Norterpenoids and Related Peroxides from the Formosan Marine Sponge *Negombata corticata*

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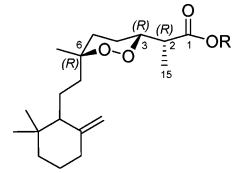
Received May 26, 2010

Six norterpenes including negombatoperoxides A and B (**4** and **5**), the inseparable epimers negombatoperoxides C and D (**6** and **7**), negombatodiol (**8**), and negombatolactone (**9**), in combination with three known compounds, (+)-nuapapuin B (**1**), (+)-nuapapuin B methyl ester (**2**), and (+)-aikupikoxide C (**3**), were isolated from the Formosan marine sponge *Negombata corticata*. In addition, 6,6-dimethylundecane-2,5,10-trione (**10**) was isolated for the first time from a natural source. Their structures, including relative configurations, were elucidated on the basis of interpretation of spectroscopic data and by the application of the empirical rule established by Capon and MacLeod. The absolute configurations of **8** and **9** were established by the application of Mosher's method and comparison of CD data with known lactones, respectively. Cytotoxicity of these isolates against human breast carcinoma, human liver carcinoma, and human lung carcinoma cell lines was evaluated.

Cyclic peroxides are of great interest due to their varied biological activities including antiparasitic activity and cytotoxicity against cancer cells.[1−3] Norterpene-related cyclic peroxides have been isolated from marine sponges of the genera *Prianos*,[4−6] *Sigmosceptrella*,[6−8] *Latrunculia*,[9−11] *Mycale*,[12−17] and *Diacarnus*.[18−24] Among them, some have been shown to exhibit antimicrobial,[9] antiviral,[14] cytotoxicity,[14] and antimalarial activities.[18,25] The wide spectrum of biological activities of cyclic peroxides prompted us to investigate the bioactive compounds from a Formosan marine sponge, *Negombata corticata*, as this organism was found to possess the known norditerpene cyclic peroxide **2**[19] by our preliminary study. The present study has led to the isolation of three known cyclic peroxides (**1**−**3**), four new norterpene-related cyclic peroxides (**4**−**7**), and three new norterpene-related compounds (**8**−**10**). The cyclic peroxides were characterized by a 2-substituted propionic acid or the corresponding methyl propionate functionality attached to a 1,2-dioxane ring at the C-3 position, like compounds **1**−**7**, of which the relative configurations at C-2, C-3, and C-6 were elucidated by applying the empirical rule introduced by Capon and MacLeod.[26] The cytotoxic activity of compounds **1**−**3** and **5**−**10** against breast carcinoma (MDA-MB-231 and MCF-7), human liver carcinoma (HepG2 and HepG3), and human lung carcinoma (A-549) cell lines was studied.

Results and Discussion

The sponge *N. corticata* was subjected to extraction with EtOH. Fractionation of the lipophilic extract by repeated silica gel column chromatography and final purification using normal-phase HPLC afforded six new (**4**−**9**) and three known compounds (**1**−**3**) along with 6,6-dimethylundecane-2,5,10-trione (**10**), which was isolated for the first time from a natural source.[27] The new compounds were given the trivial names negombatoperoxides A−D (**4**−**7**), negombatodiol (**8**), and negombatolactone (**9**). The known compounds



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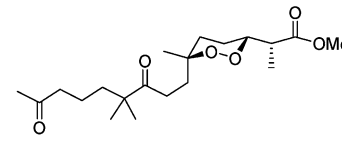
2 R = Me



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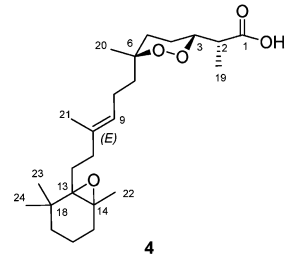

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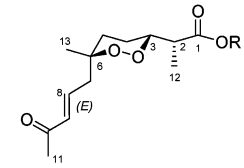
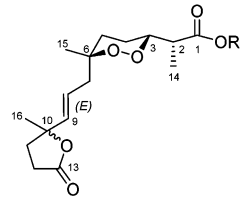
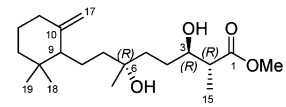


5 R = H
5a R = Me



6/7 epimers R = H
6a/7a epimers R = Me



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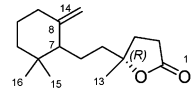

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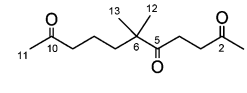

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10.1021/np100353x
Published on Web 08/18/2010

