(33)[22] (1.0 g), $Ph_3P{=}CHCO_2Me$ (2.56 g), and THF (5 mL) were stirred for 1 h. Evaporation and chromatography (silica gel, Et_2O/hexane, 1:3) gave methyl 4-(2-methyl-1,3-dioxalan-2-yl)-2-butenoate (1.22 g, 85%) as a colorless oil. TLC and NMR spectroscopy indicated the presence of both *E* and *Z* isomers, in approximately a ratio of 3:1: R_f 0.55 (silica gel, Et_2O/hexane 1:4); IR ν_{max} (CH_2Cl_2) 2990, 1720, 1680, 1620 cm^{-1}; ^1H NMR (90 MHz) δ 6.85 (m, 1 H), 5.80 (d, 1 H, J = 15 Hz), 3.91 (s, 4 H), 3.65 (s, 3 H), 3.05 (m, 0.5 H), 2.53 (m, 1.5 H), 1.35 (s, 3 H); mass spectrum, m/e 171 ($M^+ - 15$), 99, 87 (base), 43, 32. The crude product was used directly without purification. The oil (610 mg) was dissolved in Me_2CO (8 mL) and reacted with $TsOH{\cdot}H_2O$ (63 mg). The solution was gently refluxed for 7 h. The solution was cooled to room temperature, diluted with Et_2O (50 mL), and treated with solid $NaHCO_3$ (1 g). The suspension was filtered, the solvent removed, and the crude product chromatographed (silica gel, Et_2O/hexane, 3:7) to give a 1:1 mixture of **31a** and **31b** (370 mg, 80%) as a pale yellow oil: R_f 0.5 (silica gel, Et_2O/hexane, 2:3); bp 60-63 °C at 0.5 mmHg; IR ν_{max} (CH_2Cl_2) 2984, 1730-1720, 1680, 1620 cm^{-1}; ^1H NMR (270 MHz) δ 6.9 (m, 1 H), 6.05 (m, 1 H), 3.78 (s, 3 H), 3.30 (dt, 2 H, J = 2.0, 8.0 Hz), 2.30 (s, 1.5 H), 2.20 (s, 1.5 H); mass spectrum, m/e 142 ($M^{\cdot+}$), 127, 111, 99 (base), 43.

Methyl 11-Hydroxy-5,7-dioxoundec-2-enoate (34a) and Methyl 11-Hydroxy-5,7-dioxoundec-3-enoate (34b). To LDA (4.44 mmol) in THF (12 mL) at -78 °C was added methyl 5-oxohexenoate **31** (300 mg) in THF (0.50 mL) over 5 min. The solution was stirred for a further 20 min before being warmed to -45 °C for 15 min. The dark orange solution was recooled to -78 °C and δ-valerolactone (**11a**) (100 mg) in THF (0.50 mL) added. After 30 min at -78 °C, the reaction mixture was warmed to -42 °C for 10 min and then to 0 °C for 10 min. The system was cooled to -78 °C and quenched with AcOH (0.30 mL) in THF (2 mL). The reaction mixture was slowly warmed up to room temperature, H_2O (3 mL) was added, and the products were extracted into Et_2O (4 × 20 mL). After drying, the solvent was removed and the resultant yellow oil chromatographed (silica gel, Et_2O) to give **34** (123 mg, 51%) as a colorless oil: R_f 0.40 (silica gel, Et_2O); IR ν_{max} ($CHCl_3$) 3400, 1720, 1660, 1595 cm^{-1}; ^1H NMR (270 MHz) δ 6.85 (2 dt, 1 H, J = 6, 14.8 Hz), 6.2, 5.95 (2 × d, 1 H, J = 14.8 Hz), 5.54 (s, 1 H), 3.72 (s, 3 H), 3.64 (t, 2 H, J = 6.1 Hz), 3.26 (2 d, 2 H, J = 6.1 Hz), 2.41 (t, 2 H, J = 7.0 Hz), 1.4-1.9 (m, 6 H); C^{13} NMR (25 MHz) δ 203, 177, 136, 131, 101, 91, 64, 54, 42, 39, 34, 23; mass spectrum, m/e 242 ($M^{\cdot+}$), 224, 169, 127, 101, 83, 69, 55. Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.47; H, 7.49. Found: C, 59.39; H, 7.43.

Attempted Spiroketalization of Methyl 11-Hydroxy-5,7-dioxoundecenoate (34). Method 1: Base-Catalyzed Procedure. NaOMe in MeOH (1 M, 20 μL) was added to **34** (50 mg) in dry methanol (1 mL). Stirring for 5 h at room temperature resulted in no reaction. After reflux for 48 h, HOAc in THF (1 M, 10 drops) was added, the solution absorbed onto silica gel (100 mg), and the solvent evaporated. Chromatography (silica gel, Et_2O/hexane, 4:1) gave **35** (40 mg, 86%) as a white crystalline solid: mp 71-73 °C (from Et_2O-hexane); IR ν_{max} ($CHCl_3$) 3500, 3200, 1701, 1605, 1585, 1254, 1140 cm^{-1}; UV λ_{max} (EtOH) 255 nm (ϵ 10 000); ^1H NMR (90 MHz) δ 7.85 (d, 1 H, J = 9 Hz), 6.96 (m, 1 H, OH), 6.65 (m, 2 H), 3.80 (s, 3 H), 3.79 (m, 3 H), 3.00 (m, 2 H), 1.79-1.42 (m, 4 H); mass spectrum, m/e 224 ($M^{\cdot+}$), 206, 192, 147, 135, 107, 91, 77. Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.25; H, 7.20. Found: C, 64.50; H, 7.20.

