(sh), 283 (shoulder tailing to ~385 nm); mass spectrum *m/e* 96 (M^+, 0.77), 68 ($C_4H_4O^+$, 1.0).

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Registry No.—**2**, 68781-88-4; **4**, 5530-96-1; **5**, 68781-89-5; **6**, 14224-63-6; **7**, 68781-90-8; **8**, 68781-91-9; dimethyl acetylenedicarboxylate, 762-42-5.

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Metabolites of the Marine Sponge *Chondrosia collectrix*

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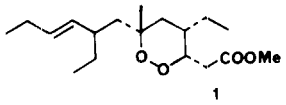
The Caribbean sponge *Chondrosia collectrix* contained antimicrobial metabolites when freshly collected, but these compounds were absent after storage in ethanol. The ethanol extracts contained five major metabolites, the ketal **3**, hemiketals **4** and **5**, the α,β-unsaturated ester **6**, and the diol **7**. A dichloromethane extract of lyophilized sponge contained a mixture of peroxide acids **17** and **18** together with the corresponding methyl esters **15** and **16** and the hemiketal **4**. The peroxides **15–18** were responsible for mild antibacterial activity.

During a study of Caribbean sponges, we have encountered a number of compounds which appear to be derived from fatty acids with methyl and ethyl side chains. The first example of these compounds were plakortin (**1**) and a related ketone **2** from a sample of *Plakortis halichondrioides*.[1] In this



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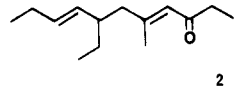

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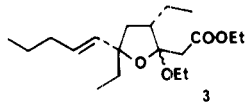

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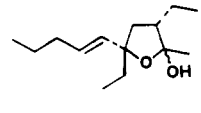

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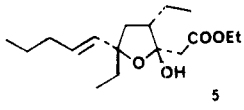

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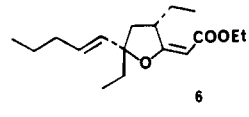

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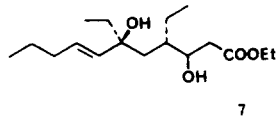

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paper we wish to report the identification of several metabolites from ethanol extracts of *Chondrosia collectrix* and different metabolites from a dichloromethane extract of the lyophilized sponge.

The ether-soluble portion of the ethanolic extract (3 months at 0 °C) of *Chondrosia collectrix*[2] was chromatographed on Florisil to obtain, in order of increasing polarity, the ketal **3** (0.4% dry weight), the hemiketal **4** (0.4% dry weight), the hemiketal ester **5** (0.9% dry weight), the ester **6** (0.2% dry weight), and the diol **7** (1.2% dry weight). The structure of the hemiketal ester **5** was determined by analysis of spectral data followed by a degradation sequence.

The hemiketal **5** had the molecular formula $C_{17}H_{30}O_4$. As expected for a hemiketal, dehydration occurred in the mass spectrometer to give $M - H_2O$ as the highest observed ion. The infrared spectrum contained hydroxyl and ester bands at 3600 and 1725 cm^{-1}, respectively. The ^{13}C NMR spectrum contained signals for an ester carbonyl at δ 171.8, two olefinic carbons at δ 135.5 (d) and 128.0 (d), a hemiketal carbon at δ 103.7 (s), two carbons bearing oxygen at δ 84.8 (s) and 60.6 (t), and 11 other carbon atoms. The ^1H NMR spectrum contained signals for an ethyl ester at δ 1.29 and 4.24 and a disubstituted olefin at δ 5.35 (d, 1 H, J = 15 Hz) and 5.55 (dt, 1 H, J = 15, 7, 7 Hz), an AB quartet at δ 2.57 and 2.76 (J = 15 Hz) assigned to the protons at C-2, and three additional methyl triplets at δ 0.86, 0.90, and 0.92.

Ozonolysis of the hemiketal **5** in ethyl acetate solution at −78 °C followed by hydrogenation of the ozonide over 10% palladium on charcoal at 0 °C gave an aldehyde **8**, which rearranged on silica gel chromatography to produce the unsaturated keto ester **9**. The keto ester **9** had the molecular formula $C_{13}H_{20}O_4$, indicating that *n*-butyraldehyde had been lost during ozonolysis. The infrared spectrum of the keto ester

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9 contained bands for hydroxyl (3500 cm^{-1}), unsaturated ester (1725 cm^{-1}), and unsaturated ketone (1690 cm^{-1}). The ^1H NMR spectrum contained a signal at δ 7.32 (d, 1 H, J = 2 Hz) which was assigned to the β proton of the α,β-unsaturated ketone, which was coupled across the ring. The ^{13}C NMR spectrum confirmed the presence of an alcohol (δ 70.7). Dehydration of the tertiary alcohol functionality in **9** gave ethyl 4,6-diethylsalicylate (**10**). The ^1H NMR spectrum clearly showed three ethyl groups with the methylene signals at δ 2.57, 2.66, and 4.39 and two aromatic proton signals at δ 7.18 (d, 1 H, J = 2 Hz) and 7.51 (d, 1 H, J = 2 Hz), which were shifted to δ 7.26 and 7.57 in the corresponding acetate **11**. The relatively small shift of the aromatic protons on acetylation, together with the 2-Hz coupling constant, indicated that both protons were meta to the phenol group. There were two possible tertiary alcohols from which ethyl 4,6-diethylsalicylate (**10**) could have been formed, but only 2-(carboethoxy)-4,6-diethyl-4-hydroxycyclohex-2-enone (**9**) was compatible with the spectral data. The aldehyde **8** was therefore the hemiketal of β-keto ester **12**. The ozonolysis was repeated in methanol solution at −78 °C to obtain an aldehyde **13** in which the hemiketal had been converted into a ketal. The spectral data of aldehyde **13** supported this structure. The loss of *n*-butyraldehyde during ozonolysis required that the hemiketal ester had the structure **5** in which only the stereochemistry of the olefinic bond was known. Attempts to perform a lanthanide-induced shift study on the hemiketal gave inconclusive results.

