quenched by dropwise addition of water. The product was diluted with 2 M aqueous sodium hydroxide solution (50 mL) and extracted with dichloromethane (2 × 50 mL). The combined dichloromethane extracts were washed with water (50 mL), dried over sodium sulfate, and filtered, and the solvent was evaporated to obtain the amine **3** (140 mg, quantitative) that was essentially pure by TLC: ^{1}H NMR ($CDCl_3$) δ 0.55 (s, 3 H), 0.78 (s, 3 H), 3.19 (br s, 1 H), 5.17 (br s, 1 H), 5.19 (m, 2 H); ^{13}C NMR ($CDCl_3$) (see Table I).

3α-(*N*-Methylamino)-5α-ergosta-7,22-diene (8). Formic anhydride was prepared by addition of formic acid (440 mg) in cold chloroform (5 mL) to a cold solution of dicyclohexyl carbodiimide (700 mg) in chloroform (5 mL). The mixture was stirred vigorously at 0 °C for 5 min as dicyclohexylurea precipitated. Approximately half of the solution was added dropwise to a stirred solution of the amine (49 mg, 0.12 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was stirred at 0 °C for 6 h. The solvents were removed in vacuo, and the residue taken up in dichloromethane and filtered to remove the urea. The solution was washed with water, dried over sodium sulfate, filtered, and concentrated to obtain the formamide, still slightly contaminated with dicyclohexyl urea.

Lithium aluminum hydride (60 mg, excess) was added to a solution of the formamide in dry tetrahydrofuran (10 mL), and the mixture was stirred under dry nitrogen for 6 h at 25 °C. The excess reagent was quenched by careful addition of water and the product partitioned between 0.5 M sodium hydroxide solution (50 mL) and dichloromethane (2 × 50 mL). The combined dichloromethane extracts were dried over sodium sulfate, and filtered, and the solvent evaporated to obtain a mixture of the *N*-methylamine **8** (19 mg, 37.5% theoretical) and unreacted formamide (21 mg, 40% theoretical) that were easily separated by silica gel chromatography.

3α-(*N*-Methylamino)-5α-ergosta-7,22-diene: ^{1}H NMR ($CDCl_3$) δ 0.54 (s, 3 H), 0.79 (s, 3 H), 2.36 (s, 3 H), 2.73 (br s, 1 H), 5.16 (br s, 1 H), 5.19 (m, 2 H); ^{13}C NMR ($CDCl_3$) (see Table I).

Acknowledgment. We thank Dr. N. Boury-Esnault for identifying the sponge sample and Mary Kay Harper for antimicrobial analyses. This research was supported by grants from the National Institutes of Health (AI-11969) and the Sea Grant College Program (NA80AA-D-00120).

Two New Metabolites of the Sponge *Dysidea amblia* and Revision of the Structure of Ambliol B

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Ambliol C (**6**), ambliofuran (**4**), pallescensin A (**7**), and pallescensolide (**8**) were isolated from a sample of *Dysidea amblia* collected at Pt. Loma, San Diego, CA. The structure of ambliol C (**6**) was determined from a single-crystal X-ray diffraction study performed on the ester **13** and that of pallescensolide (**8**) from ^{1}H NMR and other spectral data. The structure of ambliol B (**9**), one of five metabolites obtained from *D. amblia* collected at Scripps Canyon, La Jolla, CA, was reassigned. X-ray diffraction analysis of the acid **14** showed that ambliol B (**9**) contained a trans-fused decalin ring system rather than the cis ring junction proposed previously.

We previously reported the structural elucidation of five diterpenes (**1**–**5**, Chart I) from a sample of *Dysidea amblia* collected at Scripps Canyon, CA.[1] A second collection of *D. amblia* from Pt. Loma, CA, contained a different array of metabolites that included the known diterpene ambliofuran (**4**) and a known sesquiterpene pallescensin A (**7**).[3] Two new metabolites, ambliol C (**6**) and pallescensolide (**8**), related to pallescensin A (**7**), were isolated. Comparison of the spectral data and chemical reactions of ambliol B (**5**) and ambliol C (**6**) led us to review the previously proposed structure for ambliol B (**5**). In this paper we report the structural determination of ambliol C (**6**), the revised structural assignment for ambliol B (**9**), and the structural elucidation of pallescensolide (**8**).