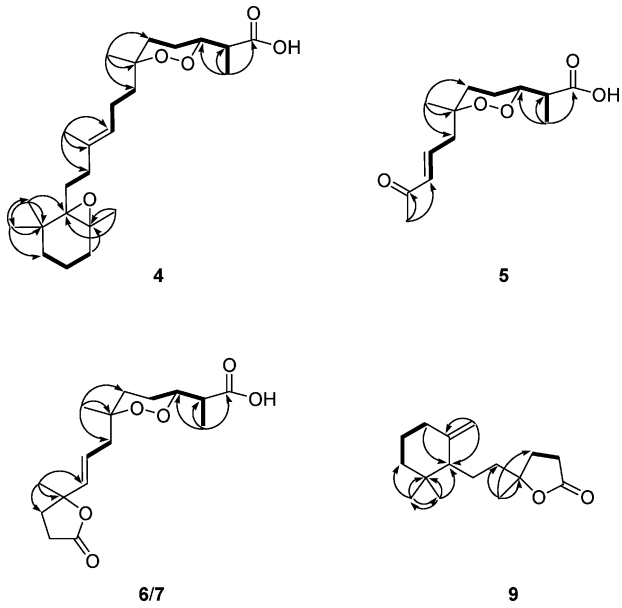


Figure 1. Selected $^1H-^1H$ COSY (—) and HMBC (→) correlations of **4**−**7** and **9**.

were identified as (+)-nuapapuin B (**1**),[19] (+)-nuapapuin B methyl ester (**2**),[19] and (+)-aikupikoxide C (**3**).[21]

Negombatoperoxide A (**4**) was obtained as a colorless oil. The HRESIMS of **4** exhibited a pseudomolecular ion peak at *m/z* 431.2776 $[M + Na]^+$ and established a molecular formula of $C_{24}H_{40}O_5$, implying five degrees of unsaturation. Its ^1H and ^{13}C NMR spectra clearly revealed the presence of a carboxylic acid (δ_C 178.5, C), a trisubstituted double bond (δ_C 135.9, C and 123.9, CH), and a tetrasubstituted epoxide containing a methyl group (δ_C 69.6, C; 63.7, C; 22.1, CH_3, and δ_H 1.28, s). In addition, the above data and the characteristic ^{13}C NMR signals at δ_C 81.3 (CH) and 80.2 (C) suggested **4** to be a member of the norterpene peroxide class.[19,23,24,26]

The gross structure of **4** was established by 2D NMR spectroscopic analysis. To establish the proton sequences in **4**, the $^1H-^1H$ COSY spectrum showed the connectivities from H-2 to H_3-19, H-2 to H_2-5, H_2-7 to H-9, H_2-11 to H_2-12, and H_2-15 to H_2-17. These results, together with the $^1H-^{13}C$ long-range correlations observed in an HMBC experiment (Figure 1), established the planar structure of **4**. Furthermore, according to the empirical rule introduced by Capon and MacLeod[26] and previous related studies,[19,21,22] the configuration at C-2 relative to C-3 for the cyclic peroxy analogues can be determined on the basis of the ^1H and ^{13}C NMR chemical shifts of 2-Me. If the ^1H NMR shift for 2-Me is observed at δ_H 1.12−1.15 and δ_C 12.4−12.8, the C-2/C-3-*erythro* configuration is assigned,[19,21,22,26] while 2-Me of a corresponding *threo* configuration should resonate at δ_H 1.22−1.28 and δ_C 13.2−13.5.[22,26] The orientation for 6-Me is determined to be axial if the ^{13}C NMR shift of this methyl appears at δ_C 20.5−20.9, while an equatorial 6-Me is assigned if this methyl carbon resonates between δ_C 23.5 and 24.0.[26] The axial or equatorial nature of H-3 in the peroxide ring was determined by the $^1H-^1H$ coupling constant value of $^3J_{3,4ax}$ ($J = 8-9$ Hz for axial H-3; $J = 3-4$ Hz for equatorial H-3).[22,24,26] Although the multiplicity of H-3 in **4** was not elucidated, a comparison of the ^1H and ^{13}C NMR spectroscopic data of the C-1−C-6 moiety in **4** (Table 1) with those in diacarperoxide D[23] and on the basis of the above rule, allowed the establishment of a C-2/C-3-*erythro* configuration and an equatorial 6-Me.

Negombatoperoxide B (**5**) was isolated as a colorless oil. Its HRESIMS spectrum exhibited a pseudomolecular ion peak at *m/z* 279.1207 $[M + Na]^+$, corresponding to a molecular formula of $C_{13}H_{20}O_5$. The molecular formula requires four degrees of unsaturation, comprising a carboxylic acid (δ_C 179.1, C), a conjugated methyl enone (δ_C 198.7, C, 143.4, CH, 134.3, CH, 26.7, CH_3), and a 1,2-dioxane ring (δ_C 81.4, CH, 79.7, C). The gross structure of **5** was established by the assistance of extensive 2D NMR analysis ($^1H-^1H$ COSY, HMQC, and HMBC). The $^1H-^1H$ COSY spectrum was used to establish the proton sequences from H_3-12 to H-2, from H-2 to H_2-5, and from H_2-7 to H-9 (Figure 1). The methyl group (δ_H 1.21 d, $J = 7.5$ Hz) attached at C-2 was confirmed by the HMBC correlations from H_3-12 to C-1, C-2, and C-3. The H_3-13 attached at C-6 was deduced by the HMBC correlations from H_3-13 to C-5, C-6, and C-7. Thus, the planar structure of **5** was established.