Treatment of the hemiketal ester **5** with hydrochloric acid in aqueous methanol at room temperature for 2 days gave, in addition to recovered starting material, a mixture of the hemiketal **4** and the α,β-unsaturated ester **6**, both identical with the materials isolated from the sponge. The hemiketal **4** lacked the carboethoxy group and contained a new methyl group which gave rise to a signal at δ 1.46 (s, 3 H) in the ^1H NMR spectrum. There was no evidence to suggest that two diastereoisomers had been formed. The α,β-unsaturated ester **6**, formed by dehydration of **5**, showed a UV absorption at λ_{max} 244 nm (ϵ 11 500). The ^1H NMR spectrum contained a new vinyl proton signal at δ 4.74 (d, 1 H, J = 1 Hz), which is close to the calculated value[3] (δ 4.76) for the vinyl proton in the ester **6** having the 2*E* geometry. The ketal **3** did not show a hydroxyl band in the infrared spectrum. The ^{13}C NMR spectrum contained signals for 19 carbon atoms, including a ketal carbon signal at δ 106.5 (s). The ^1H NMR spectrum contained signals at δ 1.13 (t, 3 H, J = 7 Hz), 1.26 (t, 3 H, J = 7 Hz), 3.50 (m, 1 H, J = 14, 7, 7, 7 Hz), 3.66 (m, 1 H, J = 14, 7, 7, 7 Hz), and 4.12 (q, 2 H, J = 7 Hz) for the protons of two ethoxy groups. Treatment of the ketal **3** with hydrochloric acid in methanol also gave the α,β-unsaturated ester **6**.

The diol **7** had the molecular formula $C_{17}H_{32}O_4$. The mass spectrum did not contain a molecular ion peak; the high-resolution mass measurement (*m/e* 282.219) reflects loss of water from the molecular ion. However, the ^{13}C NMR spectrum showed signals for an ester carboxyl at δ 172.4 and three carbons bearing oxygen at δ 74.0 (s), 67.9 (d), and 59.9 (t), which implied that the diol **7** contained four oxygen atoms. Since the ^{13}C NMR spectral data contained signals at δ 135.1 (d) and 128.9 (d) due to a disubstituted olefin, the diol **7** must be acyclic. The ^1H NMR spectrum contained four methyl signals at δ 0.86, 0.88, 0.89, and 1.24 (all triplets), two mutually coupled signals at δ 2.32 (dd, 1 H, J = 16, 2 Hz) and 2.46 (dd, 1 H, J = 16, 10 Hz) assigned to the protons at C-2, a multiplet at δ 4.14 which consisted of signals for the methylene protons of the ethyl ester which covered the proton at C-3, and two olefinic proton signals at δ 5.22 (d, 1 H, J = 16 Hz) and 5.60 (dt, 1 H, J = 16, 7, 7 Hz). We assigned the structure of the diol **7** by analogy with the carbon skeleton of hemiketal **5**. In order to confirm this assignment, we reduced the hemiketal **5** with sodium borohydride in methanol solution to obtain a diol **14** which was *not* identical with diol **7**. However, there was sufficient similarity between the spectral data of **7** and **14** to assume that the diols were diastereoisomers. The stereochemistry of these diols will be discussed later.

Since the sponge sample had been stored in ethanol for 3 months, we suspected that ethyl esters might have resulted from addition of ethanol to the corresponding acids. We therefore lyophilized a sample of sponge that had been frozen since collection, powdered the dry sponge, and Soxhlet extracted the powder with dichloromethane. The extract was evaporated to an oil which was partitioned to obtain an acid fraction and a neutral fraction. The neutral fraction contained the ketal **4** (0.26% dry weight) together with an inseparable 4:1 mixture of the methyl esters **15** and **16** (1.2% dry weight). The acid fraction contained an inseparable 1:1 mixture of acids **17** and **18** (4.8% dry weight). Neither the acid mixture nor the ester mixture could be separated on LC, although the positions and multiplicities of the ^1H NMR signals for the protons at C-3 clearly indicated that mixtures were present. The key to the structural elucidation of the peroxides **15–18** was the recognition of the C-2 and C-3 proton signals in the ^1H NMR spectrum of the major methyl ester **15** as being almost identical with those of plakortin (**1**) (Figure 1). The signals in the methyl ester **15** were δ 2.40 (dd, 1 H, J = 16, 3.5 Hz), 3.06 (dd, 1 H, J = 16, 9.5 Hz), and 4.48 (m, 1 H, J = 9.5, 6, 3.5 Hz), while those in plakortin (**1**) were at δ 2.35 (dd, 1 H, J = 16, 3.5 Hz), 3.05 (dd, 1 H, J = 16, 9.5 Hz), and 4.49 (m, 1 H, J = 9.5, 6, 3.5 Hz). These data strongly suggested a six-membered peroxide ring with the same stereochemistry at C-3 and C-4 as plakortin (**1**). The carbon skeleton was established by reduction of the mixture of methyl esters **15** and **16** with lithium aluminum hydride in anhydrous ether to obtain a crude triol mixture which was acetylated with acetic anhydride in pyridine to obtain a diacetate **19** as the major product. The diacetate **19** was identical in all respects with the diacetate obtained by lithium aluminum hydride reduction of the hemiketal **5** followed by acetylation. Thus, the major ester **15** must have the carbon skeleton of the hemiketal **5**. The ste-

