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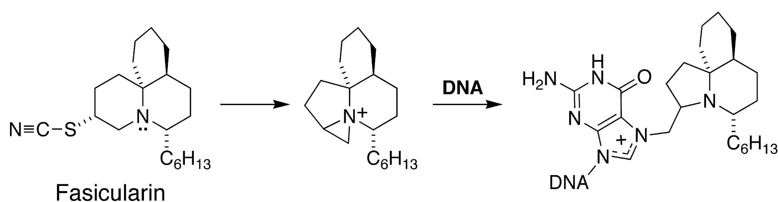

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DNA Damage by Fasicularin

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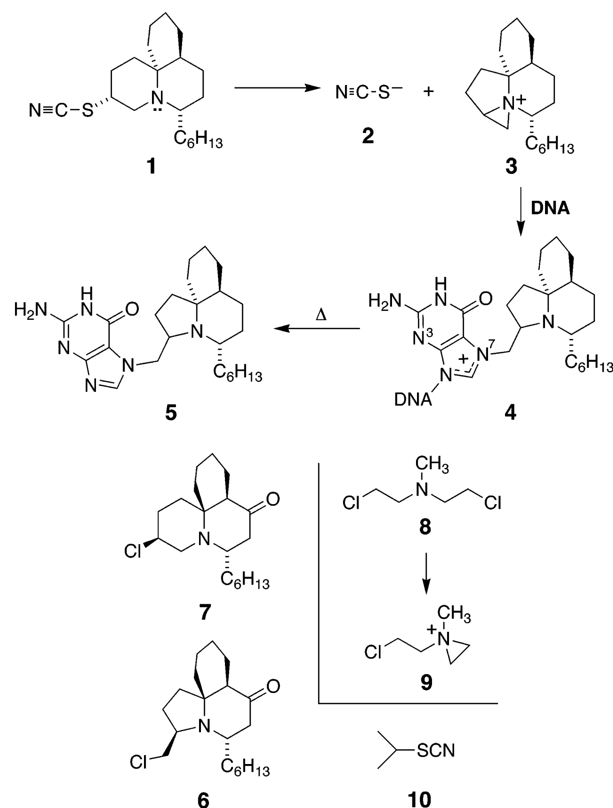
Fasicularin (**1**, Scheme 1) is a thiocyanate-containing alkaloid isolated from the ascidian (sea squirt) *Nephteis fasicularis*.[1] This natural product displays cytotoxic properties (IC_{50} of 14 μg/mL against Vero cells),[1] and experiments showing that a DNA repair-deficient cell line is hypersensitive to fasicularin suggest that an ability to damage DNA underlies the biological activity of this agent.[1] Along these lines, it is noteworthy that natural products structurally related to fasicularin, cylindricines A and B (**6** and **7**, respectively), undergo interconversion via a putative aziridinium ion intermediate.[2] Aziridinium ions are well-known DNA-alkylating species.[3] Accordingly, we set out to investigate whether the biological properties of fasicularin might stem from the ability of this natural product to generate a DNA-alkylating aziridinium ion (**3**) via intramolecular displacement of the thiocyanate group, as shown in Scheme 1.

In this work, we utilized a small sample (<1 mg) of synthetic (±)-fasicularin prepared as described previously.[4] Treatment of a 5′-^{32}P-labeled DNA duplex with fasicularin in pH 7.0 aqueous buffer, followed by Maxam−Gilbert workup (200 mM piperidine, 90 °C, 30 min)[5] and sequencing gel analysis, reveals selective strand cleavage at guanine residues. Alkylation at the N7-position of guanine residues in DNA yields this type of base-labile strand scission.[5−7] The N7-atom of guanine residues is the most nucleophilic site in DNA and, as such, is a common alkylation site for a variety of electrophilic species, including aziridinium ions.[3,7−11,12]

While the piperidine-labile cleavage at guanine residues seen in Figure 1 is consistent with a mechanism involving *alkylation* of DNA by the aziridinium ion **3**, it is important to consider alternative mechanisms that could potentially give rise to this type of DNA damage. For example, *oxidative* damage at guanine residues can also generate base-labile lesions.[13,14] In the context of our studies related to fasicularin, it is especially relevant to point out that thiocyanate-derived radicals can cause guanine-specific oxidative DNA damage.[15,16] Therefore, we felt it was important to examine whether thiocyanate radicals resulting from autoxidation of the thiocyanate anion (**2**, Scheme 1) could be responsible for the guanine-specific DNA damage caused by fasicularin. In addition, when considering alternative mechanisms for fasicularin-mediated DNA damage, it is worth recognizing that simple organic thiocyanates can alkylate nitrogen nucleophiles.[17] Therefore, we also designed experiments to shed light on whether the secondary thiocyanate functional group of fasicularin might alkylate DNA directly, rather than via the aziridinium ion **3**.

Accordingly, a ^{32}P-labeled DNA fragment was treated with NaSCN (**2**, 800 μM−10 mM) or isopropyl thiocyanate (**10**, 800 μM−10 mM) under reaction conditions identical to those utilized for the experiments with fasicularin. Maxam−Gilbert workup of the DNA, followed by sequencing gel analysis, shows that neither of these agents generates significant levels of base-labile strand cleavage at guanine residues (or any other sites). These results suggest that neither the thiocyanate anion (**2**) nor direct alkylation by the organic thiocyanate functional group is responsible for the DNA damage caused by fasicularin.

