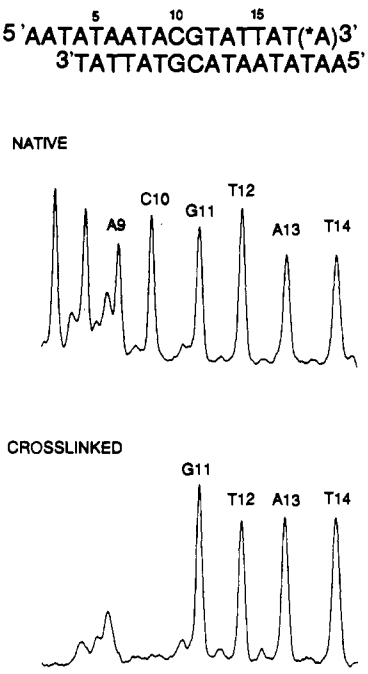

Figure 1. Partial fragmentation patterns (iron(II)/EDTA/ascorbic acid/H_2O_2) for radiolabeled (* ≈ ^{32}P) native and interstrand cross-linked 5'-d(AATATAATACGTATTAT*A). Lettering indicates residue cleaved.

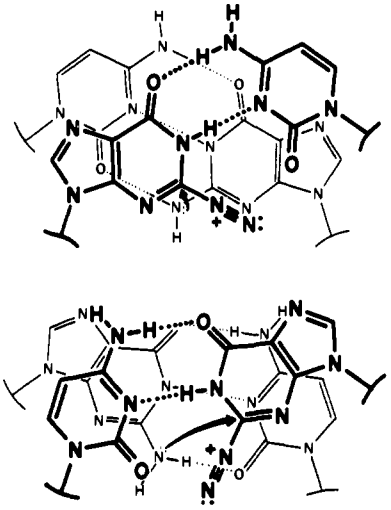

Figure 2. View down the helix axis at the duplex DNA sequences 5'-CG (upper) and 5'-GC (lower) in the B conformation. In each case, one exocyclic amino group of a deoxyguanosine residue has been replaced by a diazonium group; the bold arrows indicate the nucleophilic addition reactions required for cross-linking as in **1**.

may enable preparation of structurally homogeneous samples critical for the determination of the three-dimensional structure and mechanism of enzymatic repair of this lesion.[16]

Acknowledgment. We thank the NIH (GM35466; GM32681; AG00417), NSF (DIR-8220099), and Sloan Foundation for financial assistance.

Supplementary Material Available: Fe(II)/EDTA fragmentation analyses of DNAs III-VI (6 pages). Ordering information is given on any current masthead page.

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Octalactins A and B: Cytotoxic Eight-Membered-Ring Lactones from a Marine Bacterium, *Streptomyces* sp.

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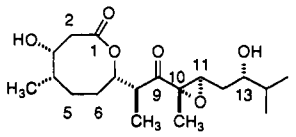
Received January 14, 1991

Although marine plants and invertebrates have been the subject of extensive chemical investigations,[1] studies of marine microorganims are rare.[2] This lack of attention is surprising given the central role soil bacteria play in the development of clinically important pharmaceutical agents and the immense diversity of bacterial species found in the marine environment.[3] Marine sediments—the effective equivalent of terrestrial soils—have diverse bacterial populations, and marine plants and animals provide host surfaces for specific bacteria.[4] We have initiated a program to explore the chemistry of marine microorganisms,[5] and we now report our findings on a marine-derived actinomycete of the genus *Streptomyces*, collected from the surface of the Sea of Cortez gorgonian octocoral *Pacifigorgia* sp. In culture, this streptomycete produces numerous metabolites including two closely related novel compounds, octalactins A (**1**) and B (**2**), with fully saturated eight-membered lactone ring functionalities.[6-8]



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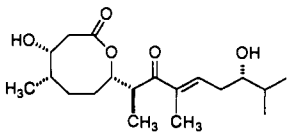

2

Ethyl acetate extracts of the culture broth of *Streptomyces* sp., isolate PG-19,[9] showed significant in vitro cytotoxicity toward B-16-F10 murine melanoma and HCT-116 human colon tumor

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(9) Marine isolate PG-19, an actinomycete of the genus *Streptomyces*, was isolated from a dilution series innoculum derived from the surface of an unidentified gorgonian of the genus *Pacifigorgia*. The gorgonian collection and microbial isolation work were performed on the research vessel *ORV John Isaacs* (SIO) as part of an expedition to the Sea of Cortez (Gulf of California, Mexico) in 1987. The bacterium was cultured on a marine medium consisting of the following constituents: starch (10 g), yeast extract (4 g), peptone (2 g), Tris buffer (1 M, pH 8.0, 10 mL), seawater (750 mL), and deionized water (250 mL). Cultures were grown in shake flasks at 23 °C for 8-12 days prior to product isolation. The octalactins were isolated by repetitive extraction of medium with EtOAc.