Method 2: Acid-Catalyzed Procedure. CF_3CO_2H in THF (1 M, 20 μL) was added to **34** (50 mg) in THF (3 mL) and the mixture was stirred at room temperature. Over several hours the starting material was observed to fragment to produce methyl 5-oxohexenoate **31** and **11a** as judged by TLC (silica gel, Et_2O). The reaction was quenched after 10 h and the products isolated by chromatography were proven to be **31** and **11a** by NMR spectroscopy.

Acknowledgment. We thank Pfizer Central Research, the Science and Engineering Research Council, the Wolfson Foundation, The Royal Society, and Northwestern University for most generous support of our research program. We are additionally grateful to Drs. Michael R. G. Leaming and Bernard Banks for most helpful discussions, Dr. N. S. Mani for completing the characterization of several compounds, Tony Raynham and Sven Taylor at Northwestern University, and Dr. David J. Williams at Imperial College for determining the X-ray crystallographic structures for **24** and **26c**. Several mass spectral determinations were performed at the Midwest Center for Mass Spectrometry, an NSF Regional Instrumentation Facility (Grant CHE-8211164), and we are most grateful for access to this facility.

Supplementary Material Available: X-ray crystal structure analysis of **24** (6 pages); table of calculated and observed structure factors of **24** (17 pages). Ordering information is given on any current masthead page.

Heterocycles from the Marine Sponge *Xestospongia* sp.†

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Received February 7, 1986

The cytotoxic nonpolar extract of a Fiji sponge contains a simple chiral butenolide, 2-oxo-2,5-dihydrofuran-5-acetic acid methyl ester (**1**) which is accompanied by epimeric substituted 3,6-dihydro-1,2-dioxins, xestin A (**5**) and xestin B (**6**). A 5*R* absolute stereochemistry is proposed for **1** based on CD results. The relative stereochemistry of dioxin ring substituents is assigned by ^1H NMR J's, and from optical properties of reduction products. Among the three metabolites, xestin A is the most in vitro active compound against P388 cells.

Soft bodied marine sponges whose extracts are physiologically active are a prime target in our chemical study of taxa from south Pacific coral reefs.[1] During a 1984 expedition to the Island of Viti Levu, Fiji, we encountered thick sheets of a soft *Xestospongia* sp. whose crude extracts at 5 μg/mL were toxic (in vitro) to greater than 75% of P388 murine leukemia cells. The broad array of me-

† Dedicated to Prof. J. F. Bunnett (UCSC) on the occasion of his 65th birthday.

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(1) (a) Myers, B. L.; Crews, P. *J. Org. Chem.* **1983**, *48*, 3583. (b) Manes, L. V.; Bakus, G. J.; Crews, P. *Tetrahedron Lett.* **1984**, *25*, 931. (c) Manes, L. V.; Naylor, S. N.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1985**, *50*, 284. (d) Crews, P.; Manes, L. V.; Boehler, *Tetrahedron Lett.* **1986**, *25*, 2797.

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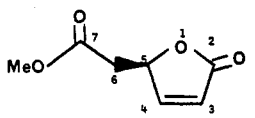
tabolites known from this genus include halogenated C_{16} polyacetylenes,[2a] a brominated C_{18} dienediynoic acid,[2j] unsaturated fatty acids,[2c] novel steroids,[2b,d,k] 1-oxaquinolizidines,[2e] and pentacyclic hydroquinones.[2f-i] None of these natural product classes were represented among the abundant components of the sponge we collected, but instead they consisted of a mildly cytotoxic but-3-enolide, 2-oxo-2,5-dihydrofuran-5-acetic acid methyl ester (1), accompanied by two cytotoxic epimeric 3,6-dihydro-1,2-dioxins, xestin A (5) and xestin B (6).

Results and Discussions

A ^{13}C NMR spectrum of the crude sponge extract was surprisingly simple and showed an intense methyl ester resonance at δ 51.8, which was later attributed to 1, and a much smaller set of even intensity peaks at δ 101.2 and 100.7, which were later attributed to 5 and 6. The isolated amounts of 1 (0.45 g), 5 (0.055 g), and 6 (0.051 g) agreed with the relative intensity of the methoxy ^{13}C NMR signals observed for the crude extract.