Dysidea amblia (de Laubenfels)[2] was collected by hand using SCUBA (−20 m). The hexane-soluble material from a methanolic extract of the homogenized sponge was chromatographed on silica gel to obtain pallescensin A (**7**, 0.4% dry weight),[3] ambliofuran (**4**, 0.6% dry weight),[1] ambliol C (**6**, 1.0% dry weight), and pallescensolide (**8**, 0.03% dry weight).

Ambliol C (**6**), $[\alpha]_D$ −37.8° (*c* 2.0 $CHCl_3$), had the molecular formula $C_{20}H_{32}O_2$, isomeric with ambliols A and B. The infrared band at 3500 cm^{-1} indicated that ambliol C was an alcohol. The ^{13}C NMR spectrum contained signals at δ 142.8 (d), 137.8 (d), 126.2 (s), and 111.3 (d) due to a β-substituted furan and at δ 76.0 (s) due to a tertiary carbinol. Both the ^{13}C and ^{1}H NMR spectra of ambliol C (**6**) were sufficiently similar to those of ambliol B (**5**) to suggest that the molecules were stereoisomers.

The same chemical reactions that had been used in the prior structural assignment of ambliol B (**5**) were now performed on ambliol C (**6**). Dehydration of ambliol B (**5**) with phosphorus oxychloride in pyridine gave trisubstituted olefin **10** while acid-catalyzed dehydration produced tetrasubstituted olefin **11** (Chart II). Dehydration of ambliol C (**6**) with either phosphorus oxychloride in pyridine or *p*-toluenesulfonic acid in benzene gave a trisubstituted olefin, dehydroambliol C (**12**). Dehydroambliol C (**12**) was not identical with the trisubstituted olefin **10** from ambliol B (**5**), although it was subsequently determined that the olefinic bond was in the same position in both molecules.[4] These results were perplexing particu-

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[1] Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1098.

[2] De Laubenfels, M. W. *Proc. U.S. Nat. Mus.* **1982**, *81*, 123.

[3] Cimino, G.; De Stefano, S.; Guerriero, A.; Minale, L. *Tetrahedron Lett.* **1975**, 1425.

[4] Walker, R. P. Ph.D. Thesis, University of California, San Diego, CA, 1981.

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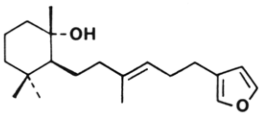
Chart I



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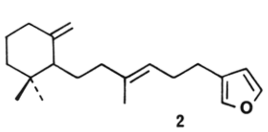

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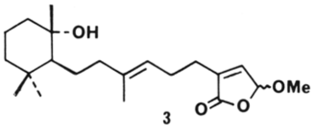

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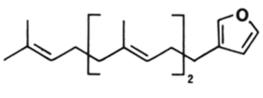

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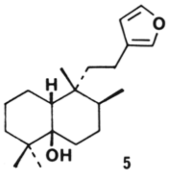

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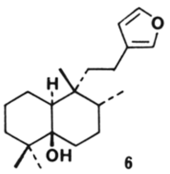

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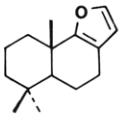

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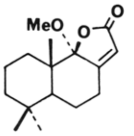

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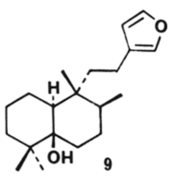
Chart II



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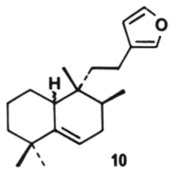

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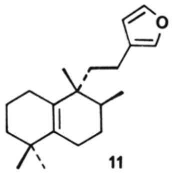

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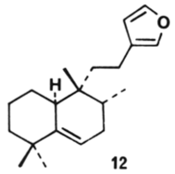

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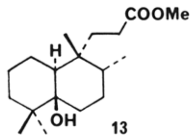

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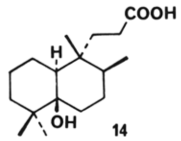

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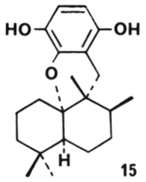

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larly since we had previously used a mechanistic interpretation of the difference in dehydration products to assign a cis ring junction to ambliol B (**5**). The structures of ambliol C (**6**) and ambliol B (**9**) were therefore determined by X-ray diffraction analysis.

Ozonolysis of ambliol C (**6**) in acetone solution at −78 °C followed by oxidation of the ozonide with Jones reagent and methylation of the resulting acid with diazomethane in ether gave the ester **13**, which crystallized from hexane, mp 85–86 °C. The results of a single-crystal X-ray diffraction study of ester **13** are presented in Figure 1. The X-ray experiment defined only the relative configuration, and the enantiomer shown was chosen on biogenetic grounds. The decalin is trans fused with the bridgehead hydrogen at C-10 in the α-configuration and the bridgehead hydroxyl at C-5 in the β-configuration. The methyl groups at C-8 and C-9 are both axial. All bond distances and angles agree well with generally accepted values.