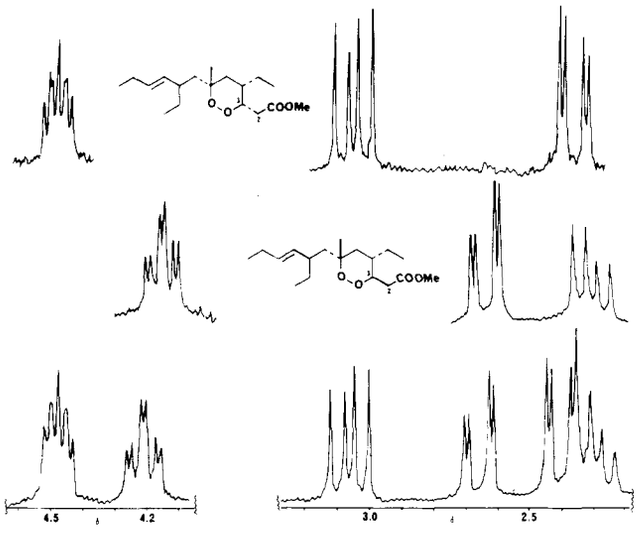

Figure 1. Selected signals from the ^1H NMR spectrum of the 1:1 mixture of acids **17** and **18** (bottom trace) compared with the same regions of the ^1H NMR spectra of plakortin (**1**) (top trace) and 3-*epi*-plakortin (center trace).

reochemistry about the six-membered ring of the major methyl ester **15** was assumed to be the same for plakortin (**1**), with the largest chain at C-6 in the equatorial conformation.

Esterification of the 1:1 mixture of acids **17** and **18** with diazomethane in ether gave a 1:1 mixture of the methyl esters **15** and **16.** We could now clearly distinguish the signals in the ^1H NMR spectrum which were associated with the minor isomer **16** of the original mixture of methyl esters. The ^1H NMR spectrum of **16,** deduced by subtraction, contained signals at δ 2.40 (dd, 1 H, J = 16, 8 Hz), 2.64 (dd, 1 H, J = 16, 3 Hz), and 4.21 (td, 1 H, J = 8, 8, 3 Hz) which were almost identical with the proton signals at C-2 and C-3 in 3-*epi*-plakortin[4] (Figure 1). Although the six-membered peroxide ring does not have the same geometry as a cyclohexane ring, examination of Dreiding models suggests that 8 Hz is reasonable for the C-3 (axial), C-4 (axial) coupling constant compared with 6 Hz for the C-3 (equatorial), C-4 (axial) coupling constant. We therefore deduced that the esters **15** and **16** and the acids **17** and **18** were mixtures of cyclic peroxides which were epimeric at C-3.

Esterification of the 1:1 mixture of acids **17** and **18** with *p*-toluenesulfonic acid in ethanol gave a mixture of ethyl esters which was hydrogenated over 10% palladium on charcoal to obtain a mixture of alcohols **20** and **21** which was converted to a mixture of the corresponding monoacetates **22** and **23** by treatment with acetic anhydride in pyridine. The mixture of acetates **22** and **23** was separated by LC on μ-Porasil. Since the hemiketal **5** had already been related to the ester **15** through the diacetate **19,** we were able to prepare one of the acetates **22** by reduction of the hemiketal with sodium borohydride in methanol at 0 °C (at 25 °C the ester group was also reduced), hydrogenation of the olefin over 10% palladium on charcoal, and acetylation with acetic anhydride and pyridine. The second acetate **23** was prepared by hydrogenation of the alcohol **7** over 10% palladium on charcoal and acetylation with acetic anhydride and pyridine. Since the reduction of the hemiketal **5** with sodium borohydride in methanol was a stereospecific reaction, we propose that the keto alcohol form of the hemiketal must be reduced with the hydride ion approaching from the face of the carbonyl opposite that of the leaving alcohol, so that the hemiketal **5** has the stereochemistry shown. We could not define the stereochemistry at C-3 of ketal **3** or C-2 of hemiketal **4.**

The peroxide esters **15** and **16** and the peroxide acids **17** and **18** were both mildly antibacterial. We have frequently encountered samples which showed activity in field screening but had lost activity by the time the sample was returned to the laboratory, usually in solvent. In this paper we have provided an example where one may presume that the compounds **3–7** were all derived from the peroxides **17** and **18,** with a resulting loss of antibacterial activity.