The *E* geometry of the C-8/C-9 double bond was deduced from a 16.0 Hz coupling constant between H-8 and H-9. By applying the aforementioned empirical rule, the equatorial orientation for 6-Me was readily assigned on the basis of its ^{13}C NMR shift (δ_C 24.3, C-13), the axial 6-Me being expected to resonate at δ_C 20.5 to 20.9. The large coupling constant $J_{3,4ax}$ (9.5 Hz) of the H-3 signal established an equatorial carbon substituent at C-3. The carbon resonance for the C-2 methyl group at δ_C 12.7 (C-12) might require a C-2/C-3-*erythro* configuration; however, its proton resonance at δ_H 1.21 (H_3-12) suggested a C-2/C-3-*threo* configuration. This result disclosed that the C-2/C-3 relative configuration could not be determined by this empirical rule for this case. On inspection of the empirical rule, it was found that most model compounds are the methyl esters.[26] Thus, the methyl ester **5a** was prepared using a mild and modified esterification method.[28] The carbon and proton resonances of the 2-Me (δ_C 12.7; δ_H 1.16) in the methyl ester **5a** required a C-2/C-3-*erythro* configuration (Experimental Section).

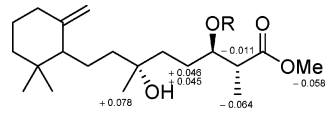
Compounds **6** and **7** were isolated as an inseparable epimeric mixture that gave a $[M + Na]^+$ peak at *m/z* 335.1472 in the HRESIMS spectrum, corresponding to a molecular formula of $C_{16}H_{24}O_6$. It is difficult to clearly distinguish between these two epimers from the ^1H NMR spectrum; however, the ^{13}C NMR spectrum displayed two sets of signals with partial overlap, and the intensity indicated that the epimers were present in a ratio close to 1:1. Analysis of the NMR data revealed that compounds **6** and **7** shared the same C-1−C-6 moiety. In addition, the carbon resonances around C-10 were observed in pairs at δ 125.2 (CH)/125.0 (CH), 135.6 (CH)/135.7 (CH), 26.8 (CH_3)/26.6 (CH_3), and 34.2 (CH_2)/34.1 (CH_2), suggesting that they might be C-10 epimers. A γ-lactone was preliminarily deduced according to the carbon resonances at δ 85.5 (C) and 176.9 (C)[29] as well as the IR absorption band appearing at 1769 cm^{-1}. The planar structure of **6/7**, established as shown in Figure 1, was further confirmed by the interpretation of $^1H-^1H$ COSY and HMBC spectra. The *E* geometry for the C-8/C-9 double bond was deduced by a 15.8 Hz coupling constant (measured in C_6D_6, Experimental Section) between H-8 and H-9. Compounds **5** and **6/7** were suggested to share the same relative configuration at C-2, C-3, and C-6 due to a high degree of similarity in ^{13}C NMR shifts between these compounds. In addition, according to the aforementioned empirical rule,[26] the orientation of 6-Me (δ_C 24.2, C-15) was assigned as equatorial in **6/7**. However, similar to compound **5**, the ^1H NMR shift of 2-Me (δ_H 1.19, $J = 6.6$ Hz, H_3-14) resonated at an indistinct region while applying this rule on **6/7**. Thus, a mixture of **6** and **7** was esterified with methanol to afford the corresponding methyl esters **6a/7a**. Consequently, the relative configuration for C-2/C-3 was readily assigned as *erythro* on the basis of the ^1H NMR shift of 2-Me (δ_H 1.15 d, $J = 6.8$ Hz, H_3-14) in **6a/7a**.

Compound **8** was isolated as an amorphous, white powder. Its molecular formula was found to be $C_{20}H_{36}O_4$, as deduced from HRESIMS and ^{13}C NMR data, indicating three degrees of unsaturation. The NMR spectroscopic data of **8** were similar to those of **2**.[19] However, the shifts for C-3 from δ_C 81.0 in **2** to the upper field, δ_C 73.8, in **8** and for H-3 from δ_H 4.23 in **2** to δ_H 3.66 in **8** indicated that the cyclic peroxide in **2** was reduced to a C-3/C-6