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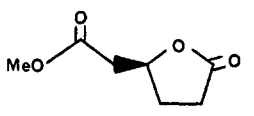

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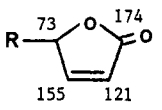

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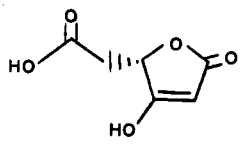

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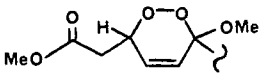
The $C_7H_8O_4$ formula of 1 was derived from the M^+ = 156 along with the C and H count obtained from ^{13}C NMR data. The elucidation of its structure was straightforward given the similarities in the ^{13}C and ^1H NMR data of 1 vs. that of 2(5*H*)-furanones 3.[3,4] The most revealing NMR data of 1 were the ^1H NMR couplings (Hz) $^3J_{3,4}$ = 5.7, $^3J_{4,5}$ = 1.5, and $^4J_{3,5}$ = 2.1, the ABX pattern of protons H-5, H-6, and H-6′, and the following ^{13}C NMR shifts (in ppm): C-2 (171.9), C-3 (121.9), C-4 (155.3), and C-5 (78.8). As expected, hydrogenation of 1 was uncomplicated and yielded 2. An absolute configuration of 5*R* is tentatively suggested for 1. The $[\alpha]^{20}_D$ −41.6° of (5*S*)-2-oxo-4-hydroxy-2,5-dihydrofuran-5-acetic acid (4)[5] hints that it and 1 are enantiomeric at C-5. More important is the negative CD peak ($[\pi - \pi^*]_{205}$ = −12°) of 1 which implies a 5*R* configuration according to an empirical correlation developed by Uchida.[6]

Table I. Proton NMR Parameters

compd (solv)	chem shifts, ppm H-3	H-4	H-5	*J*, Hz[a] 3,4	3,5	4,5	4,6	5,6	ref
5 ($CDCl_3$)	5.00	6.11	5.84	1.2	2.1	10.2			this work
(CD_3CN)				1.2	2.1	10.2			this work
6 ($CDCl_3$)	4.78	6.17	5.85	4.5	1.5	10.2			this work
(CD_3CN)				4.2	1.8	10.2			this work
9 ($CDCl_3$)	4.76	6.22	5.68	4.5	1.5	10			7
10								4.0	14
11				1.9	2.1	10.2	0.5	5.3	15
12				4.5	1.1	10.1	1.1	4.5	15

[a] Coupling constants given as absolute values.

The isomeric compounds xestin A (5) (white solid, mp 55–56 °C) and xestin B (6) (viscous oil) were unstable to regular phase chromatography. Purification was accomplished by repeated reverse-phase chromatography. In comparison to 1, these compounds were more complex, as a molecular formula of $C_{26}H_{44}O_5$ could be established by a mass spectrometry M^+ = 436 and further confirmed by NMR data. The partial structure A, present in chondrillin



A

(9), a C_{24} metabolite isolated by Wells from a sponge in the genus *Chondrilla*,[7] was evident in 5 and 6 from ^{13}C and ^1H NMR resonances. One additional functionality, a $CH_2CH{=}CHCH{=}CHCH_3$ [5: UV, 227 nm; ^{13}C, four HC= between δ 127 and 132, 35 (t), 18 (q); ^1H, δ 1.72 (d, J = 6, 3 H)] was present in both compounds. Gross structures 5 and 6 could be written and chemical evidence in their support of was sought next. Treatment of 5 with $LiAlH_4/EtOH$[8] afforded selective reduction of the carbomethoxyl group because alcohol 7 was obtained in quantitative yield. Hydrogenation of 5 to the hydroxy keto ester 8 is described below.

The relative stereochemical features of xestin A (5) and xestin B (6) were elucidated next. The *E,E* acyclic double bond stereochemistry was assigned on the basis of characteristic ^{13}C NMR shifts at the terminal CH_3 (5, δ 18.1; 6, δ 17.8) and at C-19 (5, δ 34.9; 6, 34.8).[9] It was initially obvious that 5 and 6 were epimers, because the most outstanding differences between the spectroscopic data of both compounds were the chemical shift variations at H-3 (5, δ 5.00; 6, δ 4.78), the differences in chemical shifts for the diastereotopic geminal protons at C-2 (5, 0.11 ppm; 6, 0.33 ppm), and the variation in J's to H-3 which are summarized in Table I. Catalytic hydrogenation of 5 with 10% Pd/C in ethyl acetate yielded a chiral 3,6-dihydro-1,2-dioxin ring degradation product, 8, in 89% yield whose $[\alpha]^{20}_D$ = −10.9°. Correspondingly, the hydrogenation of 6 yielded the same 8 enantiomer because its $[\alpha]^{20}_D$ = −14.0°. Both samples of 8 gave spectroscopic data that were in accord with its structure. For example, a ^1H–^1H COSY NMR spectrum of 8 verified the connectivities of

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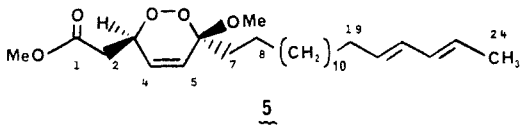

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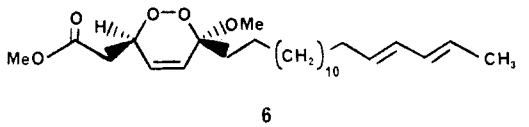

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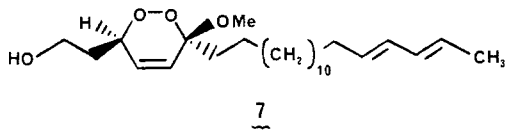

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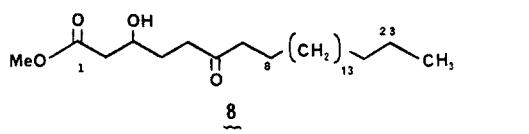