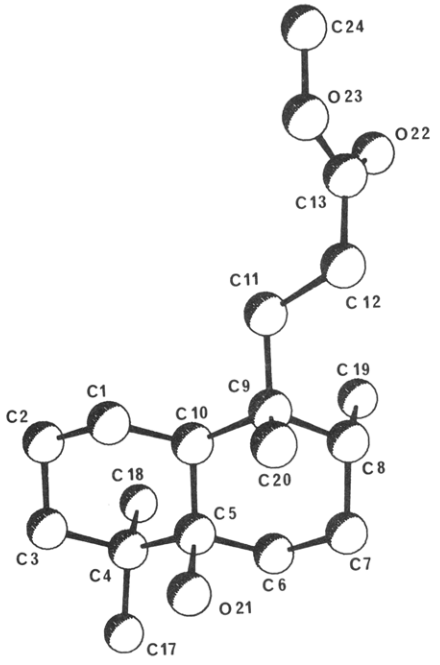

Figure 1. A computer-generated perspective drawing of the final X-ray model of ester **13**. Hydrogens are omitted for clarity and no absolute configuration is implied.

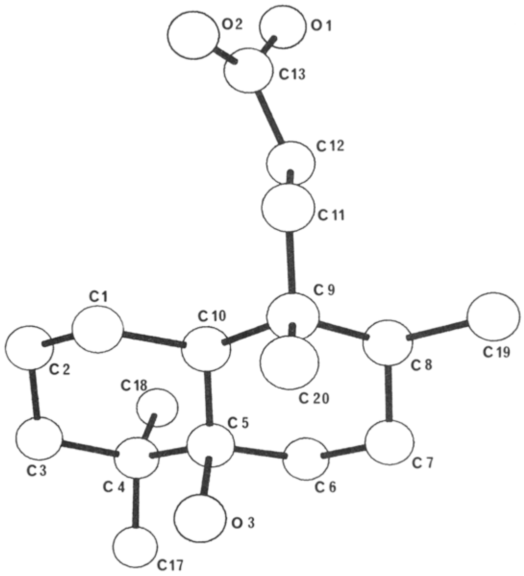

Figure 2. A computer-generated perspective drawing of the final X-ray model of acid **14**. Hydrogens are omitted for clarity.

Ozonolysis of ambliol B (**9**) followed by oxidation of the resulting ozonide gave crystalline acid **14**, mp 123–125 °C.[5] A computer-generated perspective drawing of the final

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[5] This experiment was reported in detail in ref 1 and is not repeated in the Experimental Section.

X-ray model of acid **14** is given in Figure 2. Both independent molecules in the asymmetric unit have the same configuration and conformation, and only one is shown. Hydrogens have been omitted for clarity and the absolute configuration (see below) is depicted.

The structures of ambliol C (**6**) and ambliol B (**9**) differ only in the stereochemistry at C-8; ambliol C (**6**) has an axial methyl substituent while the orientation of the methyl group in ambliol B (**9**) is equatorial. The revised structure of ambliol B (**9**) differs from the previously proposed structure **5** at C-10 only and the decalin ring system is trans-fused.[6] The previously reported[1] interconversion of the tetrasubstituted olefin **11** with aureol (**15**),[7] a molecule of known absolute configuration, allows the absolute configuration of ambliol B (**9**) to be defined as 5*R*, 8*S*, 9*R*, 10*S*.

