Acknowledgment. This work was supported in part by National Science Foundation Grant DMR-8719302 and the Committee on Research, UCB. We also thank Dr. Fred Hollander for solving the crystal structure of **4b**.

Supplementary Material Available: Tables of atomic coordinates, intramolecular distances, intramolecular angles, torsion angles, anisotropic thermal parameters, and root-mean-square amplitudes of anisotropic displacement and a stereo figure for **4b** (17 pages). Ordering information is given on any current masthead page.

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Nitrous Acid Cross-Links Duplex DNA Fragments through Deoxyguanosine Residues at the Sequence 5′-CG

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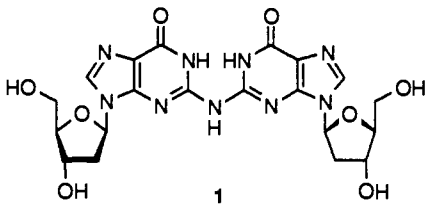
Defining the impact of nitrates, nitrites, and *N*-nitroso compounds on human health is important, given dietary and environmental exposure to these substances.[1,2] Sodium nitrite, for example, is a common additive to cured meats, contributing to their characteristic color and flavor, as well as protecting consumers against botulism.[1-3] Of several mechanisms suggested to account for the in vitro mutagenicity of nitrous acid,[4] one involves the creation of DNA interstrand cross-links.[5] Thus, in addition to providing insights into the chemical reactivity of duplex DNA, this reaction may be of biochemical significance. We report herein that treatment of duplex DNA fragments with nitrous acid forms thermally stable and base-stable interstrand cross-links preferentially through deoxyguanosine residues at the nucleotide sequences 5′-CG and 5′-GC, with a preference for the former.

Compound **1** is isolated on enzymatic hydrolysis of nitrous acid treated DNA and is a candidate for the nucleus of heat- and base-stable cross-links.[6] This suggests that spatially proximal



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deoxyguanosine residues on opposite strands might be cross-linked. To test this prediction, seven radiolabeled DNA duplexes[7] I–VII, 5′-d[AATATAAT(N_4)ATTAT], N_4 = AGCT (I), ACGT (II), TGCA (III), TCGA (IV), GGCC (V), CCGG (VI), and TATA (VII), in pH 4.5 sodium acetate (0.3 M) at 25 °C were treated with 0.5 M sodium nitrite for 1.5 h. DNA was isolated by ethanol precipitation and was evaluated by denaturing polyacrylamide gel electrophoresis (DPAGE). All experiments returned predominantly single stranded (high-mobility) DNA. The yields (Cerenkov counting) of the least mobile,[9] interstrand cross-linked products were as follows: AGCT (I, 3.2%), CCGG (VI, 2.4%) > ACGT (II, 0.76%), TCGA (IV, 0.62%) > TGCA (III, 0.16%), GGCC (V, 0.09%) ≫ TATA (VII, <0.05%).

The cross-linked DNAs ACGT (II), TGCA (III), TCGA (IV), GGCC (V), and CCGG (VI) were stable to base (1 M aqueous piperidine, 90 °C, 0.5 h). For cross-linked DNAs ACGT (II), TCGA (IV), and CCGG (VI), analyses of the cross-link position at nucleotide resolution[8,10-12] revealed a single dG to dG cross-link at the central 5′-CG sequence (for example, Figure 1), consistent with **1** as the nucleus of the cross-links. Similar analyses of cross-linked TGCA (III) and GGCC (V) indicated heterogeneity of cross-link position,[8] but in both cases linkage was predominantly dG to dG at 5′-GC. In contrast, the cross-link formed by the most efficiently cross-linked DNA, AGCT (I), was thermally labile (H_2O, 90 °C), reverting under these conditions predominantly to single strands (gel mobility assay).[13] It is thus likely that this cross-link is structurally distinct and its formation mechanistically distinct from those at the other 5′-CG and 5′-GC sequences.