Scheme 1



We next sought direct evidence for the formation of the expected fasicularin−DNA adduct **4** (Scheme 1). Toward this end, mixed-sequence duplex DNA was treated with fasicularin, followed by thermal workup to release alkylated bases. The mixture was filtered to remove large DNA fragments and analyzed by LC-ESI/MS, where the mass spectrometer was operated in the selected-ion(+) monitoring mode. A single compound whose mass corresponds to the fasicularin−guanine adduct (**5**, $[M + H]^+$ at m/z 427.2; calcd 427.3) elutes at 35 min from a C18 reverse-phase column employing a gradient of 20−90% acetonitrile in 0.5% aqueous formic acid (see Supporting Information for details). Further MS/MS analysis of the 35 min peak (Figure 2) reveals that collision-induced dissociation of the parent ion produces the neutral loss of guanine (m/z 427→276) and the fasicularin fragment (m/z 427→152), similar to the fragmentations seen previously for other N7-alkylguanine adducts.[18,19] In addition, the small peak at m/z 410, corresponding to loss of the exocyclic amine group from the fasicularin−guanine adduct, is typical of N7-alkylguanine adducts.[20,21] Finally, the

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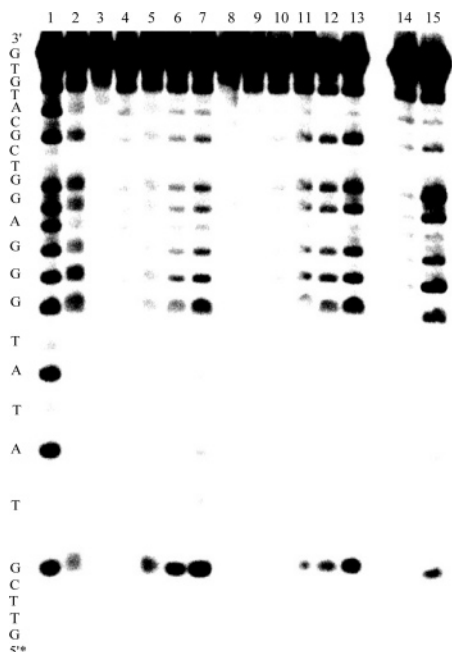

Figure 1. DNA damage by fasicularin (**1**). The 5′-^{32}P-labeled oligonucleotide 5′-GTTCGTATATGGGAGGTCGCATGTG-3′ was treated with **1** at concentrations comparable to its IC_{50} value. Reactions were conducted in 50 mM MOPS buffer, pH 7.0, containing 30% acetonitrile at 37 °C for 48 h, followed by Maxam−Gilbert workup and analysis using 20% denaturing polyacrylamide gel as described previously.[11] Lane 1, A+G sequencing reaction; lane 2, G sequencing reaction. Lanes 3−7 employed duplex DNA, where the underlined region of the oligonucleotide shown above is double-helical: lane 3, untreated duplex; lane 4, duplex with Maxam−Gilbert workup (no fasicularin); lanes 5−7, duplex treated with **1** (400 μM, 800 μM, 2 mM), followed by Maxam−Gilbert workup. Lanes 8−13 employed single-strand oligonucleotide: lane 8, untreated oligonucleotide; lane 9, oligonucleotide with Maxam−Gilbert workup; lanes 10−13, oligonucleotide treated with **1** (40, 100, 400, and 800 μM), followed by Maxam−Gilbert workup; lane 14, duplex with Maxam−Gilbert workup; lane 15, duplex treated with 40 μM **8**, followed by Maxam−Gilbert workup. Reactions analyzed in lanes 14 and 15 were conducted in 50 mM MOPS buffer, pH 7.0, containing 10% methanol at 37 °C for 2 h.

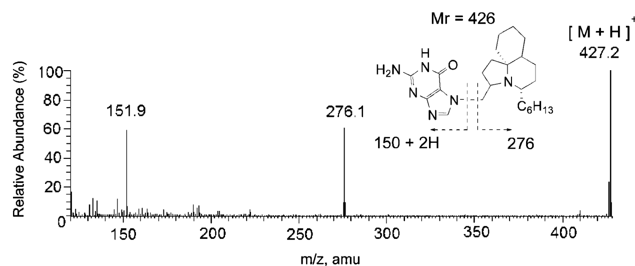

Figure 2. Mass spectrum of the fasicularin−guanine adduct **5** obtained by LC/MS/MS of *m*/*z* 427 (M + H).

proposed regiochemistry for attack of guanine on the fasicularin-derived aziridinium ion follows literature precedents regarding the preferred site of nucleophilic attack on structurally analogous aziridinium ions.[22]

In conclusion, it is well known that alkylation of guanine residues in cellular DNA can yield potent biological effects, including cytotoxicity.[7,23,24] Thus, the DNA-alkylating properties reported here offer a reasonable chemical basis for fasicularin's biological activity. Finally, this work presents fasicularin as the first natural product found to generate a DNA-alkylating aziridinium ion via a chemical mechanism analogous to the clinically used anticancer drugs mechlorethamine (**8**), melphalan, and chlorambucil.[3,23,25] Further studies of fasicularin may reveal useful insights regarding Nature's strategies for the delivery of aziridinium ions to cellular DNA.

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Supporting Information Available: Experimental procedures for polyacrylamide gel electrophoresis and LC/MS analysis of DNA damage by fasicularin. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Interestingly, **1** appears to represent the first case in which an aziridinium ion has been generated via intramolecular displacement of a thiocyanate group under physiologically relevant conditions. The thiocyanate leaving group (pK_a of HSCN = −1.9) may provide an appropriate balance of stability and reactivity in this type of system. Regarding the leaving group ability of the thiocyanate group, see ref 17 and Rappoport, Z.; Avramovitch, B.; Karni, M.; Apeloig, Y. *Isr. J. Chem.* **1989**, *29*, 267−279.

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