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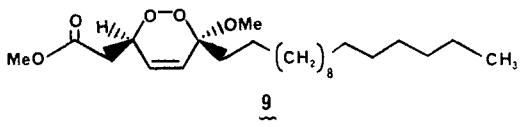

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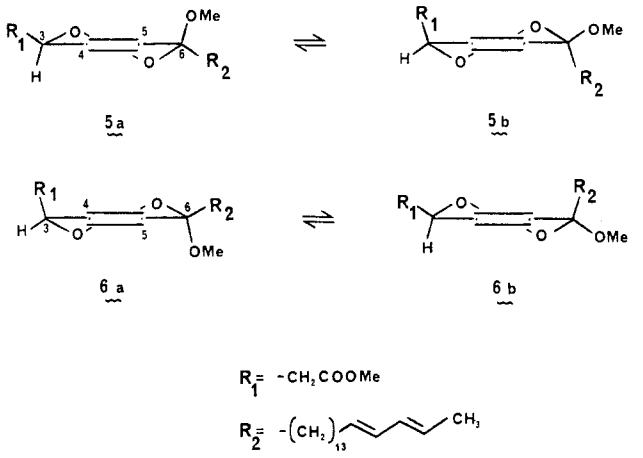
protons attached from C-2 through to C-5, and this was analogous to results that were also obtained for **5**. Finally both synthetic samples of **8** showed identical incremental ^1H NMR shifts for the OMe when the chiral shift reagent Eu(hfc)$_3$ was added to their $CDCl_3$ solutions. This established that the configuration at C-3 is identical in **5** and **6**; therefore, C-6 must be the epimeric center.

The conformational possibilities for the dioxene ring in **5** and **6** are depicted in Scheme I. Dreiding models indicated a similar half-chair conformation and almost identical proton proton dihedral angles for cyclohexene and 3,6-dihydro-1,2-dioxin.[10] Consequently, similar ^1H NMR vicinal couplings are observed between the vinyl and allylic protons in cyclohexenes and in **5** and **6** (see below and Table I). Ring alkyl substituents at C-3 and C-6 ought to prefer a pseudoequatorial rather than a pseudoaxial position, and the methoxy at C-6 ought to prefer a pseudoaxial rather than a pseudoequatorial position, by analogy to the anomeric effect known to operate in 2-alkoxytetrahydropyrans. Estimates of the upper energy limit of the conformational preferences for 3,6-dihydro-1,2-dioxin substituents were made on the basis of data from cyclohexanes[11] and 2-oxotetrahydropyrans[12] as follows: axial CH_2R destabilized by 2.1 kcal/mol; axial OCH_3 destabilized by 0.7 kcal/mol, equatorial OCH_3 anomeric effect destabilized by 1.3 kcal/mol (CCl_4), or 1.1 kcal/mol (CH_3CN). Under nonpolar solvent conditions conformer **5a** should be unfavorable by an axial OCH_3 (–0.7 kcal/mol) and conformer **5b** should be unfavorable by two axial CH_2R (–4.2 kcal/mol) and an equatorial OCH_3 (–1.3 kcal/mol). Thus, a larger difference in free energy of 4.8 kcal/mol is predicted and ensures that conformer **5a** will predominate. Similarly, conformer **6a** should be destabilized by an axial OCH_3 (–0.7 kcal/mol) and an axial CH_2R (–2.1 kcal/mol), while conformer **6b** should be destabilized by an axial CH_2R (–2.1 kcal/mol) and an equatorial OCH_3 (–1.3 kcal/mol). In this case, the predicted difference in free energy is only 0.6 Kcal which indicates that **6a** might be only slightly favored (by 72%). The vicinal and allylic ^1H NMR coupling constants that were measured for both compounds seemed to verify the above trends when they were analyzed according to Karplus-type relationships.[13] For a 3,6-dihydro-1,2-dioxin half-chair conformation the estimates of proton dihedral angles and their corresponding J values between H's attached to the sp^3 C's (H_e or H_a) and sp^2 C's (H_v) include: $\angle H_e$–H_v = 43°, $^3J[H_e$–$H_v] = 5.0$ Hz, $^4J[H_e$–$H_v] = 0.5$ Hz; $\angle H_a$–H_v = 77°, $^3J[H_a$–$H_v] = 1.8$ Hz, $^4J[H_a$–$H_v] = 2.1$ Hz. The proton J's observed for **5** in $CDCl_3$ and CH_3CN, as shown in Table I, are consistent with conformer **5a** as being homogeneous. Likewise, the proton J's observed for **6** in $CDCl_3$, $^3J = 4.5$ Hz and $^4J = 1.5$ Hz, vs. those in CH_3CN, $^3J = 4.2$ Hz and $^4J = 1.8$ Hz, are consistent with **6a** as being the major conformer and with the equilibrium being slightly shifted toward **6b** in a more polar solvent such as CD_3CN. Such an equilibrium shift is in accord with what has been observed in the past for systems where an axial anomeric effect is operating. Coupling constant arguments of the type described above have also been used in the past to analyze the conformations of natural products containing a substituted cyclohexene ring. Specific examples include the use of J values appearing in Table I to verify the proton stereochemistry shown in the structures for shikimic acid (**10**),[14] conduritols **11** (conformationally homogeneous), and **12** (conformationally nonhomogeneous).[15]