Among those DNAs that form thermally stable and base-stable dG to dG linkages (as in **1**), there exists a preference for cross-linking at the nucleotide sequence 5′-CG relative to 5′-GC [4-fold in TCGA (IV) vs TGCA (III); 25-fold in CCGG (VI) vs GGCC (V)]. The mechanistic origin of this preference is unknown, but differences in ground-state DNA structure may be relevant. A plausible mechanism for cross-linking involves sequential diazotization of N2 of one dG residue, nucleophilic attack by N2 of the neighboring dG residue on C2 of the diazotized residue, and loss of N_2.[6] The sequence 5′-CG in the B conformation[14] is structurally matched to the addition step (Figure 2), with the putative reactive centers being in van der Waals contact. The isomeric sequence 5′-GC requires a 3-Å sliding of the base pairs relative to one another to bring these reactive centers into contact. Achievement of the strand-linking transition state with only slight conformational reorganization at the sequence 5′-CG might correspond to a lower transition-state energy. This is obviously not the whole story, however, given flanking sequence effects on cross-linking efficiency.[15]

Nitrous acid thus cross-links deoxyguanosine residues in duplex DNA with a preference for the sequence 5′-CG, supporting **1** as the nucleus of nitrous acid promoted, heat- and base-stable DNA interstrand cross-links. Knowledge of this sequence preference

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(7) DNA was synthesized, purified, and radiolabeled as previously described.[8]

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(9) All DNAs tested returned several interstrand cross-linked products (distinct DPAGE bands); only the least mobile band ever exceeded 0.1% yield. DPAGE mobility of cross-linked DNA is a function of cross-link position, with cross-links farthest from the duplex termini having lowest mobility.[10]

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(13) A byproduct of reversion to single strands has DPAGE mobility consistent with scission at the bold T of AGCT (I), suggesting partial cross-linking dG to dT at 5′-AG.

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(15) Other explanations, including preferential diazotization at 5′-CG or preferential hydrolysis of a diazotized dG at 5′-GC, are currently under investigation.

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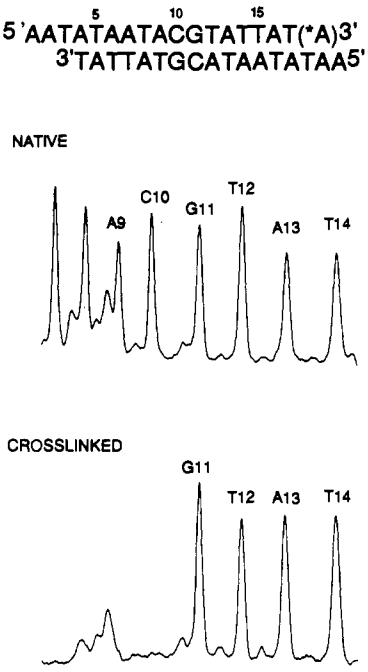

Figure 1. Partial fragmentation patterns (iron(II)/EDTA/ascorbic acid/H_2O_2) for radiolabeled (* ≈ ^{32}P) native and interstrand cross-linked 5'-d(AATATAATACGTATTAT*A). Lettering indicates residue cleaved.

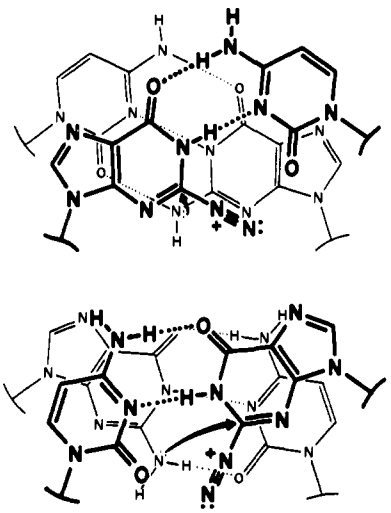

Figure 2. View down the helix axis at the duplex DNA sequences 5'-CG (upper) and 5'-GC (lower) in the B conformation. In each case, one exocyclic amino group of a deoxyguanosine residue has been replaced by a diazonium group; the bold arrows indicate the nucleophilic addition reactions required for cross-linking 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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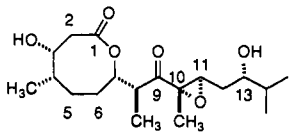
Although marine plants and invertebrates have been the subject of extensive chemical investigations,[1] studies of marine microorganims are rare.[2] This lack of attention is surprising given the central role soil bacteria play in the development of clinically important pharmaceutical agents and the 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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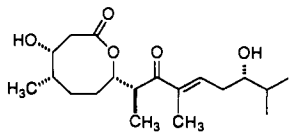

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Ethyl acetate extracts of the culture broth of *Streptomyces* sp., isolate PG-19,[9] showed significant in vitro cytotoxicity toward B-16-F10 murine melanoma and HCT-116 human colon tumor

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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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