Published in final edited form as:
J Nat Prod. 2009 August ; 72(8): 1547–1551. doi:10.1021/np900310j.

Aromatic Cyclic Peroxides and Related Keto-Compounds from the *Plakortis* sp. Component of a Sponge Consortium

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Abstract

Six unreported aromatic compounds, **1–6**, were isolated, along with the known compounds dehydrocurcuphenol and manoalide, from a sample of *Plakortis* sp., which was the main component of a Pacific sponge consortium. The new molecules were chemically characterized by spectroscopic methods. Compounds **1–4** contain a six-membered cyclic peroxide, whereas **5** and **6** display a terminal methyl ketone. The new metabolites were tested for antifungal and antibacterial properties. Compounds **1** and **4** were weakly active against *S. aureus*.

Cyclic peroxides have been described previously from a number of marine organisms, especially from sponges of the family Plakinidae.[1] After the first report of plakortin,[2] an increasing number of related compounds, exhibiting a 1,2-dioxane ring with such groups as an acetic acid moiety at C-3 and an aliphatic chain at C-6, have been characterized.[3] Minor groups include cyclic peroxides in which the alkyl chain terminates with a phenyl residue[3g,4] or keto-analogues with the peroxide ring opened.[2,5] Several biological activities including cytotoxic,[3q,4g,6,7] antimicrobial,[2,3g] and antitumor[3e,o,4a,8] properties have been described for these compounds.

We report here the isolation of six novel metabolites, compounds **1–6**, all of which are characterized by the presence of a terminal aromatic ring, from the *Plakortis* sp. component of a Pacific sponge consortium. Compounds **1–4** were cyclic peroxides, whereas compounds **5** and **6** were analogues with the opened ring. Two unrelated known molecules, dehydrocurcuphenol[9] and manoalide,[10] were also isolated from the same material.

The sponge consortium, which was constituted by *Plakortis* sp. and *Dactylospongia* sp. specimens, was collected in a twilight zone habitat of Orote Peninsula, Guam, at a depth of 96 m. The sponges were separated as well as possible, freeze-dried, and kept at −20 °C. Only the sample containing mainly *Plakortis* sp. (74 g wet weight) was considered for the chemical analysis and thus extracted exhaustively with MeOH/EtOAc. The solvent was removed, and the aqueous residue was dissolved in MeOH and extracted subsequently with *n*-hexane and CH_2Cl_2. After evaporation of the organic solvents, the *n*-hexane phase gave 0.645 g of crude residue, whereas the CH_2Cl_2 portion afforded 0.836 g of material. The two extracts were

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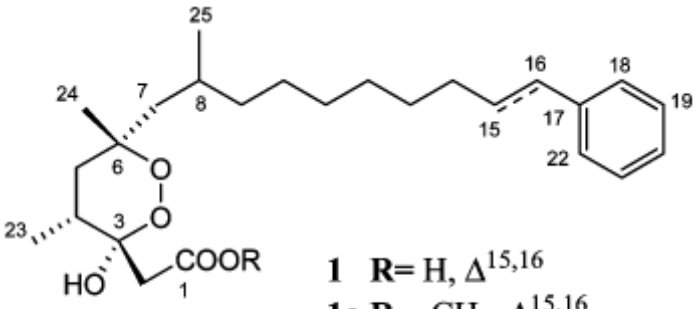
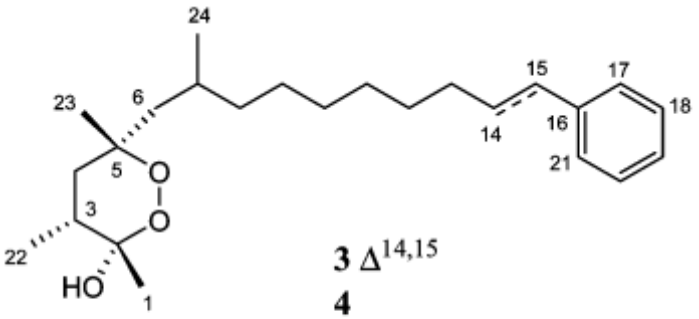
Supporting Information **Available**: 1D and 2D NMR spectra of compounds **1–3**; ^1H and ^{13}C NMR spectra of compounds **1a** and **4**; ^1H NMR and selected 2D NMR spectra of compounds **2a, 5**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

analyzed by TLC chromatography in different eluent systems, showing the presence of two UV–visible spots at R_f 0.32 and 0.35 ($CHCl_3$/MeOH, 9:1) in the CH_2Cl_2 part as well as a series of related UV–visible spots at R_f 0.35–0.55 (light petroleum ether/Et_2O, 1:1) in the *n*-hexane-soluble portion. Fractionation of the CH_2Cl_2 extract gave compounds **1** and **2** along with the sesquiterpenes dehydrocurcuphenol[9] and manoalide,[10] whereas compounds **3–6** were purified from the *n*-hexane extract.

