with about the first 10 carbons on the *sn*-2 acyl chain of phospholipids; these ten or so carbons are exposed to an environment in the enzyme that is very hydrophobic. Each methylene on the *sn*-2 acyl chain provides a binding energy of about 655 cal/mol. The hydrophobic interaction between the enzyme and the *sn*-2 chain is much more important for the monomeric inhibitors than for the micellar inhibitors. Binding of a monomeric substrate dissolved in water is more favorable the more hydrophobic (i.e., longer) its *sn*-2 fatty acid chain is, as this represents a net transfer from an aqueous environment to a hydrophobic one. In contrast, for similar substrates which are micellar, the hydrophobic *sn*-2 chain is transferred from the hydrophobic environment of a micelle to a similar (energetically) hydrophobic environment in the active site of the enzyme. The small, but significant, effect of the fatty acyl chain length on the interaction of the enzyme with the micellar substrate is not well understood. We are currently trying to model these interactions.

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Supplementary Material Available: Listings of NMR and mass spectra data (8 pages). Ordering information is given on any current masthead page.

Unmasking the Chemistry of DNA Cleavage by the Esperamicins: Modulation of 4′-Hydrogen Abstraction and Bistranded Damage by the Fucose–Anthranilate Moiety

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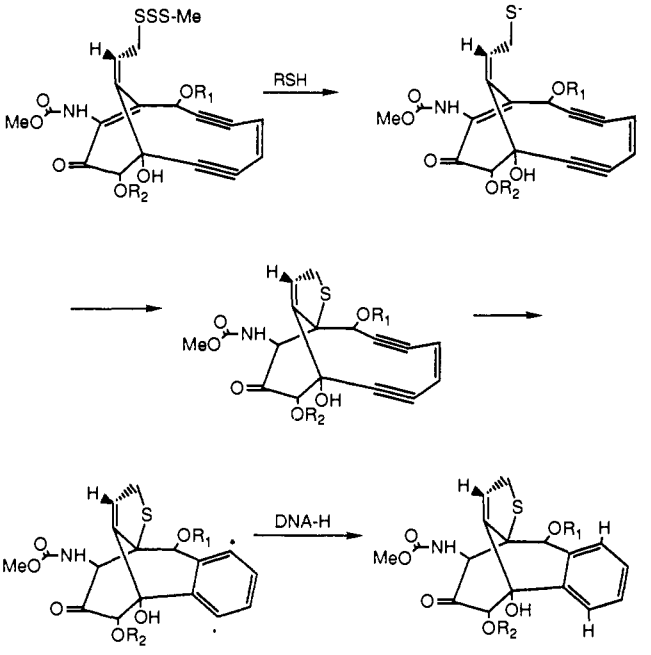
Abstract: The chemistry of DNA cleavage by the esperamicins A_1, C, D, and E (esp A, C–E) has been examined. High-resolution gel electrophoresis reveals that esp A, a known single-strand cleaver, affords fragmentation products consistent with exclusive 5′-hydrogen abstraction. In contrast, esp C–E, analogs that produce significant double-strand cleavage, generate fragmentation products consistent with both 5′- and 4′-hydrogen abstraction. On the basis of these observations and other findings reported for a related enediyne antibiotic, calicheamicin γ_1[1] (Townsend, C. A.; DeVoss, J. J.; Ding, W.-D.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669), we conclude that 4′-hydrogen abstraction and bistranded DNA cleavage are directly related to the reactivity of the C-7 radical as modulated by the fucosyl–anthranilate group.

The enediyne antibiotics, a potent class of antitumor compounds and DNA cleavers, represented by the esperamicins A_1, C, D, and E (esp A, C–E)[1] and by calicheamicin γ_1[12] (Figure 1), have been the subject of intense mechanistic and synthetic study.[3] A key mechanistic feature of the activation of these compounds toward DNA cleavage is a Bergman cyclization of the enediyne core,[4] triggered by an intramolecular conjugate addition of thiolate derived by reduction of the intrinsic trisulfide, to give the highly reactive bis radical 1,4-benzyne localized at C-7 and C-10 of the esperamicin core (Scheme I). The exquisite positioning of the benzyne moiety in the minor groove of DNA has been postulated to lead to double-strand scission by synchronous homolytic hydrogen abstractions from deoxyribose groups of nucleotide residues on opposing strands.[1-3]