Table 1. ^{13}C NMR Spectroscopic Data of Compounds **4**−**10**

position	**4**[a], δ_C, mult.	**5**[b], δ_C, mult.	**6/7**[a], δ_C, mult.	**8**[a], δ_C, mult.	**9**[a], δ_C, mult.	**10**[a], δ_C, mult.
1	178.5, C	179.1, C	179.1, C	176.5, C	177.0, C	30.1, CH_3
2	42.6, CH	42.6, CH	42.7, CH	45.4, CH	29.2, CH_2	207.6, C
3	81.3, CH	81.4, CH	81.4, CH	73.8, CH	33.4, CH_2	37.0, CH_2
4	22.6, CH_2	22.7, CH_2	22.7, CH_2	28.8, CH_2	87.0, C	31.1, CH_2
5	32.5, CH_2	32.6, CH_2	32.0, CH_2	37.8, CH_2	39.7, CH_2	214.3, C
6	80.2, C	79.7, C	79.9, C	72.6, C	20.4, CH_2	47.3, C
7	34.9, CH_2	37.9, CH_2	37.7, CH_2	41.0, CH_2	54.2, CH	39.4, CH_2
8	22.1, CH_2	143.4, CH	125.2/125.0, CH	20.5, CH_2	148.9, C	19.0, CH_2
9	123.9, CH	134.3, CH	135.7/135.6, CH	54.6, CH	32.2, CH_2	43.9, CH_2
10	135.9, C	198.7, C	85.5, C	149.4, C	23.6, CH_2	208.8, C
11	36.5, CH_2	26.7, CH_3	34.2/34.1, CH_2	32.4, CH_2	36.0, CH_2	30.0, CH_3
12	28.2, CH_2	12.7, CH_3	29.1, CH_2	23.7 CH_2	35.0, C	24.5, CH_3
13	69.6, C	24.3, CH_3	176.9, C	36.1, CH_2	25.5, CH_3	24.5, CH_3
14	63.7, C		12.8, CH_3	35.0, C	109.5, CH_2	
15	30.4, CH_2		24.2, CH_3	14.3, CH_3	28.4., CH_3	
16	17.2, CH_2		26.6/26.8, CH_3	26.9, CH_3	26.6, CH_3	
17	36.2, CH_2			109.1, CH_2		
18	34.7, C			28.4, CH_3		
19	12.7, CH_3			26.5, CH_3		
20	24.0, CH_3					
21	16.1, CH_3					
22	22.1, CH_3					
23	25.9, CH_3					
24	25.4, CH_3					
OMe				51.8, CH_3		

[a] Spectra were measured in $CDCl_3$ (75 MHz). [b] Spectra were measured in $CDCl_3$ (125 MHz).



8a R = (*S*)-MTPA
8b R = (*R*)-MTPA

$\Delta\delta = \delta(S) - \delta(R)$ MTPA ester

Figure 2. ^1H NMR chemical shift differences of MTPA esters of **8**.

diol in **8**. In order to confirm the above elucidation and the relative configuration of **8**, a reductive cleavage was applied to compound **2**, a cyclic peroxide for which the absolute configuration is known,[19] to afford the corresponding diol. The ^1H and ^{13}C NMR spectroscopic data of this synthetic diol were identical to those of **8**. In addition, the specific rotation of the synthetic diol ($[\alpha]^{25}_D$ +5) prepared from **2** showed the same positive sign as that of the isolate **8** ($[\alpha]^{25}_D$ +2); however, the values of both natural and synthetic compounds were too small to be used for conclusive determination of the absolute configuration of **8**. The absolute configuration of **8** was further determined by the application of Mosher's method.[30,31] Analysis of the ^1H NMR data of esters **8a** and **8b** resulted in the determination of a 3*R* configuration (Figure 2). Consequently, the absolute configuration of **8** was determined as 2*R*, 3*R*, and 6*R*. However, the configuration at C-9 remains unknown.

The molecular formula of **9** was found to be $C_{16}H_{26}O_2$, as deduced from HRESIMS and ^{13}C NMR data. Its IR absorption bands showed the absence of a hydroxy group and the presence of a γ-lactone (1770 cm^{-1}). The latter was confirmed by the carbon resonances at δ_C 87.1 (C) and 177.0 (C).[29] A comparison of the NMR spectroscopic data of **9** with those of **8** disclosed that both shared the same 2,2-dimethyl-6-methylenecyclohexyl moiety. The above functionality fulfilled the total four degrees of unsaturation computed from the molecular formula of **9**. Consequently, **9** was suggested to be composed of a 2,2-dimethyl-6-methylenecyclohexyl moiety linked through a C_2H_4 chain to a γ-lactone. This was confirmed by the interpretation of the $^1H-^1H$ COSY and HMBC experiments, and the planar structure of **9** was thus determined as shown in Figure 1. Its CD spectrum exhibited a negative Cotton effect ($\Delta\varepsilon$ −0.1) at 219 nm attributed to the n→π^* electronic transition of the γ-lactone.[32,33] Consequently, the absolute configuration at C-4 was assigned as *R*, while the configuration at C-7, the asymmetric carbon in the 2,2-dimethyl-6-methylenecyclohexyl moiety, remains unknown. In view of the fact that compound **8** has a 2*R*,3*R*,6*R* configuration and the known compounds **1** and **2** both possess a 2*R*,3*R*,6*R* configuration, and on the basis of biogenetic considerations, the absolute configurations of compounds **4**−**7** are tentatively assigned to be the same as those of **1**, **2**, and **8**.