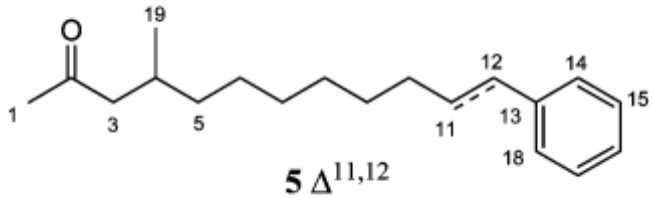
A preliminary NMR analysis of **1–6** immediately showed their structural relationship and in particular the presence in all of them of a non-terpenoid skeleton exhibiting a monosubstituted aromatic ring.



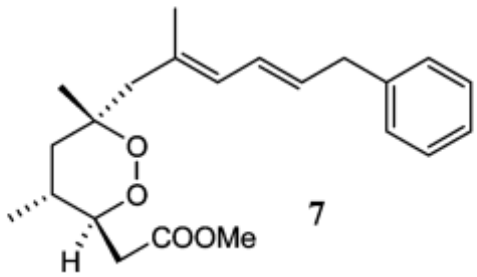
1 **R**= H, $\Delta^{15,16}$
1a **R**= CH_3, $\Delta^{15,16}$
2 **R**= H
2a **R**= CH_3



3 $\Delta^{14,15}$
4



5 $\Delta^{11,12}$
6



7

We started from the more abundant compound **1**, which was immediately revealed to be quite unstable. In fact, **1** was observed to undergo a degradation process during the workup that resulted in the conversion into the co-occurring compound **3**. The ^{1}H NMR spectrum of **1** contained signals attributable to two vinyl protons, an isolated methylene, and three methyls in addition to the aromatic signals at δ 7.19–7.33 and to a series of aliphatic multiplets between δ 2.21 and 1.14 (see Experimental Section). Analysis of the ^{13}C NMR spectrum confirmed the presence of a monosubstituted aromatic ring conjugated with a disubstituted double bond and also suggested the presence of a partial structural moiety containing two quaternary carbons linked to oxygen (see Experimental Section). Even though the corresponding signal was not detected in the carbon spectrum of compound **1**, the presence of an acid functional group was strongly suspected due to both the IR band at 1715 cm^{-1} and the polarity on Si-gel TLC (R_f 0.32, $CHCl_3$/MeOH, 9:1).

With the aim of confirming this hypothesis and also of obtaining a more stable derivative, **1** was treated with CH_2N_2 to obtain the corresponding methyl ester **1a**. An extensive spectral analysis was carried out on this derivative, leading to the complete characterization (Table 1). The HRESIMS spectrum of **1a** contained the sodiated molecular peak at *m/z* 455.2750 [M + Na]$^{+}$ corresponding to the molecular formula $C_{26}H_{40}O_5$. The ^{1}H–^{1}H COSY spectrum revealed two distinct spin systems (H_3-23/H-4/H_2-5 and the fragment from H_2-7 through H-16), along with the phenyl residue and the isolated AB methylene systems (H_2-2). Analysis of the HMBC spectrum of **1a** allowed the connection of these moieties through the quaternary carbons C-3 (δ 100.7) and C-6 (δ 81.5) as well as the location of the ester function at C-1. In fact, diagnostic long-range correlations were observed between H_2-2 and both C-1 and C-3, between H_3-23 and C-3, and between H_3-24 and C-5, C-6, and C-7 (Table 1). Having assessed the carbon sequence from C-1 to the phenyl terminal moiety, the remaining degree of unsaturation indicated by the molecular formula of **1a** had to be a ring. Thus, a peroxide moiety was introduced between the C-3 and C-6 carbons, forming a six-membered ring. The remaining oxygen was located at C-3 as a tertiary alcohol. Comparison of the carbon and proton values of **1a** (Table 1) with those reported for several cyclic peroxide models[3b,d,p] supported our hypothesis. In particular **1a** showed close structural similarities with methyl capucinoate (**7**), a metabolite isolated from the Caribbean sponge *Plakinastrella onkodes*,[11] from which it differs in the length and unsaturation degree of the alkyl chain and in the presence of an additional −OH function at C-3. The relative configuration of the chiral carbons in the peroxide ring was established by NOE experiments as well as by comparison of NMR values with those of **7**. Diagnostic NOE correlations were observed between H-4 (δ 1.99) and H_3-24 (δ 1.37), implying the same axial orientation and providing the relative configuration of C-4 and C-6, analogous to methyl capucinoate (**7**). The configuration at C-3 was suggested by the chemical shift of H-5$_{ax}$ (δ 1.70, dd, J = 13.1, 13.1 Hz), which was significantly downfield shifted with respect to the corresponding proton H-5$_{ax}$ (δ 1.39, dd, J = 12.8, 12.8 Hz) in **7**, consistent with the presence of an axially oriented −OH at C-3.