The esperamicins and calicheamicins bear a number of structural similarities and differences (Figure 1). The bicyclo[7.3.1]tridecadiynediene ring system which constitutes the reactive core is identical for the two classes with the exception of the occurrence of a hydroxyl group at C-4 of esperamicin.[5] In the fully elaborated esp A, the C-4 hydroxyl group serves as the site of attachment of a fucosyl–anthranilate moiety. Esp C–E are derived from esp A by chemical hydrolysis and lack the fucosyl–anthranilate moiety at C-4. Esp D and E are derived by additional hydrolytic deletions from the oligosaccharide group appending C-12.[5] In the calicheamicins a single oligosaccharide

Scheme I. Mechanism of Activation of the Esperamicins by Reduction of the Trisulfide



array is attached to the core via the C-8 hydroxyl. Thus, esp C–E and the calicheamicins are most closely related, although structural

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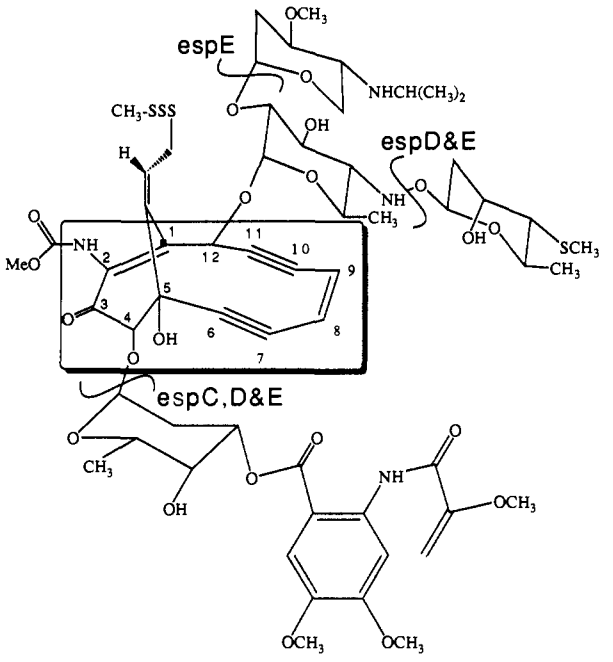
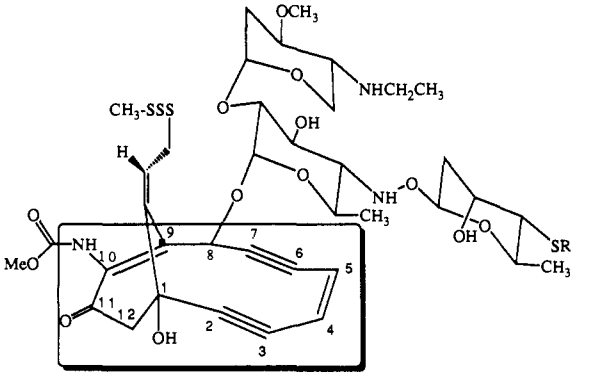
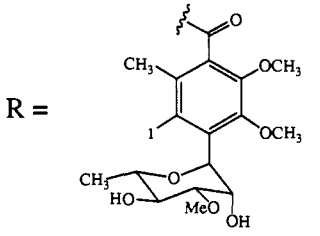


esperamicins A_1, C-E





calicheamicin γ_1^I

Figure 1. Structures of esp A and C-E and of calicheamicin γ_1^1. Carbohydrate deletions leading to esp C-E by chemical hydrolysis of esp A are noted. The enediyne cores are highlighted.

differences in the oligosaccharide groups are responsible for the greater sequence specificity of the calicheamicins.[6] The bilateral carbohydrate distribution of esp A suggests that this compound may constitute a special case with respect to the chemistry of DNA cleavage.

Recent studies suggest that esp A is indeed an exception. The cleavage of supercoiled plasmid DNA by esp A has been shown to occur predominantly via single-strand breaks.[1c,7] Cleavage by esp C-E, however, affords a substantial number of double-strand breaks, as does cleavage by the calicheamicins.[1c,2,7] The structural relationship of esp A to esp C-E suggested to us that the modulation of single- vs double-strand cleavage between the two groups of compounds might be directly related to a distinct chemical event on DNA that is affected by the removal of the fucosyl-anthranilate moiety. We here report results with esp A and esp C which suggest that the putative opposing drug radicals at C-10 and C-7 (Figure 1) effect distinct 5′- and 4′-hydrogen abstractions from opposing strands to initiate deoxynucleotide fragmentation. Moreover, the basis for the deficiency in double-strand cleavage with esp A is due to the selective suppression of 4′-hydrogen abstraction.