Pallescensolide (**8**), $[\alpha]_D$ −57.4° (*c* 0.9, CHCl$_3$), had the molecular formula $C_{16}H_{24}O_3$. The infrared spectrum indicated the presence of an α,β-unsaturated γ-lactone (1755 cm^{-1}). Since we had previously found ambliolide (**3**) accompanying ambliol A (**1**) in *D. amblia*, a similar relationship between pallescensolide (**8**) and pallescensin A (**7**) was suspected. The ^{13}C NMR spectrum contained a lactone carbonyl signal at δ 175.6, two olefinic signals at δ 125.9 and 116.7, a ketal carbon signal at δ 107.5, and a methoxyl carbon at δ 60.6, all reasonable chemical shift values for a γ-methoxy-α,β-unsaturated γ-lactone moiety.[8] The ^{1}H NMR spectrum contained three methyl signals at δ 0.81 (s, 3 H), 0.84 (s, 3 H), and 0.94 (s, 3 H), and the methoxyl signal at δ 3.08 (s, 3 H). The olefinic proton signal at δ 5.72 (d, 1 H, *J* = 2 Hz) was allylically coupled to the C-7 axial proton signal at δ 2.14 (m, 1 H, *J* = 14, 13, 6, 2 Hz), which was in turn coupled to the C-7 equatorial proton signal at δ 2.70 (ddd, 1 H, *J* = 14, 5, 2 Hz), the C-6 axial proton signal at δ 1.82 (m, 1 H, *J* = 13, 6, 3, 2 Hz), and the C-6 equatorial proton signal at δ 1.41 (m, 1 H, partially obscured); the signals at δ 1.82 and 1.41 were coupled to one another and to the axial C-5 proton signal at δ 1.70 (dd, 1 H, *J* = 13, 3 Hz). The ring-A proton signals were too complex for first-order interpretation, but the lower than average chemical shift of the C-1 axial proton at δ 1.95 (m, 1 H, *J* = 13, 10, 7 Hz) suggested that the methoxyl group at C-9 was also axial.[9]

A reviewer has questioned whether ambliolide (**3**) and pallescensolide (**8**) are natural products or artifacts resulting from air oxidation during the isolation procedure. Preliminary experiments have shown that oxidation of ambliol A (**1**) with singlet oxygen in methanol gave a number of products, none of which was ambliolide (**3**).[10] We therefore suspect that oxidation of furans can occur in the sponge but that the methoxy group might be introduced during extraction with methanol.

The sample of *D. amblia* from Pt. Loma could not be distinguished from the Scripps Canyon specimens by using classical taxonomic methods. Furthermore, single sponges contained both sesquiterpenes and diterpenes. The presence of two classes of terpenes in the same sponge might be considered unusual except that there are a few precedents among *Dysidea* species.[11]

Experimental Section[12]

Extraction and Chromatography. *Dysidea amblia* (de Laubenfels) was collected by hand using SCUBA (−20 m) at Pt. Loma, San Diego, CA, in August 1980. The sponge (88 g dry weight) was steeped in methanol at 0 °C for 3 months, then homogenized, and Soxhlet extracted with fresh methanol (2 L) for 2 days. The combined extracts were evaporated under vacuum to obtain an oily aqueous suspension that was partitioned between water (500 mL) and hexane (3 × 1 L), then dichloromethane (3 × 1 L), and finally 2:1 chloroform:isopropyl alcohol (2 × 1 L). Each extract was evaporated under vacuum and the residues were examined by TLC and ^{1}H NMR.

The hexane-soluble material (7.7 g, 8.8% dry weight) was dissolved in hexane and the solution applied to TLC-grade silica gel (246 g) in a 600-mL Buchner funnel. Elution under suction with solvents of increasing polarity from hexane through ether to 10% methanol in ether gave nine fractions. The fraction eluted with 1% ether in hexane gave pallescensin A (**7**, 351 mg, 0.4% dry weight), having identical spectral data with those reported.[3] The fraction eluted with 2% ether in hexane contained ambliofuran (**4**, 532 mg, 0.6% dry weight).[1] The fraction eluted with 10% ether in hexane was rechromatographed on a Merck Lobar "B" silica gel medium pressure column to obtain ambliol C (**6**, 871 mg, 1.0% dry weight), which was crystallized from hexane. The fraction eluted with 50% ether in hexane was rechromatographed by LC on μ-Partisil by using ether as eluant to obtain pallescensolide (**8**) (22 mg, 0.03% dry weight).

Ambliol C (6): mp (hexane) 45–46 °C; $[\alpha]_D$ −37.8° (*c* 2.0, CHCl$_3$); IR (film) 3500 cm^{-1}; UV (pentane) 211 nm (ϵ 4100); ^{1}H NMR (CCl$_4$) δ 0.83 (s, 3 H), 0.93 (d, 1 H, *J* = 7 Hz), 0.99 (s, 3 H), 1.11 (s, 3 H), 2.07 (m, 1 H, *J* = 14, 14, 4, 4 Hz), 2.29 (t, 2 H, *J* = 8 Hz), 6.15 (bs, 1 H) 7.11 (bs, 1 H), 7.29 (bs, 1 H); ^{13}C NMR (C$_6$D$_6$) δ 142.8 (d), 138.7 (d), 126.2 (s), 111.3 (d), 76.0 (s), 40.7 (d), 40.5 (t), 39.1 (s), 37.5 (s), 37.0 (t), 35.5 (d), 25.8 (t), 24.8 (t), 24.7 (q), 23.6 (q), 22.8 (t), 21.5 (t), 21.3 (q), 18.4 (t), 14.3 (q); high-resolution mass spectrum, obsd *m/z* 304.2396, $C_{20}H_{32}O_2$ requires 304.2402.