The spectroscopic data of compound **2** strongly resembled those of **1**. The ^{1}H NMR spectrum of **2** was substantially similar to that of **1** with regard to the peroxide cycle protons, whereas some differences were observed for the alkyl chain. In particular, the spectrum of **2** lacked the vinyl proton signals resonating in **1** at δ 6.21 (H-15) and 6.39 (H-16), both replaced by two methylene multiplets at δ 2.59 (H_2-16) and 1.62 (H_2-15). Accordingly, the ^{13}C NMR spectrum of **2** contained two additional sp^3 carbon signals at δ 36.0 (C-16) and 31.5 (C-15) and lacked two sp^2 carbon signals attributed to the conjugated double bond in the alkyl chain of **1** (see Experimental Section). These data suggested that **2** was the 15,16-dihydro derivative of **1**, as confirmed by the quasi-molecular ion peak in the ESIMS spectrum at *m/z* 443, with two additional mass units with respect to that of **1**. Analogously with **1**, compound **2** appeared quite unstable and its rapid conversion into co-occurring compound **4** was also observed, thus supporting the presence of the same carboxylic acid functionality. The treatment of an aliquot

of **2** with CH_2N_2 gave the corresponding methyl ester derivative **2a**, thus confirming the structural hypothesis. Compound **2a** was fully characterized by HRESIMS and 2D NMR experiments (Table 1). The relative configuration in the peroxide ring moiety of **2a** was suggested to be the same as **1a** on the basis of NOE experiments.

Compound **3** was obviously related to **1**, being observed during a rapid conversion of **1** into **3** upon workup. The HRESIMS spectrum of **3** contained the sodiated molecular peak at *m/z* 397.2725 $[M + Na]^+$ according to the molecular formula $C_{24}H_{38}O_3$, which lacked a CO_2 moiety with respect to that of **1**. Comparison of the ^1H NMR spectra of both compounds indicated that in **3** a singlet methyl (δ 1.34, s, 3H, H_3-1) replaced the methylene H_2-2 (AB system at δ 2.89 and 2.56) bearing the carboxyl function in **1**. Accordingly, the ^{13}C NMR spectrum of **3** contained a $-CH_3$ signal at δ 22.6 in place of a $-CH_2$ signal at δ 38.8. Careful analysis of 2D NMR spectra of **3** indicated that the remaining part of the molecule was identical with compound **1**, thus implying that **3** had to be the decarboxylated derivative of **1**, most likely formed during the workup. All resonances were assigned as reported in Table 2.

The HRESIMS spectrum of compound **4** displayed the sodiated molecular peak at *m/z* 399.2880 $[M + Na]^+$, representing the molecular formula $C_{24}H_{40}O_3$. Analogously with **3** deriving from **1**, compound **4** was observed to be formed by transformation of co-occurring **2**. Analysis of spectroscopic data of **4** evidenced that it was the decarboxylated derivative of **2** and consequently the 14,15-dihydro derivative of **3**. All carbon and proton resonances (Table 2) were easily attributed by 2D NMR experiments.

Compounds **5** and **6**, with the molecular formulas $C_{19}H_{28}O$ and $C_{19}H_{30}O$, respectively, as deduced by HRESIMS, were less polar with respect to the compounds above-described. Analysis of their ^1H and ^{13}C NMR spectra immediately revealed that **5** and **6** shared with the co-occurring metabolites both the terminal aromatic ring and the alkyl chain moieties, whereas the peroxide ring was lacking (Table 3). In its place, a terminal methyl ketone function was present in both compounds, as indicated by the ^{13}C NMR signals at δ_C 208.6 (qC, C-2) and 30.7 (CH_3, C-1) in **5** and at δ_C 209.0 (qC, C-2) and 30.3 (CH_3, C-1) in **6**. The difference between the two compounds was that **5** exhibited a conjugated double bond [δ_H 6.38 (1H, d, J = 15.3 Hz, H-12) and 6.22 (1H, dt, J = 7.0, 15.3 Hz, H-11)], analogously with **1** and **3**, whereas **6** had a saturated alkyl chain (Table 3), the same as **2** and **4**. All the spectral data were consistent with the proposed structures.

A biogenetic correlation between peroxides **1** and **2** and keto-derivatives **5** and **6**, respectively, could be hypothesized, analogously with that suggested by Higgs and Faulkner for similar metabolites isolated from *Plakortis halichondrioides*.[2] According to this suggestion, the pairs **1/5** and **2/6** could derive from common 1,3-diene intermediates **I** and **II**, respectively, as depicted in Scheme 1.

Compounds **1–6** were tested for antifungal (*Candida albicans*) and antibacterial (*Escherichia coli* and *Staphylococcus aureus*) activities. No growth inhibition was exhibited against *E. coli* and *C. albicans* by all metabolites. Compounds **1** and **4** showed weak activity against *S. aureus*, with MIC values of 128 and 64 μg/mL, respectively.