It is interesting to note that although this sponge was similar in appearance to *Plakortis* species, it could be distinguished by texture and by taxonomic analysis. We have been able to differentiate other *Plakortis* species in the field that would not be distinguished using preserved specimens and have later found that the chemical metabolites were quite different. These other *Plakortis* samples have also yielded cyclic peroxides, whose structures will be reported elsewhere.[5]

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Ultraviolet spectra were recorded on a Perkin-Elmer Model 124 double-beam spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. ^1H NMR spectra were recorded on a Varian HR-220 NMR spectrometer, and ^{13}C spectra were recorded on a Varian CFT-20 NMR spectrometer; all chemical shifts are reported with respect to Me_4Si (δ = 0). Low-resolution mass spectra (LRMS) were recorded on a Hewlett-Packard 5930A mass spectrometer. High-resolution mass spectra (HRMS) were supplied by the Chemistry Department at UCLA. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were either spectral grade or distilled from glass prior to use.

Collection and Extraction. The sponge *Chondrosia collectrix* was collected by hand using SCUBA (−25 m) at Lighthouse Reef, Belize. A portion of the material was stored in ethanol (2 L), while the remainder was stored at −20 °C. After 3 months, the sponge (50-g dry weight) stored in ethanol was homogenized and Soxhlet extracted with ethanol. The ethanol was evaporated in vacuo and the residue partitioned between water (200 mL) and ether (3 × 250 mL). The ether extract was dried over sodium sulfate and the solvent evaporated to yield a brown gum (4.8 g).

Chromatography. The crude extract was applied to a column (55 × 3 cm d.) of Florisil, and material was eluted with solvent mixtures of increasing polarity from hexane through ether to ethyl acetate. The fractions eluted with 5% ether in hexane were purified by LC on μ-Porasil using 1% ether in hexane as eluant to obtain the ketal **3** (200 mg, 0.4% dry weight). The earlier fractions eluted with 10% ether in hexane contained the hemiketal **4** (200 mg, 0.4% dry weight), while later fractions contained the major hemiketal **5** (450 mg, 0.9% dry weight). The α,β-unsaturated ester **6** (100 mg, 0.2% dry weight) was obtained from fractions eluted with 25% ether in hexane. Fractions eluted with ether gave the diol **7** (600 mg, 1.2% dry weight).

Ketal 3: $[\alpha]^{20}_D$ +1.34° (*c* 1.2 CCl_4); IR (film) 1750, 1460, 1220, 1110, 1050, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz), 0.89 (t, 6 H, J = 7 Hz), 1.13 (t, 3 H, J = 7 Hz), 1.26 (t, 3, H, J = 7 Hz), 2.00 (m, 2 H), 2.21 (m, 1 H), 2.72 (d, 1 H, J = 13 Hz), 2.82 (d, 1 H, J = 13 Hz), 3.50 (m, 1 H, J = 14, 7, 7, 7 Hz), 3.66 (m, 1 H, J = 14, 7, 7, 7 Hz), 4.12 (m, 2 H), 5.32 (d, 1 H, J = 15 Hz), 5.50 (dt, 1 H, J = 15, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 169.2 (s), 135.0 (d), 128.4 (d), 106.5 (s), 85.3 (s), 60.1 (t), 55.8 (t), 48.1 (t), 42.0 (t), 40.2 (d), 35.7 (t), 34.8 (t), 23.0 (t), 21.7 (t), 15.5 (q), 14.2 (q), 13.8 (q), 13.1 (q), 9.2 (q); HRMS, observed *m/e* 308.2350, $C_{19}H_{32}O_3$ (M − H_2O) requires 308.2351.

Hemiketal 4: $[\alpha]^{20}_D$ +12.3° (*c* 2.2, CCl_4); IR (film) 3500, 1460, 1380, 975 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7 Hz), 0.86 (t, 3 H, J = 7 Hz), 0.89 (t, 3 H, J = 7 Hz), 1.46 (s, 3 H), 1.98 (m, 3 H), 5.31 (d, 1 H, J = 15 Hz), 5.55 (dt, 1 H, J = 15, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 135.6, 128.0, 104.8, 84.6, 49.3, 41.5, 35.7, 34.6, 26.8, 23.0, 22.1, 13.7, 13.2, 9.2; HRMS, observed *m/e* 226.1929, $C_{14}H_{26}O_2$ requires 226.1933.

Hemiketal 5: $[\alpha]^{20}_D$ +0.86° (*c* 1.2, CCl_4); IR (film) 3600, 1725, 1195, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz), 0.90 (t, 3 H, J = 7 Hz), 0.92 (t, 3 H, J = 7 Hz), 1.29 (t, 3 H, J = 7 Hz), 2.00 (m, 2 H), 2.57 (d, 1 H, J = 15 Hz), 2.76 (d, 1 H, J = 15 Hz), 4.24 (q, 2 H, J = 7 Hz), 4.70 (OH), 5.35 (d, 1 H, J = 15 Hz), 5.55 (dt, 1 H, J = 15, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 171.8 (s), 135.5 (d), 128.0 (d), 103.7 (s), 84.8 (s), 60.6 (t), 49.2 (d), 43.6 (t), 41.2 (t), 35.3 (t), 34.6 (t), 23.0 (t), 21.9 (t), 14.1 (q), 13.7 (q), 13.0 (q), 8.9 (q); HRMS, observed *m/e* 280.2038, $C_{17}H_{28}O_3$ (M − H_2O) requires 280.2038.

