

DNA Damage by Fascicularin

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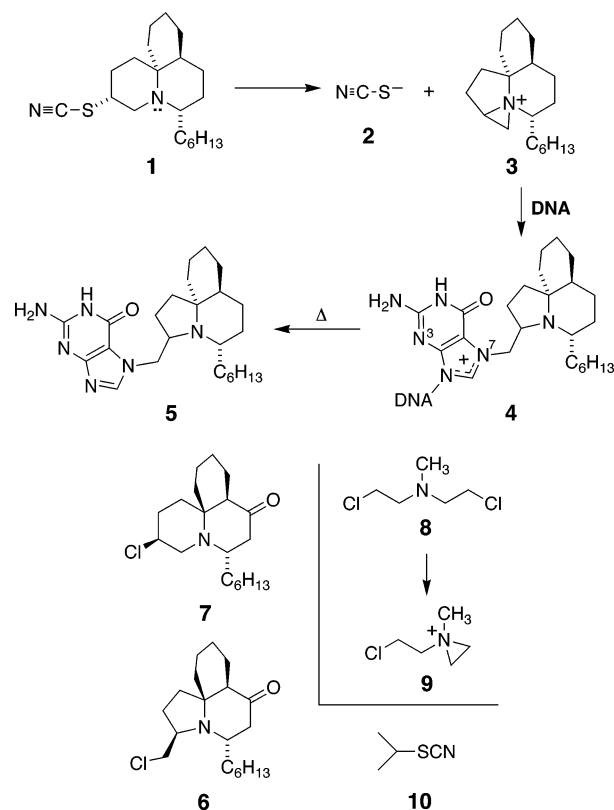
Fascicularin (**1**, Scheme 1) is a thiocyanate-containing alkaloid isolated from the ascidian (sea squirt) *Nephteis fascicularis*.¹ This natural product displays cytotoxic properties (IC₅₀ of 14 μg/mL against Vero cells),¹ and experiments showing that a DNA repair-deficient cell line is hypersensitive to fascicularin 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 fascicularin, 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 fascicularin 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 (±)-fascicularin prepared as described previously.⁴ Treatment of a 5'-³²P-labeled DNA duplex with fascicularin 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 fascicularin, 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 fascicularin. In addition, when considering alternative mechanisms for fascicularin-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 fascicularin might alkylate DNA directly, rather than via the aziridinium ion **3**.

Accordingly, a ³²P-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 fascicularin. Maxam–Gilbert workup of the DNA, followed by sequencing gel analysis, shows that neither of these agents generates significant levels of base-labile strand

Scheme 1



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 fascicularin.

We next sought direct evidence for the formation of the expected fascicularin–DNA adduct (Scheme 1). Toward this end, mixed-sequence duplex DNA was treated with fascicularin, 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 fascicularin–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 fascicularin 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 fascicularin–guanine adduct, is typical of N7-alkylguanine adducts.^{20,21} Finally, the

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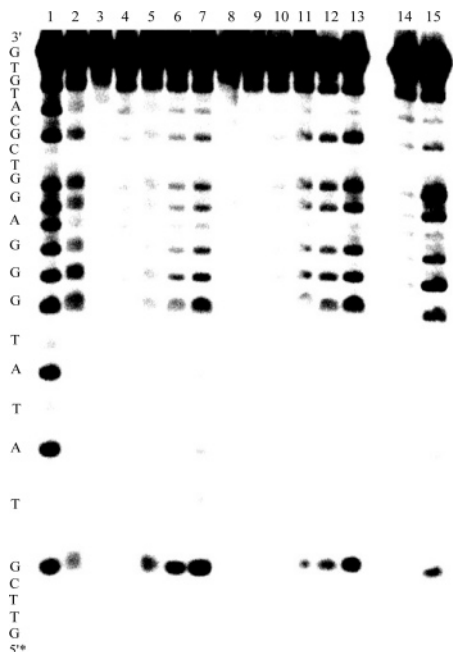


Figure 1. DNA damage by fascicularin (**1**). The 5'-³²P-labeled oligonucleotide 5'-GTTTCGTATATGGGAGGTCGCATGTG-3' was treated with **1** at concentrations comparable to its IC₅₀ value. Reactions were conducted in 50 mM MOPS buffer, pH 7.0, containing 30% acetonitrile at 37 °C for 48 h, followed by Maxam–Gilbert 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 fascicularin); 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.