Experimental Section

General Procedures. All commercially available chemicals and reagents were of the highest quality available and were used without further purification. [γ-^{32}P]ATP (>5000 Ci/mmol) was purchased from Amersham, and restriction enzymes were obtained from Bethesda Research Laboratories. Esp C-E were prepared by chemical hydrolysis as previously described.[1b]

Preparation of DNA Fragments. The *Pst*I-*Bam*HI region of pBR322 was amplified from 1 ng of target DNA via the polymerase chain reaction (PCR) in a reaction containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM dGTP, dATP, dCTP, and dTTP, 1 μM each of two primers, and 2.5 units of *Taq* polymerase in 100 μL. The double-stranded product ($\sim$10 μg) was extracted,[8] purified, and washed using Centricon Microconcentrators (Amicon) to a final concentration of 0.5 μg/μL.

The double-stranded DNA (10 μg) was digested for 1 h at 37 °C with either 50 units of *Hin*dIII or 50 units of *Eco*RI in 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 10 mM $MgCl_2$. The *Hin*dIII-digested sample was used for 5′-^{32}P-end-labeling, and the *Eco*RI-digested sample was 3′-^{32}P-end-labeled.

In order for 5′-^{32}P-end-labeling to be carried out, the 5′-phosphate ends of the *Hin*dIII-digested DNA were hydrolyzed with 0.4 unit of calf intestine phosphatase (Boehringer) in 50 mM Tris (pH 9.0), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 1 mM spermidine at 37 °C for 1 h. The reaction was quenched by addition of 4 μL of 0.5 M EDTA, and the sample was heated at 65 °C for 10 min. The DNA was extracted and precipitated according to established procedures[8] and redissolved to a concentration of 1.0 μg/μL.

5′-^{32}P-End-labeling was accomplished with T_4 polynucleotide kinase. The reaction (45 μL) contained the phosphatase-treated DNA (7 μg) in 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, BSA (10 μg/μL), 20 units of T_4 kinase, and 180 μCi of [γ-^{32}P]ATP. The reaction was incubated at 37 °C for 1 h and subsequently terminated by addition of 4.0 M ammonium acetate (200 μL) and cold absolute ethanol (750 μL). After 20 min in a dry ice/acetone bath the sample was precipitated, washed, and lyophilized, and the pellet was redissolved in H_2O. The 782-bp *Hin*dIII-*Pst*I fragment was released from the labeled fragment by digestion with 10 units of *Pst*I in 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 100 mM NaCl at 37 °C for 1 h. The fragment was purified on a 5% disulfide cross-linked polyacrylamide gel.[9] Sequence verification was performed by the method of Maxam and Gilbert.[10]

The *Eco*RI-digested DNA was used for 3′-^{32}P-end-labeling. The reaction (30 μL) contained DNA (5 μg), 50 mM Tris-HCl (pH 8.0), 10

(1) (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461. (b) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Kriishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462. (c) Long, B. H.; Golik, J.; Forenza, S.; Ward, B.; Rehfuss, R.; Dabrowiak, J. C.; Catino, J. J.; Musial, S. T.; Brookshire, K. W.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2. (d) Sugiura, Y.; Uesawa, Y.; Takahashi, Y.; Kuwahara, J.; Golik, J.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7672.

(2) Lee, M. D.; Ellestad, G. A.; Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235 and references therein.

(3) Nicolaou, K. C.; Dai; W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387 and references therein.

(4) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25 and references therein.

(5) We have chosen to retain the separate numbering systems for the enediyne core of the esperamicins[1] and of the calicheamicins[2] derived by the original investigators. Thus, C-4 and C-12 of esperamicin, the sites of carbohydrate attachment, correspond to C-12 and C-8 of calicheamicin, respectively (see Figure 1).

(6) Walker, S.; Valentine, K. G.; Kahne, D. *J. Am. Chem. Soc.* **1990**, *112*, 6428.