α,β-Unsaturated Ester 6: $[\alpha]^{20}_D$ −116° (*c* 1.4, CCl_4); UV (MeOH) 244 nm (ϵ 11 500); IR (film) 1715, 1690, 1660, 1205, 1050, 975 cm^{-1};

^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7 Hz), 0.94 (t, 3 H, J = 7 Hz), 0.96 (t, 3 H, J = 7 Hz), 1.26 (t, 3 H, J = 7 Hz), 1.80 (m, 3 H), 2.05 (m, 2 H), 2.14 (dd, 1 H, J = 11, 7 Hz), 2.79 (m, 1 H), 4.14 (q, 2 H, J = 7 Hz), 4.74 (d, 1 H, J = 1 Hz), 5.36 (d, 1 H, J = 15 Hz), 5.59 (dt, 1 H, J = 15, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 174.1 (s), 165.4 (s), 131.6 (d), 130.4 (d), 90.8 (s), 87.7 (d), 58.7 (t), 44.4 (t), 39.1 (d), 34.5 (t), 33.5 (t), 25.1 (t), 22.6 (t), 14.7 (q), 13.7 (q), 11.6 (q), 8.5 (q); HRMS, observed m/e 280.2038, $C_{17}H_{28}O_3$ requires 280.2038.

Diol 7: $[\alpha]^{20}_D$ +0.32° (c 1.3, CCl_4); IR (film) 3450, 1740, 1175, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz), 0.88 (t, 3 H, J = 7 Hz), 0.89 (t, 3 H, J = 7 Hz), 1.24 (t, 3 H, J = 7 Hz), 2.06 (m, 2 H), 2.32 (dd, 1 H, J = 16, 2 Hz), 2.46 (dd, 1 H, J = 16, 10 Hz), 4.14 (m, 3 H), 4.28 (OH), 5.22 (d, 1 H, J = 16 Hz), 5.60 (dt, 1 H, J = 16, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 172.4 (s), 135.1 (d), 128.9 (d), 74.0 (s), 67.9 (d), 59.9 (t), 40.4 (two c), 36.7 (t), 35.7 (t), 34.3 (t), 26.1 (t), 22.5 (t), 13.7 (q), 13.5 (q), 12.1 (q), 7.7 (q); HRMS, observed m/e 282.2191, $C_{17}H_{30}O_3$ (M − H_2O) requires 282.2195.

Extraction of Lyophilized Sponge. The frozen sponge was transferred directly from the freezer to a freeze-dryer. When dry, the sponge (50 g) was powdered and Soxhlet extracted using dichloromethane (1.5 L). Evaporation of the solvent gave a brown gum (5.0 g, 10% dry weight). The gum was partitioned between ether (200 mL) and saturated sodium bicarbonate solution (3 × 150 mL). The ether layer was dried over sodium sulfate and the solvent evaporated to obtain a gum (1.4 g, 2.8% dry weight). The aqueous extracts were acidified at 0 °C and extracted with ether (3 × 200 mL). The combined ether extracts were dried over sodium sulfate, and the solvent was evaporated to obtain an acid fraction (3.6 g, 7.2% dry weight).

Chromatography of Neutral Material. The neutral fraction (1.4 g) was applied to a column (25 × 3 cm d.) of Florisil, and materials were eluted with mixtures of hexane and ether of gradually increasing polarity. Fractions eluted with 5% ether in hexane gave a 4:1 mixture of two peroxide esters **15** and **16** (600 mg, 1.2% dry weight), which could not be separated by LC on μ-Porasil or C-18 Bondapak. Elution with 15% ether in hexane gave the hemiketal **4** (130 mg, 0.26% dry weight).

Peroxide Esters 15 and 16: IR (film) 1740, 1430, 1160, 970 cm^{-1}. **Major isomer 15:** ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3 H, J = 7 Hz), 0.91 (t, 3 H, J = 7 Hz), 0.92 (t, 3 H, J = 7 Hz), 2.08 (m, 2 H), 2.40 (dd, 1 H, J = 16, 3.5 Hz), 3.06 (dd, 1 H, J = 16, 9.5 Hz), 3.70 (s, 3 H), 4.48 (m, 1 H, J = 9.5, 6, 3.5 Hz), 5.50 (m, 2 H). **Minor isomer 16:** ^{1}H NMR (selected signals only) δ 2.40 (dd, 1 H, J = 16, 8 Hz), 2.64 (dd, 1 H, J = 16, 3 Hz), 3.68 (s, 3 H), 4.21 (dt, 1 H, J = 8, 8, 3 Hz).

Chromatography of Acid Fraction. A portion of the acid fraction (1.2 g) was applied to a column (25 × 3 cm d.) of silica gel. Elution with ether gave a 1:1 mixture of two peroxide acids **17** and **18** (800 mg, 4.8% dry weight), which could not be separated by LC.

An ethereal solution of diazomethane (excess) was added to an ethereal solution of the acids **17** and **18** (51 mg, 0.14 mmol). After 15 min, the solvent was evaporated to obtain a 1:1 mixture of esters **15** and **16** (54 mg, quantitative).

Peroxide Acids 17 and 18: IR (film) 2950 (broad), 1700, 960 cm^{-1}; ^{1}H NMR, see Figure 1.