2-Oxo-2,5-dihydrofuran-5-acetic acid methyl ester (**1**) appears to be the smallest molecular weight α,β-butenolide observed from a marine sponge.[16] A cytotoxic C_{11} butenolide, epilolide, was reported from *Tedania ignis*.[17] Larger butenolides with a 4-oxygen group (e.g., a tetronic acid subunit) are occasionally observed from Dictyoceratid

Scheme I



(10) Cyclohexene is known to be slightly more puckered than cyclohexane. For example, NMR "R" value analysis reveals that R = 1.9–2.2 for a perfect chair and R increases with increasing puckering. Thus, R = 2.2 for cyclohexane and R = 3.83 for cyclohexene. See: Lambert, J. B.; Shurvell, H. F.; Verbit, L.; Cooks, R. G.; Stout, G. H. In *Organic Structural Analysis*; Macmillan: New York, 1976; Chapter 4.

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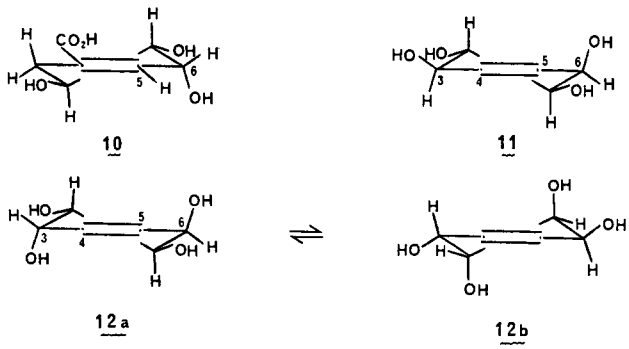
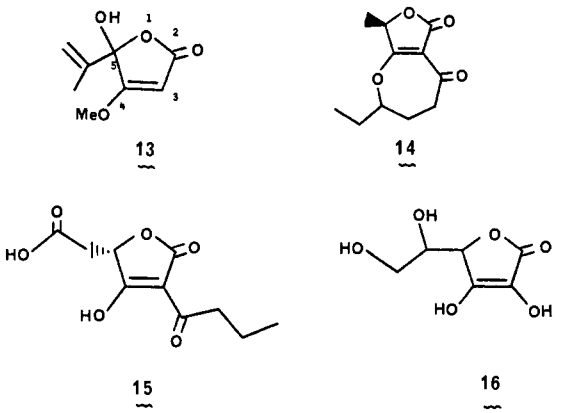


10 11 12a ⇌ 12b

marine sponges, and the tetronic acid subunits are always a part of a terpene skeleton.[18] There are several small molecular weight α,β-butenolides known from terrestrial organisms, and they are all microbial metabolites,[19] which usually have a 4-hydroxyl or 4-alkoxyl group. Examples of these include penicillic acid (**13**), terrestric acid (**14**), (*S*)-carlosic acid (**15**), and ascorbic acid (**16**). Chondrillin



13 14 15 16

(**9**), a counterpart to xestin A (**5**) and xestin B (**6**), was reported without C-3 vs. C-6 relative stereochemistry, though chiroptical data on a degradation product led to the suggestion, without substantiation, of *S* stereochemistry at C-3. We are now able to confidently assign these relative stereochemical elements in **9** on the basis of the nearly identical NMR data between it and **6** (see Table I). We have also observed **1** from one other unidentified Fiji sponge,[20] but it was accompanied by only fatty esters and steroids. The cooccurrence of **1** and **5**, **6** from *Xestospongia* tempts us to speculate that biological singlet oxygen cycloaddition to the methoxy diene chromophore in a tetraene ester generates compounds **5** and **6**. The dioxene ring could then undergo peroxide bond cleavage followed by scission of the C-6 to C-7 bond and finally lactonization to generate **1**. Other examples of cyclic peroxides of a polyketide biosynthetic origin are rather rare from marine sponges but do include several compounds from the genus *Plakortis*.[16a]

The heterocycles reported above are responsible for the *Xestospongia* crude extract cytotoxicity but xestin A (**5**) is the most active. The results from in vitro assay against P388 cells are as follows: butenolide **1** was 50% active at 50 μg/mL and completely inactive at 5 μg/mL; xestin A (**5**) exhibited an IC_{50} of 0.3 μg/mL; xestin B (**6**) exhibited an IC_{50} of 3 μg/mL. In addition, xestin A exhibited strong in vitro activity at 5 μg/mL against other tumor cells including lung (A549), colon (HCT-8), and mammary (MDAMB), whereas xestin B was completely inactive at this concentration.[21]

Experimental Section

Our general analytical, chemical, and chromatographic methods have been described previously.[22] The NMR spectra were recorded on a JEOL FX-100 PFT spectrometer operating at 99.5 MHz for ^1H and 25.1 MHz for ^{13}C. High-field ^1H and ^{13}C NMR were recorded on a GN-300 spectrometer. Multiplicities of ^{13}C NMR peaks were determined from APT or DEPT data. Mass spectrometry data were obtained on a Finnigan 4000 (6000 LS7 computer system). High-performance liquid chromatography (HPLC) was done on a Waters ALC-201, using columns which include a Waters μ-Porasil, Whatman Partisil, or a Rainin Microsorb C-18. Rotations were measured on a Perkin-Elmer 141 polarimeter.