Experimental Section

General Experimental Procedures

Optical rotations were measured on a Jasco DIP 370 digital polarimeter; IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer; 1D and 2D NMR spectra were recorded on a Bruker 400 AMX (400.13 MHz) in $CDCl_3$ (δ values are reported referred to $C\underline{H}Cl_3$ at 7.26 ppm), and ^{13}C NMR were recorded on a 300 AMX Bruker (75.47 MHz) (δ

values are reported to $\underline{C}DCl_3$, 77.0 ppm); HRESIMS were carried out on a Micromass Q-TOF micro; HPLC Waters 501 pump with a refractometer detector was used equipped with direct-phase Kromasil silica column, 5 μ (250 × 4.60 mm, Phenomenex), and with a reversed-phase Kromasil C-18 column, 5 μ (250 × 4.60 mm, Phenomenex); TLC plates (silica gel 60 F254) were from Merck (Darmstadt, Germany); silica gel powder (silica gel 60 0.063–0.200 mm) was from Merck (Darmstadt, Germany); Sephadex LH-20 was from Amersham Pharmacia Biotech (Uppsala, Sweden).

Animal Material

The sponge was collected from a rock wall at 96 m depth, near Blue Hole, Orote Peninsula, Guam. The collected specimen was actually a mix of two sponges growing on one another, with one of the sponges exhibiting an unusual growth form with numerous small fistules of about 2–3 cm in length protruding from the sponge consortium. The fistule-forming sponge belongs to the genus *Dactylospongia* (Thorectidae, Dictyoceratida), forming small connected globular cushions with the aforementioned small fistules. The consistency is very firm and the color beige-yellow in alcohol. The surface is smooth and unarmored. The skeleton is composed of interconnecting fibers without a clear distinction between primaries and secondaries. Only two species belonging to the genus *Dactylospongia* have been described so far, and this specimen does not fit either of them.

The other sponge was originally described as *Plakinastrella clathrata* Kirkpatrick, 1911 by Funafuti. However distinct size classes of the spicules were not observed; thus this species better fits the genus *Plakortis*. The specimen forms small lobate encrustations across *Dactylospongia*. The surface is smooth, but feels rough to touch; the color is light yellow-pink in alcohol. The skeleton is confused, dense, with many subdermal spaces and composed of calthrops and derivates. The size range of the abundant diods is 35–130 × 2–2.5 μm; triods are scarce and irregular with two rays (together 75–90 μm) in line with a shorter third ray (20–25 μm), forming an angle; regular calthtrops with rays (20–40 μm).

The two sponges were separated as well as possible; then they were freeze-dried and kept at −20 °C. A voucher of both specimens was deposited at the National Museum of Natural History, Leiden, under code number RMNH POR 4821.

Extraction and Isolation

The sample containing mainly *Plakortis* sp. (74 g wet weight) was extracted with a solution of MeOH and EtOAc (1:1, 350 mL × 4) using ultrasound. Filtration and evaporation of the homogenate gave a residue that was suspended in MeOH (400 mL) and extracted subsequently with hexane (400 mL × 4) and $CHCl_3$ (400 mL × 4). The evaporation of hexane and $CHCl_3$ extracts gave a gummy residue (0.645 and 0.836 g, respectively). The $CHCl_3$ extract was subjected to a Sephadex LH-20 chromatography eluting with $CHCl_3$/MeOH (1:1), to give three fractions, A (0.230 g), B (0.160 g), and C (0.295 g). Fractions A and C were phthalate mixtures, and fraction B showed interesting UV–visible spots on TLC (R_f 0.30–0.40, $CHCl_3$/MeOH, 90:10). Fraction B was purified on reversed-phase HPLC (MeOH/H_2O, 9:1, flow rate 3 mL/min) to afford **1** (6.0 mg, 0.72%) and **2** (4.5 mg, 0.54%) along with the known dehydrocurcuphenol (2.0 mg, 0.24%) and manoalide (9.0 mg, 1.08%). Half of the hexane extract was directly purified on reversed-phase HPLC (MeOH, flow rate 3 mL/min) to afford **3** (2.0 mg, 0.62%), **4** (3.5 mg, 1.09%), **5** (1.0 mg, 0.31%), and **6** (1.5 mg, 0.47%) along with lipid and sterol fractions.

Compound 1—colorless oil, R_f 0.32 ($CHCl_3$/MeOH, 90:10); $[\alpha]_D$ +42 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (3.49) nm; IR (liquid film) ν_{max} 3418, 2925, 2830, 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33 (2H, bd, J = 7.6 Hz, H-18, H-22), 7.28 (2H, bt, J = 7.4 Hz,