Ozonolysis of Hemiketal 5. A stream of ozone in oxygen was bubbled into a solution of the hemiketal **5** (30 mg, 0.1 mmol) in ethyl acetate (5 mL) at −78 °C until a blue-colored solution resulted. Excess ozone was removed in a stream of nitrogen. After 10% palladium on charcoal catalyst (2 mg) was added the solution was stirred under an atmosphere of hydrogen for 15 min. The catalyst was removed by filtration and the solvent evaporated to yield an unstable aldehyde **8**. After standing overnight, the aldehyde **8** was chromatographed on a short column of silica gel to obtain the unsaturated keto ester **9** (21 mg, 88% theoretical): UV ($CHCl_3$) 239 nm (ϵ 7100); IR (CCl_4) 3500, 1725, 1690, 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 7 Hz), 1.06 (t, 3 H, J = 7 Hz), 1.31 (t, 3 H, J = 7 Hz), 1.48 (m, 1 H), 2.30 (m, 2 H), 4.26 (q, 2 H, J = 7 Hz), 7.32 (d, 1 H, J = 2 Hz); ^{13}C NMR (C_6D_6) δ 195.7, 165.0, 157.2, 132.4, 70.7, 61.3, 46.4, 46.3, 40.2, 22.6, 14.1, 11.1, 7.7; HRMS, observed m/e 240.1366, $C_{13}H_{20}O_4$ requires 240.1361.

p-Toluenesulfonic acid (2 mg) was added to a solution of keto ester **9** (10 mg, 0.042 mmol) in benzene (5 mL), and the solution was boiled under reflux for 30 min. The cooled solution was filtered, and the solvent was evaporated to yield an oil (9 mg) which was purified on a silica gel plate to obtain ethyl 4,6-diethylsalicylate (**10**) (8 mg, 86% theoretical): UV (MeOH) 314 nm (ϵ 3300), 242 (9300), 209 (21 000); IR (CCl_4) 3210, 1680, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 6 H, J = 7 Hz), 1.41 (t, 3 H, J = 7 Hz), 2.57 (q, 2 H, J = 7 Hz), 2.66 (q, 2 H, J = 7 Hz), 4.39 (q, 2 H, J = 7 Hz), 7.18 (d, 1 H, J = 2 Hz), 7.51 (d, 1 H, J = 2 Hz); HRMS, observed m/e 222.1254, $C_{13}H_{18}O_3$ requires 222.1256.

Acetate 11: ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3 H, J = 7 Hz), 1.27 (t, 3 H, J = 7 Hz), 1.38 (t, 3 H, J = 7 Hz), 2.36 (s, 3 H), 2.58 (q, 2 H, J = 7 Hz), 2.67 (q, 2 H, J = 7 Hz), 4.34 (q, 2 H, J = 7 Hz), 7.26 (d, 1 H, J = 2 Hz), 7.67 (d, 1 H, J = 2 Hz).

Treatment of Hemiketal 5 with Hydrochloric Acid. A solution of the ketal **5** (20 mg, 0.067 mmol) in aqueous methanol (1:1, 5 mL) containing 5% hydrochloric acid (1 drop) was stirred at 25 °C for 2 days. The solvent was evaporated in vacuo to obtain an oil which was chromatographed on a silica gel plate to obtain the ketal **5** (2 mg, 10% recovery), the hemiketal **4** (5 mg, 33% theoretical), and the α,β-unsaturated ester **6** (7 mg, 37% theoretical).

Treatment of Ketal 3 with Hydrochloric Acid. A solution of the ketal **3** (10 mg, 0.029 mmol) in aqueous methanol (1:1, 3 mL) containing 5% hydrochloric acid (1 drop) was stirred at 25 °C for 2 days. After the solvents were evaporated, the product was chromatographed on a silica gel plate to obtain the hemiketal **5** (3 mg, 33% theoretical) and the α,β-unsaturated ester **6** (3 mg, 35% theoretical).

Reduction of Hemiketal 5 with Sodium Borohydride. Sodium borohydride (20 mg, 0.52 mmol) was added to a cooled solution of the hemiketal **5** (8 mg, 0.28 mmol) in ethanol, and the solution was stirred at 0 °C for 2 h. The solvent was evaporated and the residue partitioned between water (5 mL) and ether (2 × 10 mL). The combined ether extracts were dried over sodium sulfate, and the solvent was evaporated to give an oil which was purified on a silica gel plate using 1:1 ether–hexane as eluant to obtain the diol **14** (5 mg, 62% theoretical): IR (CCl_4) 3400, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H, J = 7 Hz), 0.88 (t, 3 H, J = 7 Hz), 0.91 (t, 3 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz), 1.81 (dd, 1 H, J = 13, 6 Hz), 2.06 (m, 2 H), 2.39 (dd, 1 H, J = 16, 10 Hz), 2.64 (dd, 1 H, J = 16, 2 Hz), 3.82 (m, 2 H), 4.18 (q, 2 H, J = 7 Hz), 5.26 (d, 1 H, J = 15 Hz), 5.65 (dt, 1 H, J = 15, 7, 7 Hz); ^{13}C NMR (C_6D_6) δ 171.8, 134.9, 128.2, 73.1, 69.9, 59.1, 42.5, 41.4, 40.2, 36.3, 34.7, 25.9, 23.1, 14.1, 13.9, 10.8, 8.3.