Collection and Isolation Procedures. A *Xestospongia* sp. (# 84-17) was collected from the Island of Viti Levu, Fiji at −30 ft by SCUBA during August, 1984. The freshly collected sponge (7.0 kg, wet weight) was immediately extracted with CH_2Cl_2, and the viscous crude oil concentrate (7.75 g) was returned to UCSC for further examination. The crude oil was examined by ^{13}C NMR, and then it was successively partitioned between equal volumes of methanol (wet, % of water adjusted to give a biphase solution of equal volume) and a solvent series of hexanes, carbon tetrachloride, and methylene chloride. Each solvent fraction was next examined by ^{13}C NMR which revealed that **1** was concentrated in the methylene chloride fraction, whereas both **5** and **6** were concentrated in the carbon tetrachloride fraction. Further purification was by flash chromatography followed by HPLC and the isolated yields, based on the wet sponge weight were **1** (0.45 g, 0.0064%), **5** (0.055 g, 0.00078%), and **6** (0.051 g, 0.00073%).

Sponge Identification. The encrusting sponge was identified as a *Xestospongia* sp. [Order Nepheliospongida; Family Nepheliospongiidae],[23] and this was based on the following characteristics. It grows on dead coral and measures about 1 cm thick by 5 cm in diameter. The sponge is fleshy yet condensed and is dark chocolate brown with medium brown interior. The dermis has small pore platelike openings. Oxeas are strewn randomly throughout the choanosome. The oxeas are often slightly curved, measure about 120 × 4 μm, and display incipient roughness in the center or centrotyloty. This sponge may have been originally described as specimen No. 410 of *Neopetrosia pandora* from Ponapê.[24]

2-Oxo-2,5-dihydrofuran-5-acetic Acid Methyl Ester (1). Purification, after solvent partitions (see above), was by flash chromatography (200–325 mesh silica, 1:1 ethyl acetate–hexane) and yielded an oil, $[\alpha]^{20}_D$ +80.3° (*c* 0.27, $CHCl_3$). Physical properties are were as follows: NMR (assignments by ^{13}C NMR APT and INEPT, ^1H–^1H COSY, and ^1H–^{13}C COSY); ^{13}C NMR (75 MHz, $CDCl_3$) 171.9 (C-2 or C-7), 169.2 (C-7 or C-2), 155.3 (C-4), 121.9 (C-3), 78.8 (C-5), 51.9 (O-CH_3), 37.6 (C-6) ppm; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (dd, J = 5.7, 1.5 Hz, H-4), 6.09 (dd, J = 5.7, 2.1 Hz, H-3), 5.34 (tt, J_{ax+bx} = 14 Hz, J_{1r} = 2, 2 Hz, H-5), 3.7 (s, OCH_3), 2.63/2.74 (8-line mult, J_{ab} = 16.5, H-6, H-6′); mass spectrum (CI, methane), *m/z* (fragment, %) 157 (M^+ + 1, 55), 156 (M^+, 18), 125 (M^+ − OMe, 8), 96 (M^+ − H − CO_2Me, 92), 83 (M^+ − CH_2CO_2Me, 100); IR (neat) 1750 cm^{-1}; UV (MeOH) λ_{max} 205 nm.

Hydrogenation of 2-Oxo-2,5-dihydrofuran-5-acetic Acid Methyl Ester (1). A microhydrogenation using Adams catalyst in ethanol yielded pure **2**: ^1H NMR (300 MHz, $CDCl_3$) δ 4.85 (br mult, H-5), 3.7 (s, OCH_3), 2.4–2.8 (mult, H-3, H-3′, H-6, H-6′),

(18) Only five marine tetronic acid containing metabolites are known having C-5 as sp^3. All are sponge sesterterpenes and include in ref 16b compounds **15–17**, **28**, and **29**.

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2.0 (br mult, H-4), 1.79 (br mult H-4'); mass spectrum (CI, methane), m/z (fragment, %) 159 ($M^+ + 1$, 52), 158 (M^+, 15), 127 ($M^+ - OMe$, 20), 98 ($M^+ - H - CO_2Me$, 16), 85 ($M^+ - CH_2CO_2Me$, 100); IR (neat) 1770, 1750 cm^{-1}; UV (MeOH λ_{max} 205 nm.