H-19, H-21), 7.19 (1H, bt, *J* = 7.2 Hz, H-20), 6.39 (1H, d, *J* = 15.9 Hz, H-16), 6.21 (1H, dt, *J* = 7.0, 15.9 Hz, H-15), 2.89 (1H, d, *J* = 15.3 Hz, H-2a), 2.56 (1H, d, *J* = 15.3 Hz, H-2b), 2.21 (2H, m, H_2-14), 2.02 (2H, m, H_2-4), 1.67 (1H, m, H-5a), 1.63 (2H, m, H_2-8), 1.48 (2H, m, H_2-13), 1.44 (1H, m, H-7a), 1.39 (3H, s, H_3-24), 1.37 (1H, m, H-5b), 1.30 (4H, m, H_2-11, H_2-12), 1.31 (1H, m, H-7b), 1.28 (2H, m, H_2-10), 1.27 (1H, m, H-9a), 1.14 (1H, m, H-9b), 0.99 (3H, d, *J* = 6.6, H_3-23), 0.92 (3H, d, *J* = 6.5, H_3-25). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138 (C, C-17), 131.1 (CH, C-15), 129.9 (CH, C-16), 128.5 (CH, C-19, C-21), 126.7 (CH, C-20), 125.9 (CH, C-18, C-22), 100.8 (C, C-3), 81.8 (C, C-6), 47.9 (CH_2, C-7), 38.8 (CH_2, C-2), 38.7 (CH_2, C-9), 38.2 (CH_2, C-5), 33.0 (CH_2, C-14), 32.9 (CH, C-4), 29.7 (CH_2, C-11), 29.4 (CH_2, C-12), 29.2 (CH_2, C-13), 28.3 (CH, C-8), 27.0 (CH_2, C-10), 21.5 (CH_3, C-25), 20.3 (CH_3, C-24), 16.1 (CH_3, C-23); ESIMS *m/z* 441 $[M + Na]^+$.

Compound 1a: Compound **1** (2 mg) was dissolved in a diethyl ether solution of CH_2N_2 and stirred for 30 min. The mixture was evaporated to isolate **1a** after silica gel column chromatography (light petroleum ether/Et_2O, 8:2). $[\alpha]_D$ +15 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR data in Table 1; HRESIMS *m/z* 455.2750 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_5Na$, 455.2773).

Compound 2—colorless oil, R_f 0.35 ($CHCl_3$/MeOH. 90:10); $[\alpha]_D$ +23 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 248 (3.46) nm; IR (liquid film) ν_{max} 3425, 2926, 2837, 1718 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (2H, m, H-18, H-22), 7.17 (2H, m, H-19, H-21), 7.16 (1H, m, H-20), 2.90 (1H, d, *J* = 15.4 Hz, H-2a), 2.59 (2H, t, *J* = 7.3 Hz, H_2-16), 2.56 (1H, d, *J* = 15.4 Hz, H-2b), 2.02 (2H, m, H_2-4), 1.68 (1H, m, H-5a), 1.62 (3H, m, H-8, H_2-15), 1.44 (1H, m, H-7a), 1.38 (3H, s, H_3-24), 1.35 (1H, m, H-5b), 1.31 (1H, m, H-7b), 1.29–1.30 (8H, m, H_2-11, H_2-12, H_2-13, H_2-14), 1.27 (3H, m, H-9a, H_2-10), 1.14 (1H, m, H-9b), 0.99 (3H, d, *J* = 6.6, H_3-23), 0.92 (3H, d, *J* = 6.5, H_3-25). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.9 (C, C-17), 128.2 (CH, C-19, C-21), 128.1 (CH, C-18, C-22), 125.5 (CH, C-20), 100.9 (C, C-3), 81.7 (C, C-6), 47.8 (CH_2, C-7), 38.9 (CH_2, C-2), 38.8 (CH_2, C-9), 38.1 (CH_2, C-5), 36.0 (CH_2, C-16), 32.7 (CH, C-4), 31.5 (CH_2, C-15), 29.7 (CH_2, C-11, C-12, C-13, C-14), 28.2 (CH, C-8), 27.0 (CH_2, C-10), 21.5 (CH_3, C-25), 20.2 (CH_3, C-24), 16.1 (CH_3, C-23); ESIMS *m/z* 443 $[M + Na]^+$.

Compound 2a: Compound **2** (2 mg) was dissolved in a diethyl ether solution of diazomethane and stirred for 30 min. The mixture was evaporated to isolate **2a** after silica gel column chromatography (light petroleum ether/Et_2O, 8:2). $[\alpha]_D$ +8.0 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR data in Table 1; HRESIMS *m/z* 457.2926 $[M + Na]^+$ (calcd for $C_{26}H_{42}O_5Na$, 457.2930).

Compound 3—colorless oil, R_f 0.81 (light petroleum ether/Et_2O, 50: 50); $[\alpha]_D$ +60 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 251 (4.56) nm; ^{1}H and ^{13}C NMR data in Table 2; HRESIMS *m/z* 397.2725 $[M + Na]^+$ (calcd for $C_{24}H_{38}O_3Na$, 397.2719).

Compound 4—colorless oil, R_f 0.85 (light petroleum ether/Et_2O, 50: 50); $[\alpha]_D$ +16 (*c* 0.3, CHCl3); UV (MeOH) λ_{max} (log ε) 248 (2.74) nm; IR (liquid film) ν_{max} 2926, 2854, 1718 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 2; HRESIMS *m/z* 399.2880 $[M + Na]^+$ (calcd for $C_{24}H_{40}O_3Na$, 399.2875).