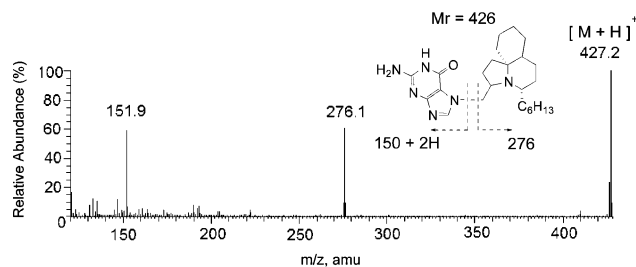


Figure 2. Mass spectrum of the fascicularin–guanine adduct **5** obtained by LC/MS/MS of m/z 427 ($M + H$).

proposed regiochemistry for attack of guanine on the fascicularin-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 fascicularin's biological activity. Finally, this work presents fascicularin 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 fascicularin 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 fascicularin. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Patil, A. D.; Freyer, A. J.; Reichwein, R.; Carte, B.; Killmer, L. B.; Faucette, L.; Johnson, R. K.; Faulkner, D. J. *Tetrahedron Lett.* **1997**, *38*, 363–364.
- (2) Blackman, A. J.; Li, C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *Tetrahedron* **1993**, *49*, 8645–8656.
- (3) Povirk, L. F.; Shuker, D. E. *Mutation Res.* **1994**, *318*, 205–226.
- (4) (a) Abe, H.; Aoyagi, S.; Kibayashi, C. *J. Am. Chem. Soc.* **2000**, *122*, 4583–4592. (b) For a recent synthesis of (–)-fascicularin via an aziridinium ion intermediate, see: Abe, H.; Aoyagi, S.; Kibayashi, C. *J. Am. Chem. Soc.* **2005**, *127*, 1473–1480.
- (5) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499–560.
- (6) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. *Biochim. Biophys. Acta* **1986**, *868*, 71–76.
- (7) Gates, K. S.; Nooner, T.; Dutta, S. *Chem. Res. Toxicol.* **2004**, *17*, 839–856.
- (8) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289–380.
- (9) Beranek, D. T. *Mutation Res.* **1990**, *231*, 11–30.
- (10) Lawley, P. D.; Phillips, D. H. *Mutation Res.* **1996**, *355*, 13–40.
- (11) Zang, H.; Gates, K. S. *Chem. Res. Toxicol.* **2003**, *16*, 1539–1546.
- (12) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. *Nucleic Acids Res.* **1986**, *14*, 2971–2987.
- (13) Muller, J. G.; Duarte, V.; Hickerson, R. P.; Burrows, C. J. *Nucleic Acids Res.* **1998**, *26*, 2247–2249.
- (14) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1151.
- (15) Milligan, J. R.; Aguilera, J. A.; Paglinawan, R. A.; Ward, J. F. *Int. J. Radiat. Biol.* **2000**, *76*, 1305–1314.
- (16) Milligan, J. R.; Tran, N. Q.; Ly, A.; Ward, J. F. *Biochemistry* **2004**, *43*, 5102–5108.
- (17) Oae, S.; Yamada, N.; Fujimori, K.; Kikuchi, O. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 248–256.
- (18) Balcome, S.; Park, S.; Quirk Dorr, D. R.; Hafner, L.; Phillips, L.; Tretyakova, N. *Chem. Res. Toxicol.* **2004**, *17*, 950–962.
- (19) Liao, P.-C.; Li, C.-M.; Hung, C.-W.; Chen, S.-H. *J. Mass. Spectrom.* **2001**, *36*, 336–343.
- (20) Chen, Y.-L.; Wang, C.-J.; Wu, K.-Y. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 893–898. Ferenc, G.; Kele, Z.; Kovacs, L. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 236–240. Catinella, S.; Rovatti, L.; Hamdan, M.; Porcelli, B.; Frosi, B.; Marinello, E. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 869–874.
- (21) Alkylation of the guanine residues at other sites such as O⁶, N1, and N3 has been considered here but deemed unlikely. Alkylation at O⁶-G and N1-G does not yield thermally labile adducts of the type observed here. In addition, N1 is not a primary site of reaction for any known alkylating agent. Alkylation at N3-G yields thermally labile adducts; however, N3-G is not a primary site of alkylation for any known agent. See ref 23 and Singer, B.; Grunberger, D. *Molecular Biology of Mutagens and Carcinogens*; Plenum: New York, 1983.
- (22) (a) Li, C.; Blackman, A. J. *Aust. J. Chem.* **1994**, *47*, 1355–1361. (b) Ikegami, S.; Uoji, K.; Akaboshi, S. *Tetrahedron* **1974**, *30*, 2077–2086. (c) Hammer, C. F.; Heller, S. R.; Craig, J. H. *Tetrahedron* **1972**, *28*, 239–253.
- (23) Gates, K. S. Covalent Modification of DNA by Natural Products. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Pergamon: New York, 1999; Vol. 7, pp 491–552.
- (24) Gates, K. S. *Chem. Res. Toxicol.* **2000**, *13*, 953–956.
- (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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