Xestin A (5). Reverse-phase flash column chromatography over 50–100 μm ODS followed by reverse-phase HPLC (10 μm ODS, 92:08 MeOH–H_2O as eluent) yielded pure **5**, whose retention time was longer than that of **6**. Physical properties were as follows: mp (MeOH) 55–56 °C; $[\alpha]^{20}_D$ +26.5° (c 0.37, CH_2Cl_2); NMR (assignments by ^{13}C NMR APT and INEPT, ^1H-^1H COSY); ^{13}C NMR (75 MHz, $CDCl_3$) 170.1 (C-1), 132.3 (C-20), 131.8 (C-21 or C-22), 130.5 (C-22 or C-21), 130.3 (C-4), 127.0 (C-23), 126.7 (C-5), 101.2 (C-6), 73.6 (C-3), 52.1 (ester OMe), 51.4 (ketal OMe), 36.4 (C-2), 34.9 (C-19), 32.6 (C-7), 29.6 (10 CH_2) 23.4 (C-8), 18.1 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 6.11 (dd; J = 1.2, 10.2 Hz, H-4), 6.00 (m, H-21, H-22), 5.84 (dd, J = 2.1, 10.2 Hz, H-5), 5.56 (m, H-20, H-23), 5.00 (m, H-3), 3.72 (s, ester OMe), 3.38 (s, ketal OMe), 2.61 (dd, J = 7.5, 16.2 Hz, H-2), 2.50 (dd, J = 6.6, 15.9 Hz, H-2'), 2.03 (4-line mult, H-19–19'), 1.72 (d, J = 6.3 Hz., Me-24), 1.64 (m, 4 H), 1.2–1.4 (10 CH_2); ^1H-^1H COSY (selected correlations), H-2 to H-2', H-2-2' to H-3, H-3 to H-4 and H-5, H-4 to H-5, Me to conjugated double bonds, double bonds to H-19–19', H-19–19' to long chain; CI (methane) mass spectrum, m/z (fragment, %) 437 ($M^+ + 1$, 20), 436 (M^+, 5), 405 ($M + 1 - O_2$, 8), 377 ($M^+ - MeOCO$, 15), 359 ($M^+ - MeOCO - H_2O$, 10), 345 ($M^+ - O_2 - CH_3OCO$, 10), 317 ($M^+ + 1 - MeOCOCH_2 - O_2 - Me$, 45), 289 (18), 171 (100), 157 (40); IR (neat) 1740 cm^{-1}; UV (MeOH) λ_{max} 227 nm (ε 29 000).

Xestin B (6). Purification was as described above for compound **5**. Physical properties were as follows: $[\alpha]^{20}_D$ +19.61° (c 0.11, Cl_2CH_2); ^{13}C NMR (25.1 MHz, $CDCl_3$) 170.7 (C-1), 132.0 (C-20, and C-21 or C-22), 130.4 (C-22 or C-21), 129.4 (C-4), 127.1 (C-23), 126.5 (C-5), 100.7 (C-6), 73.4 (C-3), 51.7 (ester OMe), 51.1 (ketal OMe), 37.3 (C-2), 34.8 (C-19), 32.6 (C-7), 29.5 (10 CH_2), 23.5 (C-8), 17.8 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 6.17 (dd, J = 4.5, 10.2 Hz, H-4), 5.97 (m, H-21, H-22), 5.85 (dd, J = 1.5, 9.9 Hz, H-5), 5.56 (m, H-20, H-23), 4.78 (m, H-3), 3.70 (s, ester OMe), 3.37 (s, ketal OMe), 2.91 (dd, J = 8.7, 15.9 Hz, H-2), 2.58 (dd, J = 9.0, 16.5 Hz, H-2'), 2.01 (4-line mult, H-19–19'), 1.70 (d, J = 6.3 Hz, Me-24), 1.62 (m, 4 H), 1.2–1.4 (10 CH_2); CI (methane) mass spectrum; m/z (fragment, %) 437 ($M^+ + 1$, 15), 436 (M^+, 5), 405 ($M^+ + 1 - O_2$, 15), 377 ($M^+ - MeOCO$, 10), 373 ($M^+ - O_2 - MeO$, 15), 359 ($M^+ - MeOCO - H_2O$, 45), 345 ($M^+ - Me - O_2 - MeOCO$, 70), 317 ($M^+ + 1 - MeOCOCH_2 - O_2 - Me$, 30), 289 (20), 171 (70), 157 (100); IR (neat) 1740 cm^{-1}; UV (MeOH) λ_{max} 227 nm (ε 29 000).

LAH Reduction of Xestin A (5) to Alcohol 7. $LiAlH_4$ (2 mg, 0.052 mmol) was dissolved in 1 mL of dry THF with 3 μL of absolute EtOH (0.052 mmol). This mixture was added to a stirred solution, blanked with nitrogen, of xestin A (21 mg, 0.048 mmol) dissolved in 2 mL of dry THF. After 1.25 h workup commenced by addition of water (3 mL) and then extraction with ethyl ether (4 × 5 mL) and CH_2Cl_2 (1 × 5 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated under low pressure, giving pure **7** (20.58 mg). Its spectroscopic properties were as follows: ^{13}C NMR (25.1 MHz, $CDCl_3$) 132.2 (C-20), 131.9 (C-21 or C-22), 131.5 (C-22 or C-21), 130.4 (C-4), 126.5 (C-5 and C-23), 101.1 (C-6), 75.4 (C-3), 59.1 (C-1), 51.2 (MeO), 35.2 (C-2), 34.9 (C-19), 32.5 (C-7), 29.3 (10 CH_2), 23.4 (C-8), 17.9 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) 6.06 (dd, J = 1.2, 10.2 Hz, H-4), 6.00 (m, H-21, H-22), 5.84 (dd, J = 2.1, 10.2 Hz, H-5), 5.56 (m, H-20, H-23), 4.80 (m, H-3), 4.12 (m, H-1), 3.77 (m, H-1', overlapped with OH), 3.39 (s, MeO), 2.03 (m, H-19–19'), 1.72 (d, J = 6.3 Hz, Me-24), 1.2–2.0 (13 CH_2); CI (methane) mass spectrum, m/z (fragment, %) 391 ($M + 1 - H_2O$, 15), 363 ($M^+ - C_2H_4OH$, 25), 348 (363 - Me, 75), 331 (363 - MeOH, 65); IR (neat) 3200–3600 cm^{-1} (OH); UV (MeOH) λ_{max} 227 nm (ε 29 000).