Compound 5—colorless oil, R_f 0.92 (light petroleum ether/Et_2O, 50: 50); $[\alpha]_D$ −2.4 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (3.36) nm; IR (liquid film) ν_{max} 2936, 2855, 1724 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 3; HRESIMS *m/z* 295.2043 $[M + Na]^+$ (calcd for $C_{19}H_{28}ONa$, 295.2038).

Compound 6—colorless oil, R_f 0.94 (light petroleum ether/Et_2O, 50: 50); $[\alpha]_D$ −4.9 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 247 (2.94) nm; IR (liquid film) ν_{max} 2936, 2861, 1718

cm^{-1}; ^{1}H and ^{13}C NMR data in Table 3; HRESIMS *m/z* 297.2190 $[M + Na]^+$ (calcd for $C_{19}H_{30}ONa$, 297.2194).

Antibacterial Assays

Antibacterial assays were performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P (NCCLS, 1992) and of the Clinical and Laboratory Standard Institute (CLSI) document M7-A7 (CLSI, 2007). The medium used to prepare the 10× drug dilutions and the inoculum suspension was liquid LB (Luria–Bertani medium: 10 g/L Bactotryptone, 5 g/L Bactoyeast, and 10 g/L NaCl, pH 7.5).[12] The bacteria suspensions were adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland (2×10^8 cfu/mL) standard at 530 nm and diluted to 1:4000 (50 000 cfu/mL) in LB medium. The bacteria suspension (0.9 mL) was added to each test tube that contained 0.1 mL of eleven 2-fold dilutions (512–0.05 μg/mL final) of each tested compound. Broth macrodilution MICs were determined after 24 h of incubation at 37 °C. MIC (minimal inhibitory concentration) was defined as the lowest compound concentration that was able to completely inhibit growth of the test bacteria. So the lowest concentration with no visible growth was determined as the MIC_{90} (90% of growth inhibition).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Mr. S. Zambardino of ICB-NMR service, Mr. M. Zampa for HRESIMS spectra, and Mr. C. Iodice for spectrophotometric measurements. This research has been partially supported by the Short Term Mobility-2007 program (CNR, Italy) to E.M. and NCI grant 5U56CA096278 to P.J.S. We thank J. Pinson for his continued assistance in collecting sponges from the twilight zone.

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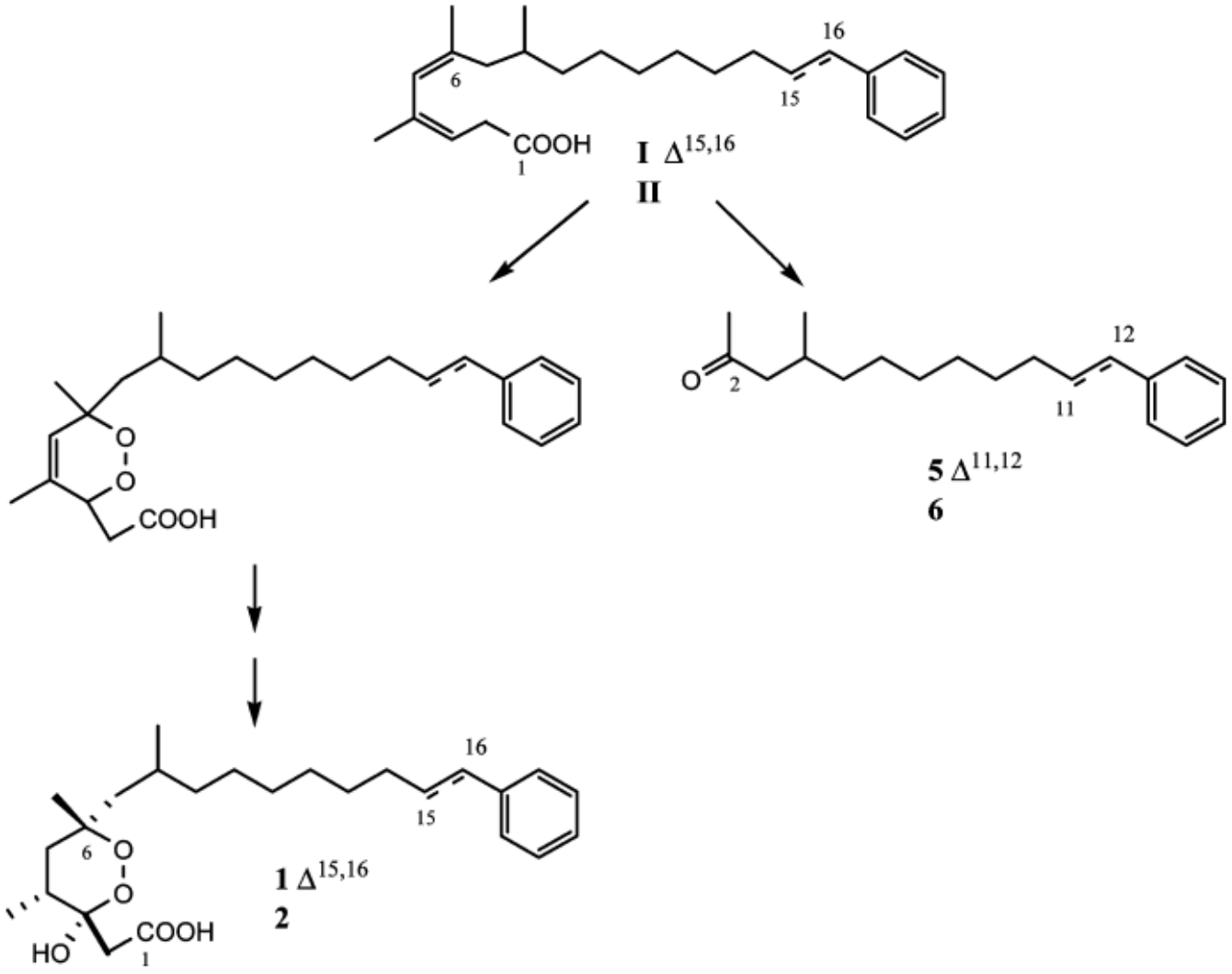