The HRFABMS data of **10** established a molecular formula of $C_{13}H_{22}O_3$, implying three degrees of unsaturation. The IR spectrum of **10** showed the presence of a carbonyl group (1715 cm^{-1}). Resonances for three sp^2 quaternary carbons at δ 207.6, 208.8, and 214.3 were ascribed to the presence of three ketones. The remaining 10 carbons were composed of four methyls, five methylenes, and one quaternary carbon. The spectroscopic data suggested the acyclic nature of **10**. Detailed interpretation of HMBC and $^1H-^1H$ COSY spectra of **10** led to the establishment of acyclic triketone **10**. Biogenetically, **10** might be derived from oxidative cleavage of epimuqubilin A[19] at the C-9/C-10 and C-13/C-14 double bonds. This compound was isolated for the first time from a natural source.[27]

The cytotoxicity of compounds **1**−**3** and **5**−**10** against MDA-MB-231, MCF-7, HepG2, Hep3B, and A-549 cancer cell lines was evaluated. The data revealed that **1** was cytotoxic toward MDA-MB-231, MCF-7, HepG2, Hep3B, and A-549 cancer cell lines with IC_{50} values of 0.3, 5.9, 0.9, 41.3, and 0.6 μM, respectively, while its methyl ester **2** showed activity against the above cancer cell lines with IC_{50} values of 3.5, 38.5, 2.9, 23.7, and 5.38 μM, respectively. Compound **3** showed weaker cytotoxicity toward three of the above cancer cell lines with IC_{50} values ranging from 11.9 to 36.7 μM. The other tested compounds were not cytotoxic (IC_{50} > 50 μM) toward the above five cancer cell lines. The diol **8** and lactone **9**, with the loss of the 1,2-dioxane ring, were inactive. In the case of **1**, **5**, and **6**, it was found that enhancement of the lipophilicity at C-6 in norterpene cyclic peroxides, like compound **1**, can increase the cytotoxicity.[34]

Previous studies and our present work revealed that the empirical rule developed by Capon and MacLeod is quite successful for the analysis of C-2, C-3, and C-6 relative configurations of norterpene

peroxide methyl esters. However, this rule showed that it is not always reliable for elucidation of the C-2/C-3 relative configuration of norterpene peroxide acids, as the ^{1}H NMR shift of 2-Me could be near or higher than 1.20 ppm, such as in the case of **5**, **6/7**, and (+)-muqubilone B (δ_H 1.20 for 2-Me),[24] exceeding the range of δ_H 1.12−1.15 for a C-2/C-3-*erythro* configuration and close to the range of δ_H 1.22−1.28 for a C-2/C-3-*threo* configuration, although these compounds all have the C-2/C-3-*erythro* configuration. Prior studies on the determination of the C-2/C-3 relative configuration for carboxylic acids of this type were accomplished mostly by preparation of the methyl ester, followed by analysis of the NMR spectroscopic data using the above empirical rule. By careful examination of the ^{13}C NMR data, we found that all of the 2-Me shifts of C2/C3-*threo* norterpene peroxide carboxylic acids appeared at δ_C 13.2−13.5, while the corresponding C-2/C-3-*erythro* acids showed signals at δ_C 12.4−12.8. Consequently, the aforementioned results revealed that the ^{13}C NMR shifts are more reliable for the determination of the C-2/C-3 relative configuration of the indicated acids than the ^{1}H NMR shifts. An overview of the literature revealed that structures of cyclic peroxide-related norterpenoids are quite diverse among different sponge species,[4−24] including the organism of our present study. Prior investigation of a Red Sea sponge *N. corticata* resulted in the isolation of a sphingolipid[35] and a latrunculin-type compound.[36] This is the first report of cyclic peroxides from the marine sponge *N. corticata*. In addition, nuapapuin A methyl ester was isolated and assigned an equatorial H-3 in 1984;[5] however, the H-3 relative configuration of this compound was eventually revised to an axial H-3 in 1999.[8] Accordingly, none of the cyclic norterpene peroxides of this class have been found to possess an equatorial H-3 to date.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR (or Varian Unity INOVA 500 FT-NMR) instrument at 300 MHz (or 500 MHz) for ^{1}H (referenced to TMS, δ_H 0.0 ppm) and 75 MHz (or 125 MHz) for ^{13}C in $CDCl_3$ (referenced to the center line of $CDCl_3$, δ_C 77.0 ppm). LRMS and HRMS spectra were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck Kieselgel 60 F_{254} 0.2 mm) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT apparatus equipped with a Shimadzu SPD-10A UV detector. A Varian Dynamax normal-phase column (Si gel, 250 × 21.4 mm, 100 Å, 5 μm) was used. *S*-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride, *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) were purchased from Aldrich, and 4-(dimethylamino)pyridine (4-DMAP) was purchased from Lancaster. 4-DMAP·HCl was prepared by adding concentrated HCl to a solution of 4-DMAP in THF.[37]

Animal Material. The sponge *Negombata corticata* was collected by hand using scuba off the southern Taiwan coast in November 2002, at a depth of 20 m, and was stored in a freezer. This sponge was identified by one of the authors (L.-H.W.). A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. DTSP).