Pallescensolide (8): mp 53–56 °C; $[\alpha]_D$ −57.4° (*c* 0.9, CHCl$_3$); IR (film) 1755 cm^{-1}; UV (pentane) 205 nm (ϵ 21 800); ^{1}H NMR (CCl$_4$) δ 0.81 (s, 3 H), 0.84 (s, 3 H), 0.94 (s, 3 H), 1.41 (m, 1 H), 1.70 (dd, 1 H, *J* = 13, 3 Hz), 1.82 (m, 1 H, *J* = 13, 6, 3, 2 Hz), 1.95 (m, 1 H, *J* = 13, 10, 7 Hz),[13] 2.14 (m, 1 H, *J* = 14, 13, 6, 2 Hz), 2.70 (ddd, 1 H, *J* = 14, 5, 2 Hz), 3.08 (s, 3 H), 5.72 (d, 1 H, *J* = 2 Hz); ^{13}C NMR (C$_6$D$_6$) δ 175.6, 125.9, 116.7, 107.5, 60.6, 49.5, 46.6, 41.9, 39.4, 33.8, 33.2, 30.8, 26.4, 22.4, 21.9, 18.6; high-resolution mass spectrum, obsd *m/z* 264.1717, $C_{16}H_{24}O_3$ requires 264.1725.

Dehydration of Ambliol C (6). (a) Phosphorus Oxychloride in Pyridine. Ambliol C (**6**, 3 mg, 0.01 mmol) was added to a solution of phosphorus oxychloride (0.5 mL) in pyridine (2 mL), and the resulting solution was stirred at 90 °C for 24 h. After the solution was allowed to cool, the reaction mixture was poured onto ice (20 g) and the organic material was extracted with dichloromethane (3 × 10 mL). The combined extracts were dried over sodium sulfate, and the solvent and pyridine were removed under vacuum to obtain an oil. The oil was chromatographed by LC on μ-Partisil to obtain dehydroambliol C (**12**, 2 mg, 70% theoretical): oil; ^{1}H NMR (CCl$_4$) δ 0.81 (d, 3 H, *J* = 7 Hz), 0.87 (s, 3 H), 1.03 (s, 3 H), 1.05 (s, 3 H), 2.29 (t, 2 H, *J* = 8 Hz), 5.29 (bs, 1 H), 6.13 (bs, 1 H), 7.09 (bs, 1 H), 7.24 (bs, 1 H); mass spectrum, *m/z* 286 (M$^+$).

(b) *p*-Toluenesulfonic Acid. A solution of ambliol C (**6**, 4 mg, 0.013 mmol) in benzene (10 mL) containing *p*-toluenesulfonic acid (1 crystal) was boiled under reflux for 24 h. After being cooled, the reaction mixture was washed with water (3 × 5 mL) and dried over sodium sulfate, and the solvent was evaporated to obtain dehydroambliol C (**12**, 2 mg, 54% theoretical).

Ozonolysis of Ambliol C (6). A stream of ozone in oxygen was bubbled through a solution of ambliol C (**6**, 90 mg, 0.29 mmol) in redistilled acetone (75 mL) at −78 °C until a pale blue solution was obtained. Excess ozone was removed in a stream of dry nitrogen as the solution was allowed to warm to 0 °C. Jones

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(6) Structures **18**, **21**, **22**, and **23** in ref 1 should be modified accordingly.

(7) Djura, P.; Stierle, D. B.; Sullivan, B.; Faulkner, D. J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435.

(8) Hochlowski, J. E.; Walker, R. P.; Ireland, C.; Faulkner, D. J. *J. Org. Chem.* **1982**, *47*, 88.

(9) Examination of molecular models revealed that the axial proton is not shielded in the alternative C-9 isomer.

(10) Kernan, M. R., unpublished results.

(11) Bergquist, P. R.; Wells, R. J. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York 1983; Vol. 5, pp 1–50.

(12) For general procedures, see: Nakatsu, T.; Ravi, B. N.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 2435.