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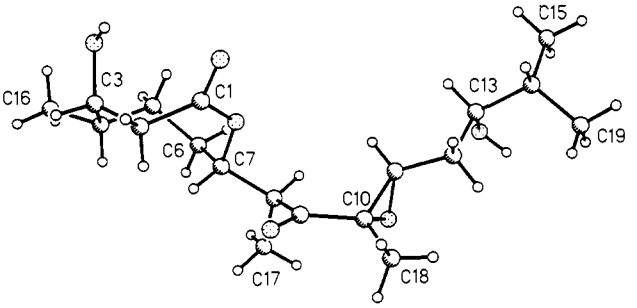

Figure 1. A computer-generated perspective drawing of octalactin A. No absolute configuration is implied.

cell lines. Fractionation of the crude extract by silica vacuum flash chromatography followed by silica HPLC (EtOAc/isooctane, 7/3) yielded octalactins A (**1**) and B (**2**) in variable ratios at less than 1% of the organic extract. Octalactin A (**1**), mp 155-157 °C, analyzed for $C_{19}H_{32}O_6$ by high-resolution mass spectrometry and ^{13}C NMR.[10] The ^{13}C NMR spectrum of **1** showed a ketone carbonyl resonance at δ 212.4 ppm and an ester carbonyl at δ 172.4 ppm. Resonances attributed to two secondary alcohols (δ 71.3 and 74.5 ppm, both CH), to the non-carbonyl carbon of the lactone (δ 79.3, CH), and to a trisubstituted epoxide (δ 62.4 ppm, C, and 58.8 ppm, CH) were also observed, thus accounting for all the oxygens in **1**. A combination of COSY ^1H NMR and XHCORR and COLOC ^{13}C NMR experiments[10] defined the planar, but not the three-dimensional, structure of **1**. The relative stereostructure of **1** was established by single-crystal X-ray crystallographic methods,[11] and a computer-generated perspective drawing is shown in Figure 1.

Octalactin A contains an unusual eight-membered lactone moiety.[6-8] As can be seen in Figure 1, the lactone ring has a boat-chair conformation with a cis lactone (C7-O1-C1-C2: -0.8 (6)°) in the solid state. Earlier molecular mechanics calculations[12,13] of eight-membered lactone rings indicated four local minima within 1.31 kcal/mol of the ground state. Calculations on a trisubstituted eight-membered lactone ring model of **1**, using Monte Carlo searching of conformation space with the BATCHMIN subroutine of MACROMODEL[14] and the MM2 force field, show six distinct minima (supplementary material) within 0.95 kcal/mol of the ground state. One of these conformations, the lower boat-chair, was that found in the X-ray study. Of the six conformations, five are cis lactones, while the sixth, with the highest calculated steric energy in vacuum, is trans. Due to the larger dipole moment of the cis conformers, they are predicted to be significantly stabilized in polar solution. Strong intermolecular hydrogen bonding between the lactone carbonyl, the C3 hydroxyl, and the C13 hydroxyl in the crystal structure could be responsible for the solid-state conformation.

Octalactin B (**2**) was isolated as a colorless oil. Analysis of ^{13}C and ^1H NMR data showed **2** to be the corresponding C10-C11 olefin derivative of **1**.[15] Octalactin A was responsible for most of the cytotoxic activity of the extract and displayed IC_{50} values of 7.2×10^{-3} μg/mL (B-16-F10) and 0.5 μg/mL (HCT-116). Octalactin B was completely inactive in the cytotoxicity assays against murine and human cancer cell lines.

Acknowledgment. This research was funded by the PHS, National Institutes of Health, and through the National Cancer Institute under Grants CA44848 (W.F.) and CA24487 (J.C.). We thank the Bristol-Myers Squibb Company for providing in vitro cancer cell cytotoxicity bioassays.

Registry No. **1**, 133473-06-0; **2**, 133473-07-1.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, bond angles, and torsional angles for octalactin A (**1**) and a summary figure of low-energy conformations (5 pages). Ordering information is given on any current masthead page.