Extraction and Isolation. The sponge *N. corticata* (1.4 kg, wet wt) was minced and extracted exhaustively with EtOH (4 × 1 L). The combined extract was concentrated to an aqueous suspension and was further partitioned between EtOAc and H_2O. The EtOAc extract (13 g) was fractionated by open column chromatography on silica gel using *n*-hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to yield 14 fractions. Fraction 4, eluted with *n*-hexane−EtOAc (7:1), was further separated by Sephadex LH-20 column chromatography with acetone as eluent to yield compound **2** (100 mg). Fraction 7, eluted with *n*-hexane−EtOAc (3:1), was subjected to normal-phase HPLC (*n*-hexane−CH_2Cl_2−EtOAc, 90:5:5) to obtain compound **9** (2.1 mg). Three subfractions (9A−9C) were afforded from fraction 9 using silica gel column chromatography with gradient elution (*n*-hexane−EtOAc, 20:1 to 10:1). Compounds **1** (7.6 mg) and **3** (1.7 mg) were obtained from subfraction 9A using normal-phase HPLC (*n*-hexane−CH_2Cl_2−acetone, 88:7:5). Similarly, compound **4** (1.2 mg) was afforded from subfraction 9B using normal-phase HPLC (*n*-hexane−CH_2Cl_2−acetone, 88:7:5). Subfraction 9C was further purified using normal-phase HPLC (*n*-hexane−CH_2Cl_2−acetone, 80:7:13) to yield compounds **8** (1.8 mg) and **10** (2.3 mg). Fraction 12 was fractionated on a Sephadex LH-20 column (acetone as eluent) and subsequently purified by normal-phase HPLC (*n*-hexane−CH_2Cl_2−acetone, 75:7:18) to afford compound **5** (4.6 mg) and an inseparable epimeric mixture of **6/7** (5.4 mg).

(+)-Nuapapuin B (1): $[\alpha]^{24}_D$ +54 (*c* 0.65, $CHCl_3$); lit. $[\alpha]_D$ +45 (*c* 0.46, $CHCl_3$).[19]

(+)-Nuapapuin B methyl ester (2): $[\alpha]^{24}_D$ +60 (*c* 0.35, $CHCl_3$); lit. $[\alpha]_D$ +39 (*c* 1.74, $CHCl_3$).[19]

(+)-Aikupikoxide C (3): $[\alpha]^{24}_D$ +71 (*c* 0.72, CH_2Cl_2); lit. $[\alpha]_D$ +88 (*c* 2.00, CH_2Cl_2).[21]

Negombatoperoxide A (4): colorless oil; $[\alpha]^{24}_D$ −71 (*c* 0.52, $CHCl_3$); IR (KBr) ν_{max} 3000−3500, 1716 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 431 $[M + Na]^+$; HRESIMS *m/z* 431.2776 $[M + Na]^+$ (calcd for $C_{24}H_{40}O_5Na$, 431.2775).

Negombatoperoxide B (5): colorless oil; $[\alpha]^{24}_D$ +80 (*c* 0.64, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (3.75) nm; IR (KBr) ν_{max} 3000−3500, 1715, 1667 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 279 $[M + Na]^+$; HRESIMS *m/z* 279.1207 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_5Na$, 279.1209).

Negombatoperoxide C (6/7): colorless oil; $[\alpha]^{24}_D$ +62 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3200−3600, 1769, 1720 cm^{-1}; ^{13}C and ^{1}H NMR data ($CDCl_3$), see Tables 1 and 2; selected ^{1}H NMR (C_6D_6, 300 MHz) 5.65 (1H, m, H-8), 5.34 (1H, d, J = 15.8 Hz, H-9), 4.26 (1H, m, H-3), 2.55 (1H, m, H-2), 1.09 (3H, s, H_3-16), 0.98 (3H, d, J = 6.1 Hz, H_3-14), 0.91 (3H, d, J = 6.1 Hz, H_3-15); ESIMS *m/z* 335 $[M + Na]^+$; HRESIMS *m/z* 335.1472 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_6Na$,335.1471).

Negombatodiol (8): amorphous powder; $[\alpha]^{24}_D$ +2 (*c* 0.14, $CHCl_3$); IR (KBr) ν_{max} 1736 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 363 $[M + Na]^+$; HRESIMS *m/z* 363.2510 $[M + Na]^+$ (calcd for $C_{20}H_{36}O_4Na$, 363.2513).

Negombatolactone (9): colorless oil; $[\alpha]^{24}_D$ +10 (*c* 0.52, $CHCl_3$); CD (2.4 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (−0.1); IR (KBr) ν_{max} 1770 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) 4.76 (1H, s, H-14a), 4.52 (1H, s, H-14b), 2.59 (2H, m, H_2-2), 2.04 (1H, m, H-3a), 2.02 (2H, m, H_2-9), 1.98 (1H, m, H-3b), 1.55 (2H, m, H-6a and H-7), 1.54 (1H, m, H-5a), 1.51 (2H, m, H_2-10), 1.45 (1H, m, H-11a), 1.44 (1H, m, H-5b), 1.43 (1H, m, H-6b), 1.40 (3H, s, H_3-13), 1.25 (1H, m, H-11b), 0.92 (3H, s, H_3-15), 0.85 (3H, s, H_3-16); ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 1; ESIMS *m/z* 273 $[M + Na]^+$; HRESIMS *m/z* 273.1828 $[M + Na]^+$ (calcd for $C_{16}H_{26}O_2Na$, 273.1834).