Reduction of Hemiketal 5 with Lithium Aluminum Hydride. Lithium aluminum hydride (20 mg) was added to a solution of the hemiketal **5** (20 mg, 0.067 mmol) in anhydrous ether (10 mL), and the reaction mixture was stirred at 25 °C for 10 min. The excess reagent was destroyed with ethyl acetate (1 mL) and the reaction mixture partitioned between 3 N hydrochloric acid (10 mL) and ether (3 × 10 mL). The combined ethereal extracts were dried over sodium sulfate, and the solvent was evaporated to obtain a triol (11 mg, 64% theoretical). A portion of the triol (5 mg, 0.019 mmol) was dissolved in acetic anhydride (0.5 mL) and pyridine (1 mL). After 20 h, the solvent was removed in vacuo to yield the diacetate **19** (4 mg, 70% theoretical) as an oil: IR (CCl_4) 3500, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz), 0.90 (t, 6 H, J = 7 Hz), 2.04 (s, 3 H), 2.06 (s, 3 H), 4.08 (m, 2 H), 5.27 (m, 2 H), 5.61 (dt, 1 H, J = 16, 7, 7 Hz).

Reduction of Peroxide Esters 15 and 16 with Lithium Aluminum Hydride. A mixture of peroxide esters **15** and **16** (20 mg, 0.07 mmol) was reduced with lithium aluminum hydride using the procedure above to obtain a mixture of triols. The triols were acetylated by the procedure above to obtain the diacetate **19** as the major product. The IR and ^{1}H NMR spectra were identical with those of the diacetate **19** from the hemiketal **5**, and the two samples showed identical retention times by gas chromatography on a 6 ft × 4 mm d. column of 3% SP 2250 at 140 °C.

Esterification and Reduction of Acids 17 and 18. p-Toluenesulfonic acid (1 mg) was added to a solution of the 1:1 mixture of acids **17** and **18** (50 mg, 0.185 mmol) in anhydrous ethanol (20 mL), and the mixture was stirred at 25 °C for 2 days. Evaporation of the solvent gave a brown gum which was purified on a silica gel plate using 1:1 ether–hexane as eluant to obtain the corresponding ethyl esters (38 mg, 69% theoretical). Palladium on charcoal catalyst (10%, 5 mg) was added to a solution of the esters in ethyl acetate (10 mL) and the reaction mixture stirred under hydrogen overnight. The catalyst was removed by filtration, the solvent was evaporated to yield an oil which was dissolved in acetic anhydride (1 mL) and pyridine (2 mL), and the reaction mixture was stirred overnight. The solvents were evaporated in vacuo to obtain a mixture of acetates **22** and **23** (40 mg, 92% theoretical). The mixture of acetates **22** and **23** was separated by LC on μ-Porasil using 1:1 ether–hexane as eluant.

Acetate 22: IR (CCl_4) 3500, 1740, 1240, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz), 0.89 (t, 3 H, J = 7 Hz), 0.92 (t, 3 H, J = 7 Hz), 1.24 (t, 3 H, J = 7 Hz), 2.03 (s, 3 H), 2.56 (m, 2 H), 4.16 (q, 2 H, J = 7 Hz), 5.47 (m, 1 H); HRMS, observed m/e 329.2320, $C_{18}H_{33}O_5$ (M − CH_3) requires 329.2328.

Acetate 23: IR (CCl_4) 3500, 1740, 1245, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7 Hz), 0.89 (t, 3 H, J = 7 Hz), 0.96 (t, 3 H, J = 7 Hz), 1.24 (t, 3 H, J = 7 Hz), 2.04 (s, 3 H), 2.50 (dd, 1 H, J = 15, 5 Hz), 2.65 (dd, 1 H, J = 15, 8 Hz), 4.14 (q, 2 H, J = 7 Hz), 5.57 (m, 1 H); HRMS, observed m/e 329.2320, $C_{18}H_{33}O_5$ (M − CH_3) requires 329.2328.

Hydrogenation of Diol 7. Palladium on charcoal catalyst (10%, 2 mg) was added to a solution of the diol **7** (30 mg, 0.10 mmol) in ethyl acetate (5 mL) and the reaction mixture stirred under hydrogen for 12 h. The catalyst was removed by filtration, and the solvent was evaporated to obtain an oil which was dissolved in acetic anhydride (0.5 mL) and pyridine (1 mL). After the solution had been stirred for 12 h, the solvent was evaporated in vacuo to obtain the acetate **23** (29 mg, 84% theoretical), identical in all respects with the sample obtained from the peroxide mixture.

Hydrogenation of Diol 14. The diol **14** (5 mg, 0.017 mmol) was hydrogenated and the product acetylated, using the procedure above, to obtain the acetate **22** (5 mg, 87% theoretical) that was identical in all respects with the sample obtained from the peroxide mixture.

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Registry No.—**3,** 68914-58-9; **4,** 68914-59-0; **5,** 68914-60-3; **6,** 68914-61-4; **7,** 68914-62-5; **8,** 68914-63-6; **9,** 68914-64-7; **10,** 68914-65-8; **11,** 68914-66-9; **12,** 68914-67-0; **13,** 68914-68-1; **14,** 68964-12-5; **15,** 68914-69-2; **16,** 68964-13-6; **17,** 68914-70-5; **18,** 68964-55-6; **19,** 68914-71-6; **20,** 68914-72-7; **21,** 68964-14-7; **22,** 68914-73-8; **23,** 68964-15-8.