Hydrogenation of Xestin A (5) to Compound 8. Xestin A (5 mg) was dissolved in 5 mL of ethyl acetate with 5 mg of Na_2CO_3 and a catalytic amount of Pd/C 10%. Hydrogenation proceeded for 3 h (1 atm of pressure). The reaction mixture was filtered and the solvent evaporated under low pressure, affording after purification **8** (4.45 mg): $[\alpha]^{20}_D$ -10.9° (c 0.036, $CHCl_3$); ^{13}C NMR (25.1 MHz, $CDCl_3$) 210.9 (C-6), 172.9 (C-1), 67.4 (C-3), 51.6 (ester OMe), 42.8 (C-2 or C-7), 41.4 (C-7 or C-2), 38.6 (C-4 or C-5), 36.0 (C-5 or C-4), 31.8 (C-22), 30.2 and 29.1 (13CH_2), 23.8 (C-8), 22.6 (C-23), 13.9 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 3.98 (m, H-3), 3.70 (s, ester OMe) 2.59 (m, H-5–5'), 2.45 (m, H-2–2' partially overlapped with H-7–7'), 2.41 (m, H-7–7'), 1.70 (m, H-4–4'), 1.55 (m, H-8–8'), 1.4–1.1 (15 CH_2), 0.87 (br t, Me-24); A ^1H-^1H COSY spectrum was consistent with the proposed structure; H-2–2' to H-3, H-3 to H-4-4', H-4-4' to H-5-5', H-7-7' to H-8-8', H-8–8' to $(CH_2)_{15}$, Me-24 to $(CH_2)_{15}$; CI (methane) mass spectrum, m/z (fragment, %) 413 ($M^+ + 1$, 10), 412 (M^+, 5), 397 ($M^+ - Me$, 40), 395 ($M^+ - 17$, 80), 381 ($M^+ - MeO$, 100), 367 (85), 253 ($C_{18}H_{37}$, 10); IR (neat), 3600–3200 (OH), 1748 (ester), 1710 (ketone) cm^{-1}.

Hydrogenation of Xestin B (6) to Compound 8. Xestin B (5 mg) was dissolved in 5 mL of ethyl acetate with 5 mg of Na_2CO_3 and a catalytic amount of Pd/C 10%. Hydrogenation proceeded for 3 h (1 atm of pressure). The reaction mixture was filtered and the solvent evaporated under low pressure, affording after purification **8** (3.9 mg), $[\alpha]^{20}_D$ -14.0° (c 0.021, $CHCl_3$). All spectral properties were identical with the other sample of **8**.

Chiral Shift Reagent Study of 8. With ^1H NMR as a monitor, each sample of **8**, obtained as described above, was titrated with tris[((3,4,5,6,7,8,9-heptafluoropropyl)hydroxymethylene)-(+)-camphorato]europium (III), $Eu(hfc)_3$. Identical concentrations of **8** (6.3 mM) in $CDCl_3$ were prepared, and the shift of the OMe was observed as $Eu(hfc)_3$ was added. Identical incremental OMe shifts were observed for both samples as the shift reagent concentration was increased (δ values for OMe relative to Me_4Si): $[Eu(hfc)_3]$ = 0 mM, δ 3.70; $[Eu(hfc)_3]$ = 0.3 mM, δ 3.70; $[Eu(hfc)_3]$ = 3.0 mM, δ 4.30; $[Eu(hfc)_3]$ = 10 mM, δ 5.0.

Acknowledgment. Partial research support to P.C. was from NOAA, National Sea Grant College Program, Department of Commerce, University of California Project R/MP-33. A grant to P.C. from the University Research Expeditions Program supported our field work in Fiji. P.C. is also grateful for the cooperation of the Fiji Government. E.Q. received a Postdoctoral Fellowship from Xunta de Galicia, Spain, and L.V.M. was a Sea Grant Trainee.

Registry No. **1**, 104532-57-2; **2**, 63545-49-3; **5**, 104532-58-3; **6**, 104597-39-9; **7**, 104532-59-4; **8**, 104532-60-7.

Structure Determination of Oligomycins A and C[1a]

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Received April 22, 1986

The structures of oligomycins A (**2**) and C (**3**) were established by chemical correlation of their respective degradation products with those derived from oligomycin B, whose structure is known. The mass spectral fragmentation behavior of the spiroketal under electron impact conditions is discussed.

The oligomycin antibiotic complex was first isolated in 1954[2] from a strain of *Streptomyces diastatochromogenes*. The complex, consisting of variable proportions of three major components A, B, and C[3]—depending on the strain

0022-3263/86/1951-4264$01.50/0