6,6-Dimethylundecane-2,5,10-trione (10): colorless oil; IR (KBr) ν_{max} 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) 2.75 (2H, m, H_2-4), 2.68 (2H, m, H_2-3), 2.41 (2H, t, J = 6.4 Hz, H_2-9), 2.20 (3H, s, H_3-1), 2.13 (3H, s, H_3-11), 1.50 (2H, m, H_2-8), 1.47 (2H, m, H_2-7), 1.13 (6H, s, H_3-12 and H_3-13); ^{13}C NMR ($CDCl_3$, 75 MHz) 214.3 (C, C-5), 208.8 (C, C-10), 207.6 (C, C-2), 47.3 (C, C-6), 43.9 (CH_2, C-9), 39.4 (CH_2, C-7), 37.0 (CH_2, C-3), 31.1 (CH_2, C-4), 30.1 (CH_3, C-1), 30.0 (CH_3, C-11), 24.5 (CH_3, C-12) 24.5 (CH_3, C-13), 19.0 (CH_2, C-8); ESIMS *m/z* 249 $[M + Na]^+$; HRESIMS *m/z* 249.1470 $[M + Na]^+$ (calcd for $C_{13}H_{22}O_3Na$, 249.1468).

Esterification of 5 and the Mixture of 6 and 7. To a stirring solution of compound **5** (3 mg) in CH_2Cl_2 (0.5 mL) were successively added DMAP (2 mg), 4-DMAP·HCl (2 mg), and EDC·HCl (4 mg). After the mixture was stirred at 0 °C for 3 h, anhydrous MeOH (0.3 mL) was added. The mixture was then warmed to room temperature and allowed to react overnight. The reaction was quenched by H_2O, and the mixture was subsequently extracted with EtOAc (5 × 6 mL). The EtOAc extract was combined and successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue, which was chromatographed on silica gel with *n*-hexane−EtOAc (1:1) as eluent to afford the corresponding methyl ester **5a** (2.9 mg): $[\alpha]^{24}_D$ +55 (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) of **5a**: δ 6.79 (1H, ddd, J = 16.3, 8.9, 5.7 Hz, H-8), 6.12 (1H, d, J = 16.3 Hz, H-9), 4.31 (1H, dd, J = 9.5, 7.5 Hz, H-3), 3.72 (3H, s, O*Me*), 2.93 (1H, dd, J = 14.3, 5.7 Hz, H-7a), 2.55 (1H, dq, J = 7.5, 7.2 Hz, H-2), 2.38 (1H, dd, J = 14.3, 8.9 Hz, H-7b), 2.27 (3H, s, H_3-11), 1.83 (2H, m, H_2-5), 1.75 (2H, m, H_2-4), 1.16 (3H, d, J = 7.2 Hz, H_3-12), 1.11 (3H, s, H_3-13).^{13}C

Table 2. ^{1}H NMR Spectroscopic Data of Compounds **4**−**8**

position	**4**, δ_H (J in Hz)[a]	**5**, δ_H (J in Hz)[b]	**6/7**, δ_H (J in Hz)[a]	**8**, δ_H (J in Hz)[a]
2	2.58, m	2.56, dq (8.5, 7.5)	2.55, m	2.56, m
3	4.25, m	4.31, dd (9.5, 8.5)	4.26, m	3.66, m
4	1.69, m	1.75, m	1.69, m	a: 1.67, m b: 1.45, m
5	a: 1.78, m b: 1.67, m	1.83, m	1.67, m	a: 1.64, m b: 1.57, m
7	a: 1.90, m b: 1.55, m	a: 2.95, dd (14.1, 5.7) b: 2.37, dd (14.1, 9.0)	a: 2.62, m b: 2.35, dd (13.7, 7.0)	a: 1.42, m b: 1.21, m
8	2.04, m	6.81, ddd (16.0, 9.0, 5.7)	5.60−5.70[c]	1.55, m
9	5.14, br t (7.0)	6.13, d (16.0)	5.60−5.70[c]	1.65, m
11	2.05 m	2.28, s	2.03−2.13[c] 2.23−2.27[c]	2.01 m
12	a: 1.81, m b: 1.66, m	1.21, d (7.5)	2.55, m	1.56, m
13		1.12, s		a: 1.50, m b: 1.23, m
14			1.19, d (6.6)	
15	a: 1.83, m b: 1.68, m		1.07, s	1.21, d (7.0)
16	1.37, m		1.51, s	1.17, s
17	a: 1.39, m b: 0.97, m			a: 4.75, s b: 4.53, s
18				0.92, s
19	1.17, d (6.8)			0.85, s
20	1.12, s			
21	1.61, s			
22	1.28, s			
23	1.02, s			
24	1.06, s			
OCH				3.69, s

[a] Spectra were measured in $CDCl_3$ (300 MHz). [b] Spectra were measured in $CDCl_3$ (500 MHz). [c] Signals are overlapped with the epimer.