Scheme 1.
Peroxides **1** and **2** and Ketones **5** and **6** Could Be Derived from Common Intermediates **I** and **II**

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Table 1

NMR Spectroscopic Data[a] for Compounds **1a** and **2a**

	compound 1a			compound 2a		
position	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**
1	171.1, qC		2, OCH_3	171.2, qC		2, OCH_3
2	39.2, CH_2	2.82, d (16.0)	4	39.2, CH_2	2.83, d (15.9)	4
		2.52, d (16.0)			2.54, d (15.9)	
3	100.7, qC		2, 23	100.9, qC		2, 23
4	32.7, CH	1.99, m	5, 23	32.7, CH	1.99, m	5, 23
5	38.2, CH_2	1.70, dd (13.1, 13.1)	23, 24	38.2, CH_2	1.71, dd (13.2, 13.2)	23, 24
		1.36, m			1.37, m	
6	81.5, qC		5, 24	81.6, qC		5, 24
7	47.8, CH_2	1.45, m	24, 25	47.8, CH_2	1.48, m	24, 25
		1.33, m			1.37, m	
8	28.3, CH	1.63, m	25	28.3, CH	1.64, m	25
9	38.7, CH_2	1.27, m	25	38.8, CH_2	1.29, m	11, 12
		1.15, m			1.18, m	
10	27.0, CH_2	1.28, m	11, 12	27.0, CH_2	1.27, m	
11	29.7, CH_2	1.30, m		29.7, CH_2	1.29–1.30, m	
12	29.4, CH_2	1.30, m.		29.7, CH_2	1.29–1.30, m	
13	29.2, CH_2	1.48, m.		29.7, CH_2	1.29–1.30, m	
14	33.0, CH_2	2.20, m	15	29.7, CH_2	1.29–1.30, m	
15	131.2, CH	6.22, dt (15.9, 7.0)	14, 16	31.5, CH_2	1.61, m	16
16	129.7, CH	6.38, d (15.9)	15	36.0, CH_2	2.60, t (7.3)	15
17	137.9, qC		16, 18	143.0, qC		16, 18, 22
18	125.9, CH	7.33, bd (7.6)	16, 19	128.2, CH	7.26, m	16, 19
19	128.5, CH	7.28, bt (7.4)	18, 20	128.4, CH	7.17, m	18, 20
20	126.7, CH	7.19, bt (7.2)	19	125.5, CH	7.16, m	19, 21
21	128.5, CH	7.28, bt (7.4)	20, 22	128.4, CH	7.17, m	20, 22
22	125.9, CH	7.33, bd (7.6)	16, 21	128.2, CH	7.26, m	16, 21

	compound 1a			compound 2a		
position	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**
23	16.1, CH_3	0.98, d (6.7)	4	16.1, CH_3	0.99, d (6.6)	4
24	20.3, CH_3	1.37, s		20.3, CH_3	1.37, s	
25	21.5, CH_3	0.92, d (6.7)	8	21.5, CH_3	0.92, d (6.7)	8
OCH_3	52.2, CH_3	3.72, s		52.2, CH_3	3.73, s	

[a] Bruker DPX Avance 400 MHz and DPX 300 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $C\underline{H}Cl_3$ (δ 7.25) and to $\underline{C}DCl_3$ (δ 77.0). Assignments determined by ^1H–^1H COSY, HSQC, and HMBC.

[b] By DEPT sequence.