(10) Octalactin A (**1**), mp 155-157 °C ($CHCl_3$/EtOAc), showed $[\alpha]_D$ -14° (*c* 1.8, $CHCl_3$), analyzed for $C_{19}H_{32}O_6$ by HRMS (obsd M^+ *m/z* 357.2264, calcd 357.2277), and showed the following spectral features: IR (neat) 3450, 1720 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$, assignments by COSY methods) δ 2.97 (br d, 13.3) and 2.72 (dd, 13.3, 6.1) both C2, 4.03 (br s) C3, 4.60 (t, 9.7) C7, 2.96 (m) C8, 3.55 (t, 6.1) C11, 1.78 (m) C12, 3.50 (m) C13, 1.13 (d, 6.8) C15, 0.92 (d, 6.8) C16, 1.44 (s) C17, 1.00 (d, 6.8) C18, 0.94 (d, 6.8) C19; unlisted protons were part of an unresolved multiplet; ^{13}C NMR (50 MHz, $CDCl_3$, assignments by XHCORR and COLOC methods) δ 172.4, 39.3, 71.3, 34.0, 22.5, 32.0, 79.3, 42.5, 212.4, 62.4, 58.8, 32.3, 74.5, 38.0, 22.0, 18.4, 12.6, 13.4, 17.6 for C1 to C19, respectively.

(11) Octalactin A (**1**) crystallized in the orthorhombic space group $P2_12_12_1$ with a = 9.965 (2) Å, b = 21.010 (4) Å, c = 9.701 (3) Å, and one molecule of composition $C_{19}H_{32}O_6$ in the asymmetric unit. Diffraction maxima were measured by using 2θ-θ scans and graphite-monochromated Cu Kα radiation to a 2θ limit of 114°. Of the 1583 reflections collected in this way, 1503 (95%) were judged observed ($|F_o| \geq 4\sigma(|F_o|)$). The structures was solved by direct methods (MULTAN) and refined by using full-matrix least-squares analysis of 25 anisotropic heavy atoms and 32 riding model, isotropic hydrogens to a standard crystallographic discrepancy ratio of 0.0525. Additional crystallographic details are available and are described under Supplementary Material Available.

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(15) Octalactin B (**2**), an oil, showed $[\alpha]_D$ -12.3° (*c* 5.6, $CHCl_3$), analyzed for $C_{19}H_{32}O_5$ by HRMS (obsd M^+ *m/z* 341.2211, calcd 341.2328), and showed the following spectral features: IR (neat 3450, 1715, 1660, 1460, 1380 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 3.05 (dd, 13.3, 1.8) and 2.73 (dd, 13.3, 6.1) both C2, 4.04 (br s) C3, 1.60 (m) C4, 1.65 (m) and 1.23 (m) both C5, 1.80 (m) and 1.70 (m) both C6, 4.76 (br t, 9.7) C7, 3.55 (dq, 7.2, 7.2) C8, 6.87 (t, 6.8) C11, 2.45 (ddd, 1.51, 6.8, 3.6) C12, 3.52 (br s) C13, 1.70 (m) C14, 1.14 (d, 7.2) C15, 0.96 (d, 6.8) C16, 1.78 (s) C17, 1.05 (d, 7.2) C18, 0.97 (d, 6.8) C19; ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.6, 39.2, 71.4, 33.9, 22.7, 32.2, 79.4, 44.3, 203.3, 137.5, 141.4, 34.1, 75.8, 38.0, 22.0, 18.7, 11.7, 14.9, 17.4 for C1 to C19, respectively.

Conformational Change of Cholesterol Side Chain in Lipid Bilayers

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Received November 7, 1990

The cholesterol-lipid interactions at the molecular level have been attributed primarily to the hydrogen-bond formation of the 3β-hydroxyl group and the hydrophobic steric contact of the condensed steroid ring.[1,2] It is not clear how the cholesterol side chain interacts with lipid molecules, although sterols containing shortened or lengthened side chains are less effective in modulating bilayer fluidity.[2] In this communication, we provide solid-state NMR evidence to indicate that the ω_4 (C_{22}-C_{23}-C_{24}-C_{25}) torsion angle of the cholesterol side chain depends on the chain length of the neighboring lysophosphatidylcholine (LPC) molecules.

Equimolar mixtures of LPC (16:0) and cholesterol are known to form lamellar structures in aqueous media as a consequence of some specific 1:1 LPC-cholesterol complex formation.[3-5] The ^{31}P NMR results shown in Figure 1A suggest a similar LPC-cholesterol complex formation for the four studied LPC/cholesterol

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