NMR ($CDCl_3$, 75 MHz) of **5a**: 198.6 (C, C-10), 174.2 (C, C-1), 143.4 (CH, C-8), 134.2 (CH, C-9), 81.4 (CH, C-3), 79.6 (C, C-6), 52.0 (CH_3, O*Me*), 42.7 (CH, C-2), 37.9 (CH_2, C-7), 32.7 (CH_2, C-5), 26.8 (CH_3, C-11), 24.3 (CH_3, C-13), 22.8 (CH_2, C-4), 12.7 (CH_3, C-12); ESIMS *m*/*z* 293 $[M + Na]^+$. The same procedure was applied on a mixture of **6/7** (1.2 mg) to prepare the corresponding methyl esters **6a/7a** (1.1 mg): $[\alpha]^{24}_D$ +47 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) of mixture **6a/7a**: δ 5.65−5.69 (2H, overlapped, H-8 and H-9), 4.26 (1H, td, J = 9.0, 3.5 Hz, H-3), 3.71 (3H, s, O*Me*), 2.60 (1H, m, H-7a), 2.58 (1H, m, H-2), 2.56 (2H, m, H_2-12), 2.36 (1H, dd, J = 14.0, 8.0 Hz, H-7b), 2.21 (1H, m, H11a), 2.07 (1H, m, H-11b), 1.75 (1H, m, H-5a), 1.67 (2H, m, H_2-4), 1.65 (1H, m, H-5b), 1.50/1.49 (3H, s, H_3-16), 1.16 (3H, d, J = 7.0 Hz, H_3-14), 1.07 (3H, s, H_3-15). ^{13}C NMR ($CDCl_3$, 125 MHz) of **6a/7a**: 176.7 (C, C-13), 174.3 (C, C-1), 135.6/135.5 (CH, C-9), 125.2/125.0 (CH, C-8), 85.3 (C, C-10), 81.5 (CH, C-3), 79.8 (C, C-6), 52.0 (CH_3, O*Me*), 42.7 (CH, C-2), 37.6 (CH_2, C-7), 34.2/34.1 (CH_2, C-11), 31.9 (CH_2, C-5), 29.0 (CH_2, C-12), 26.7/26.5 (CH_3, C-16), 24.1 (CH_3, C-15), 22.7 (CH_2, C-4), 12.9 (CH_3, C-14); ESIMS *m*/*z* 349 $[M + Na]^+$.

Reductive Cleavage of Cyclic Peroxide 2. A mixture of **2** (20 mg), EtOAc (10 mL), HOAc (1 mL), and zinc (600 mg) was stirred at room temperature overnight. The solution was filtered, evaporated, and separated on a short silica gel column with *n*-hexane−EtOAc (1:1) to afford the corresponding diol (19.8 mg), which has the identical NMR spectroscopic data and similar specific rotation, $[\alpha]^{24}_D$ +5 (*c* 0.72, $CHCl_3$), to those of natural compound **8**.

Preparation of (*S*)- and (*R*)-MTPA Esters of 8. To a solution of **8** (0.7 mg) in pyridine (0.4 mL) was added (*R*)-MTPA chloride (25 μL), and the mixture was allowed to stand overnight at room temperature. The reaction was quenched by the addition of 1.0 mL of H_2O, and the mixture was subsequently extracted with EtOAc (3 × 1.0 mL). The EtOAc-soluble layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to short silica gel column chromatography using *n*-hexane−EtOAc (7:1) to yield the (*S*)-MTPA ester, **8a** (0.7 mg). Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **8a**: δ 7.380−7.550 (5H, m, Ph), 5.381 (1H, m, H-3), 4.750 (1H, s, H-17a), 4.516 (1H, s, H-17b), 3.581 (3H, s, COO*Me*), 3.515 (3H, s, MTPA-O*Me*), 2.848 (1H, m, H-2), 1.788 (1H, m, H-4a), 1.677 (1H, m, H-4b), 1.118 (3H, d, J = 7.1 Hz, H_3-15), 1.105 (3H, s, H_3-16), 0.911 (3H, s, H_3-18), 0.833 (3H, s, H_3-19). The same procedure was used to prepare the (*R*)-MTPA ester, **8b** (0.6 mg from 0.7 mg of **8**), with (*S*)-MTPA chloride. Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **8b**: δ 7.380−7.550 (5H, m, Ph), 5.378 (1H, m, H-3), 4.753 (1H, s, H-17a), 4.514 (1H, s, H-17b), 3.639 (3H, s, COO*Me*), 3.537 (3H, s, MTPA-O*Me*), 2.859 (1H, m, H-2), 1.742 (1H, m, H-4a), 1.632 (1H, m, H-4b), 1.182 (3H, d, J = 7.1 Hz, H_3-15), 1.027 (3H, s, H_3-16), 0.917 (3H, s, H_3-18), 0.841 (3H, s, H_3-19).

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection. Cytotoxicity assays were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[38,39] Doxorubicin was employed as positive control, which exhibited cytotoxic activity toward MDA-MB-231, MCF-7, HepG2, Hep3B, and A-549 cancer cell lines with IC_{50} values of 0.24, 0.59, 0.19, 0.14, and 0.36 μM, respectively.

Acknowledgment. This work was supported by grants from the National Science Council of Taiwan (NSC98-2113-M-110-002-MY3) and Ministry of Education (97C031702) awarded to J.-H.S.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and HRESIMS spectra for **4**−**10**; ^{1}H and ^{13}C NMR spectra of **1**, **2**, **5a**, and **6a/7a**; and ^{1}H NMR spectra of MTPA esters **8a** and **8b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP100353X