[c] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

Table 2

NMR Spectroscopic Data[a] for Compounds **3** and **4**

	compound 3			compound 4		
position	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**
1	22.6, CH_3	1.34, s		22.7, CH_3	1.35, s	
2	101.2, qC		22	101.2, qC		22
3	33.4, CH	2.02, m	22	33.4, CH	2.00, m	22
4	38.8, CH_2	1.46, m	22,23	38.8, CH_2	1.45, m	22,23
		1.28, m			1.28, m	
5	81.6, qC		6, 23	81.6, qC		6, 23
6	48.0, CH_2	1.43, m	23, 24	48.1, CH_2	1.44, m	23, 24
		1.25, m			1.24, m	
7	28.3, CH	1.63, m	6, 24	28.3, CH	1.64, m	6, 24
8	38.8, CH_2	1.26, m	24	38.8, CH_2	1.27, m	24
		1.15, m			1.16, m	
9	27.0, CH_2	1.30, m	8	27.0, CH_2	1.29–1.30, m	8
10	29.7,[d] CH_2	1.30, m		29.8,[d] CH_2	1.29–1.30, m	
11	29.3,[d] CH_2	1.30, m		29.6,[d] CH_2	1.29–1.30, m	
12	29.2, CH_2	1.46, m		29.5,[d] CH_2	1.29–1.30, m	
13	33.0, CH_2	2.20, m	14	29.3,[d] CH_2	1.29–1.30, m	14
14	131.2, CH	6.22, dt (15.7,6.9)	13, 15	31.4, CH_2	1.62, m	13, 15
15	129.7, CH	6.38, d (15.7)	14	36.0, CH_2	2.60, t (7.4)	14
16	137.8, qC		15, 17	143.2, qC		15, 17
17	125.9, CH	7.33, bd (7.3)	15, 18	128.2, CH	7.26, m	15, 18
18	128.5, CH	7.28, bt (7.2)	19	128.4, CH	7.17, m	19
19	126.7, CH	7.18, bt (7.1)	18, 20	125.5, CH	7.17, m	18, 20
20	128.5, CH	7.28, bt (7.2)	21	128.4, CH	7.17, m	21
21	125.9, CH	7.33, bd (7.3)	15, 20	128.2, CH	7.26, m	15, 20
22	16.2, CH_3	0.97, d (6.5)	3	16.2, CH_3	0.96, d (6.7)	3

	compound 3			compound 4		
position	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**
23	20.1, CH_3	1.39, s		20.1, CH_3	1.39, s	
24	21.5, CH_3	0.93, d (6.7)		21.6, CH_3	0.92, d (6.4)	

[a] Bruker DPX Avance 400 MHz and DPX 300 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $C\underline{H}Cl_3$ (δ 7.25) and to $\underline{C}DCl_3$ (δ 77.0). Assignments determined by ^1H–^1H COSY, HSQC and HMBC.

[b] By DEPT sequence.

[c] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

[d] Interchangeable.

J Nat Prod. Author manuscript; available in PMC 2010 August 1.

Table 3

NMR Spectroscopic Data[a] for Compounds **5** and **6**

	compound 5			compound 6		
position	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**	**δ_C, mult.[b]**	**δ_H (*J* in Hz)**	**HMBC[c]**
1	30.7, CH_3	2.12, s	3	30.3, CH_3	2.12, s	3
2	208.6, qC		1, 3	209.0, qC		1, 3
3	51.2, CH_2	2.40, dd (6.1–16.2)	1, 19	51.3, CH_2	2.39, dd (5.7–15.6)	1, 19
		2.21, m)			2.21, dd (8.0–15.6)	
4	28.9, CH	1.97, m	19	28.9, CH	1.98, m	19
5	36.7, CH_2	1.28, m	19	37.1, CH_2	1.27, m	19
		1.17, m			1.15, m	
6	29.7,[d] CH_2	1.29–1.30, m		29.7,[d] CH_2	1.29–1.31, m	
7	29.3,[d] CH_2	1.29–1.30, m		29.7,[d] CH_2	1.29–1.31, m	
8	29.3,[d] CH_2	1.29–1.30, m		29.4,[d] CH_2	1.29–1.31, m	
9	29.3, CH_2	1.47, m	10	29.4,[d] CH_2	1.29–1.31, m	
10	32.8, CH_2	2.20, m	11	29.4,[d] CH_2	1.29–1.31, m	
11	131.1, CH	6.22, dt (15.3,7.0)	10, 12	30.7, CH_2	1.63, m	12
12	129.7, CH	6.38, d (15.3)	11	36.0, CH_2	2.60, t (7.4)	11
13	138.0, qC		12, 14	142.6, qC		12, 14
14	125.8, CH	7.33, bd (7.0)	12, 15	128.2, CH	7.28, m	12, 15
15	128.3, CH	7.29, bt (7.3)	14	128.5, CH	7.18, m	14
16	126.6, CH	7.19, bt (7.4)	15, 17	125.6, CH	7.18, m	15, 17
17	128.3, CH	7.29, bt (7.3)	16, 18	128.5, CH	7.18, m	16, 18
18	125.8, CH	7.33, bd (7.0)	12, 17	128.2, CH	7.28, m	12, 17
19	19.7, CH_3	0.89, d (6.5)		19.8, CH_3	0.89, d (6.7)	

[a] Bruker DPX Avance 400 MHz and DPX 300 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ 7.25) and to $CDCl_3$ (δ 77.0). Assignments determined by ^1H–^1H COSY, HSQC, and HMBC.

[b] By DEPT sequence.

[c] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

[d] Interchangeable.