References and Notes

(1) M. D. Higgs and D. J. Faulkner, *J. Org. Chem.*, **43,** 3454 (1978).
(2) This sponge was collected as one of four samples which appeared in the field to be similar to a preserved sample of *Plakortis halichondrioides*. It is a dark grey-brown (almost black) massive sponge with a white interior.
(3) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, Oxford, 1969, p 185.
(4) 3-*epi*-Plakortin was isolated from a sample of *P. halichondrioides* collected in Belize and was characterized in a similar manner to plakortin.
(5) D. B. Stierle, research in progress.

Acyclic Diterpenes from the Marine Sponge *Didiscus* sp.

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The marine sponge *Didiscus* sp. contains six acyclic diterpenes: (3*E*,5*E*,10*E*)-7-hydroxy-13-keto-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**4**), (3*Z*,5*E*,10*E*)-7-hydroxy-13-keto-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**5**), (3*E*,5*E*,10*E*)-7-ethoxy-13-keto-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**9**), (3*Z*,5*E*,10*E*)-7-ethoxy-13-keto-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**10**), (3*E*,5*E*,10*E*)-7-ethoxy-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**11**), and (3*Z*,5*E*,10*E*)-7-ethoxy-3,7,11,15-tetramethylhexadeca-1,3,5,10,14-pentaene (**12**).

Few diterpenes have been isolated from marine sponges.[1] The only linear diterpene that had previously been obtained from a sponge was the isonitrile **1** from a Hawaiian *Halichondria* species.[2] Other linear diterpenes such as phytol from *Gracilaria andersoniana*,[3] crinitol (**2**) from *Cystoseira crinita*,[4] and elaganolone (**3**) from *Cystoseira elegans*[5] had been found in marine algae. In this paper we wish to describe six closely related linear diterpenes from the marine sponge *Didiscus* sp.

During a routine thin-layer chromatographic screening of Caribbean sponges collected at Belize we observed that the crude ethanolic extract of *Didiscus* contained several compounds which exhibited strong ultraviolet absorption. The ethyl acetate soluble material from the ethanolic extract was chromatographed on Florisil to obtain three bands containing diterpenes. The major band was rechromatographed on silica gel to obtain a 3:1 mixture of two isomers, **4** and **5,** which could be separated by LC on μ-porasil.

The major diterpene **4** had the molecular formula $C_{20}H_{30}O_2$. The infrared spectrum indicated that the diterpene **4** contained a hydroxyl group (3470 cm^{-1}) and an unsaturated ketone (1690, 1620 cm^{-1}). The ^{13}C NMR spectrum contained signals for a carbonyl group at δ 207.1, the carbon of a tertiary alcohol at 73.0 (s), and ten olefinic carbons, indicating that **4** was acyclic. Every signal in the ^1H NMR spectrum could be assigned (Table I) and the assignment confirmed by decoupling. The terminal vinyl group gave signals at δ 6.42 (dd, 1 H, J = 17, 11 Hz), 5.22 (bd, 1 H, J = 17 Hz), and 5.07 (bd, 1 H, J = 11 Hz). The vinyl proton signal at δ 6.64 (dd, 1 H, J = 15, 11 Hz, C-5) was coupled to signals at 6.07 (d, 1 H, J = 11 Hz, C-4) and 5.82 (d, 1 H, J = 15 Hz, C-6). The vinyl proton signal at δ 6.13 (bs, 1 H) was coupled ($J < 1$ Hz) to two methyl singlets at 2.14 and 1.87, suggesting a β,β-dimethyl, α,β-unsaturated ketone group.[6] The remaining vinyl proton signal at δ 5.27 (bt, 1 H, J = 7 Hz) was coupled to a methyl singlet at 1.59 ($J < 1$ Hz) and to a methylene signal at 2.09 (m, 2 H), which was in turn coupled to a methylene signal at 1.66 (t, 2 H, J = 6.5 Hz). The remaining signals were a two-proton singlet at δ 3.05 and methyl singlets at 1.87 and 1.33. Since the UV spectrum [λ_{max} 282 nm (ϵ 41 200), 272 (ϵ 52 200), 261 (ϵ 41 200), 253 (ϵ 30 000)] suggested the presence of a triene, only two structures, **4** and **6,** can account for these data. The chemical shift data for the vinyl protons clearly favor structure **4.**

Treatment of ketone **4** with 4-phenyl-1,2,4-triazoline-3,5-dione in dichloromethane solution at room temperature gave an adduct **7.** The ^1H NMR spectrum of the adduct **7** contained an ABX system at δ 4.01 (m, 1 H, J = 16, 3, 3, Hz), 4.34 (dd, 1 H, J = 16, 4 Hz), and 5.64 (m, 1 H, J = 4, 3 Hz) with the signal at 4.01 long-range coupled to a signal at 4.73 (dd, 1 H, J = 8, 3 Hz) which was in turn coupled to a vinyl signal at 5.61 (dd, 1 H, J = 15, 8 Hz). These signals were assigned to the protons on the diazine ring and clearly eliminated structure **6.**

The minor isomer **5** appeared to be a geometrical isomer of **4.** It gave the same adduct **7** on treatment with 4-phenyl-1,2,4-triazoline-3,5-dione and both compounds could be hydrogenated over 10% palladium on carbon catalyst to obtain the same ketone **8** in high yield. The ketones **4** and **5** were therefore isomeric about the $^3\Delta$ double bond. The ^1H NMR

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