.78 (1H, brd, *J* = 12.8 Hz, H-6*eq*), 1.70 (3H, s, H_3-19), 1.62 (3H, s, H_3-20), 1.61 (3H, s, H_3-16), 1.56 (2H, m, H_2-8), 1.45 (1H, m, H-1*ax*), 1.35 (2H, m, H_2-9), 1.19 (1H, m, H-1b), 1.15 (1H, m, H-6*ax*), 0.90 (3H, s, H_3-17); ^{13}C NMR (see Table 1); EIMS *m*/*z* 272 $[M]^+$ (95), 257 (30), 229 (32), 187 (20), 161 (92), 109 (70), 69 (100); HREIMS *m*/*z* 272.2488 (calcd for $C_{20}H_{32}$, 272.2504).

Lobophytumin E (5): colorless oil; $[\alpha]^{25}_D$ +61.5 (*c* 0.53, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 244 (4.3) nm; IR (KBr) ν_{max} 2917, 1762, 1714, 1652, 1460, 1380, 957 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.03 (1H, s, H-14), 4.72 (1H, brs, H-16a), 4.69 (1H, brs, H-16b), 2.52 (1H, m, H-3a), 2.44 (1H, dd, *J* = 15.2, 2.6 Hz, H-12a), 2.41 (1H, m, H-5), 2.32 (1H, brd, *J* = 8.1 Hz, H-1), 2.26 (1H, m, H-3b), 2.13 (3H, s, H_3-20), 2.09 (1H, m, H-12b), 1.91 (1H, m, H-2a), 1.86 (3H, s, H_3-19), 1.85 (1H, m, H-8a), 1.72 (1H, m, H-11), 1.63 (1H, m, H-7), 1.60 (1H, m, H-8b), 1.59 (1H, m, H-2b), 1.56 (2H, m, H-9), 1.52 (1H, m, H-6), 1.00 (3H, s, H_3-17), 0.85 (3H, d, *J* = 6.2, H_3-18); ^{13}C NMR (see Table 1); EIMS *m*/*z* 286 $[M]^+$ (3), 205 (24), 188 (44), 173 (12), 159 (16), 108 (100), 93 (18), 83 (64), 55 (18); HREIMS *m*/*z* 286.2296 (calcd for $C_{20}H_{30}O$, 286.2297).

Lobophytumin F (6): colorless oil; $[\alpha]^{25}_D$ +52.2 (*c* 0.43, $CHCl_3$); IR (KBr) ν_{max} 1759, 1659, 1460, 1380, and 960 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.73 (1H, brs, H-16a), 4.69 (1H, brs, H-16b), 2.53 (1H, m, H-3a), 2.44 (1H, m, H-5), 2.43 (1H, dd, *J* = 15.3, 2.7 Hz, H-12a), 2.32 (1H, m, H-1), 2.27 (1H, m, H-3b), 2.24 (2H, d, *J* = 6.6 Hz, H-14), 2.12 (1H, m, H-12b), 1.92 (1H, m, H-2a), 1.82 (1H, m, H-8a), 1.74 (1H, m, H-11), 1.63 (1H, m, H-7), 1.60 (1H, m, H-8b), 1.57 (2H, m, H-9), 1.55 (1H, m, H-2b), 1.46 (1H, m, H-6), 0.99 (3H, s, H_3-17), 0.90 (3H, d, *J* = 6.5, H_3-20), 0.89 (3H, d, *J* = 6.5, H_3-19), 0.84 (3H, d, *J* = 6.4, H_3-18); ^{13}C NMR (see Table 1); EIMS *m*/*z* 288 $[M]^+$ (2), 207

(36), 188 (28), 173 (10), 159 (10), 108 (100), 85 (20), 81 (30), 57 (16); HREIMS *m/z* 288.2459 (calcd for $C_{20}H_{32}O$, 288.2453).

Cytotoxicity Bioassays. The cytotoxicities of compounds **1**−**6** against human lung adenocarcinoma A-549 and human colon carcinoma HCT-116 cell lines were evaluated by using the MTT[22] and SRB[23] methods, respectively, according to the protocols described in previous literature. Nocodazole was used as the positive control, with IC_{50} values of 0.087 ± 0.013 μM for the HCT-116 cell line and 0.173 ± 0.044 μM for the A549 cell line, respectively.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR and HR-EIMS spectra of compounds **1**−**6** and ^{13}C NMR data for **3**, **4**, and the model compounds **8**, **9**, and **11**. This information is available free of charge via the Internet at http://pubs.acs.org.

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