(13) The coupling constants are not first order.

reagent (2 mL of 1.38 M) was added and the solution was stirred at 0 °C for 1 h. The reaction mixture was extracted with ether (4×25 mL), and the combined extracts were washed with water (3×25 mL) and then 5% sodium hydroxide solution (3×25 mL). The base extracts were acidified with 3 N hydrochloric acid and extracted with ether (3×25 mL). The combined ether extracts were washed with water and dried over sodium sulfate, and the solvent was evaporated to give a mixture of carboxylic acids. The acid mixture was esterified with excess ethereal diazomethane solution. Evaporation of the solvent gave an oil that was chromatographed by LC on μ-Partisil by using 50% ether in hexane as eluant to obtain the ester **13** (70 mg, 82% theoretical): mp 85–86 °C; IR (film) 3500, 1725 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.83 (s, 3 H), 0.90 (d, 3 H, $J = 7$ Hz), 1.00 (s, 3 H), 1.04 (s, 3 H), 2.13 (t, 2 H, $J = 8$ Hz), 3.59 (s, 3 H); mass spectrum, m/z 296 (M$^+$).

Single-Crystal Diffraction Study of Ester 13. The lattice parameters were $a = 7.678$ (5) Å, $b = 9.256$ (5) Å $c = 12.345$ (4) Å, and $\beta = 102.01$ (4)°. Systematic absences and density considerations were accommodated by space group $P2_1$ with 1 molecule of $C_{18}H_{32}O_3$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 45°$ were collected on a computer-controlled diffractometer using graphite monochromated Mo Kα (0.71069 Å) radiation and an ω-scan technique. After correction for Lorentz, polarization, and background effects, 1278 (92%) of the 1395 reflections surveyed were considered observed ($|F_o| \geq 3\,\sigma\,(F_0)$). The structure was solved uneventfully by using a multisolution weighted tangent formula approach.[14] Hydrogens were located on a difference map following partial refinement of the non-hydrogen atoms. Full-matrix least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens have currently converged to a conventional crytallographic residual of 0.071.[15]

Single-Crystal X-ray Diffraction Analysis of Acid 14. Preliminary X-ray photographs displayed orthorhombic symmetry, and accurate lattice constants of $a = 7.944$ (1) Å, $b = 18.411$ (3) Å, and $c = 50.325$ (10) Å were determined from a least-squares fit of 15 moderate angle 2θ values. The systematic extinctions, density considerations, and optical activity were accommodated by space group $C222_1$ with 2 molecules of composition $C_{17}H_{30}O_3$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kα radiation (1.54178 Å) and variable 1° ω scans. After correction for Lorentz, polarization, and background effects, 1689 (60%) of these reflections were judged observed ($|F_o| \geq 3\sigma(F_o)$).[16] A phasing model was found by using a multisolution tangent formula approach. After partial refinement of the structure, hydrogens were located in a difference map. The final model with anisotropic non-hydrogen atoms and isotropic hydrogens has refined to a conventional crystallographic discrepancy index of 0.074 for the observed reflections.[15]

Acknowledgment. We thank Dr. Janice E. Thompson for identifying the sponge and comparing sponge samples. This research was supported by grants from the State Sea Grant Programs of California (NOAA 04-8-MO1-198) and New York and from the National Institutes of Health (CA-24487 to J.C.) and National Science Foundation (INR 817327 to J.C.).

Supplementary Material Available: Tables of fractional coordinates and temperature factors, bond distances, bond angles, and torsional angles for ester **13**; tables of fractional coordinates, thermal parameters, bond distances, bond angles, and torsional angles for acid **14** (15 pages). Ordering information is given on any current masthead page.

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(14) All crystallographic calculations were done on a PRIME 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE, data reduction programs, (Leonowicz, M. E., Cornell University, 1978); BLS78A, anisotropic block-diagonal least-squares refinement (Hirotsu, K.; Arnold, E., Cornell University, 1980); XRA76, the X-ray system of crystallographic programs, (Stewart, J. M., ed.; University of Maryland, Technical Report TR-445, March 1976); ORTEP crystallographic illustration program (Johnson, C. K.; Oak Ridge, ORNL-3794); MULTAN-78 (locally modified) (Main, P. et al. "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data"; University of York, England). For literature description of MULTAN, see: Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sec. B* **1970**, *B26*, 274–285.

(15) Additional crystallograpic details are available and are described in the paragraph at the end of the paper.

(16) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN-78, a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, Univeristy of York, England, 1978; BLS78A, an anisotropic block-diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.