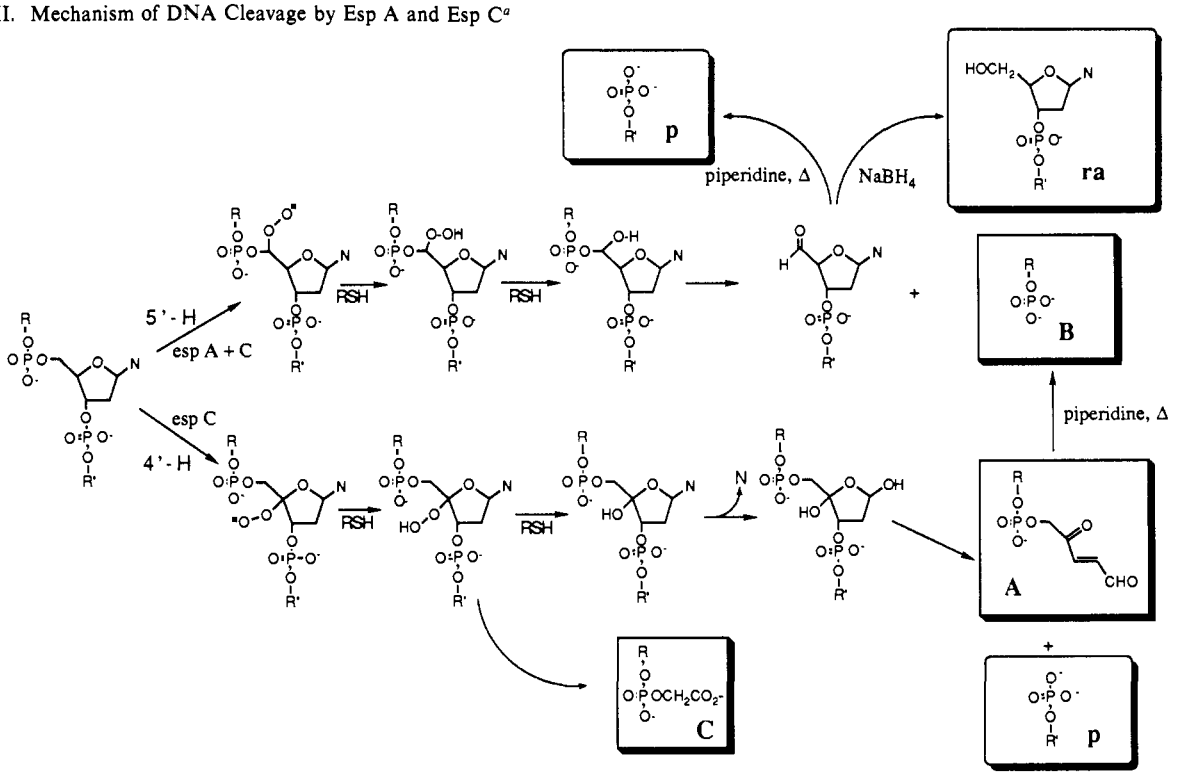
(7) Kishikawa, H.; Jiang, Y.-P.; Goodisman, J.; Dabrowiak, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 5434.

(8) (a) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463. (b) Messing, J. *Methods Enzymol.* **1983**, *101*, 20.

(9) Hensen, J. N. *Anal. Biochem.* **1981**, *116*, 146.

(10) (10) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.

Scheme II. Mechanism of DNA Cleavage by Esp A and Esp C[a]



[a] Rectangles highlight products observed by 5'-^{32}P-end-labeling (see Figure 2). Rounded rectangles highlight products observed by 3'-^{32}P-end-labeling (see Figure 3).

mM $MgCl_2$, 50 mM NaCl, 0.25 mM dGTP, dCTP, and dTTP, 90 μCi of [α-^{32}P]dATP, and 2.5 units of the Klenow fragment. The sample was held at 22 °C for 15 min and a second aliquot of the four nonradioactive deoxynucleotides added. The sample was held at 22 °C for an additional 15 min and then heated to 75 °C for 10 min. The 375-bp *Eco*RI–*Bam*HI fragment was released from the labeled fragment by digestion with 10 units of *Bam*HI added directly to the labeling mixture after cooling to room temperature. The liberated DNA was purified as described previously.

Reactions of DNA with Esp A, C–E. Esperamicin reactions contained ^{32}P-end-labeled DNA (~150000 cpm) treated with 2 μM esp A or C, 50 μM esp D, or 375 μM esp E in a solution containing 0.14 μg/μL salmon sperm DNA, 50 mM HEPES (pH 7.5), and 100 μM EDTA. The reaction was initiated by addition of 1 mM dithiothreitol and allowed to continue at room temperature for 7 min. The reaction was terminated by the addition of a "stop solution" (20 μL) containing 1 M sodium acetate and salmon sperm DNA (24 μg).

For 3'-end-labeled samples, the sample was divided into two equal portions and reacted with $NaBH_4$ or piperidine. $NaBH_4$ treatment (1 M, 100 μL) was for 30 min at 0 °C. The reaction was terminated by addition of 0.5 M sodium acetate in 1 M acetic acid (200 μL). The DNA was precipitated with absolute ethanol (175 μL) at −70 °C for 10 min and centrifuged at 14000 rpm for 30 min at 4 °C. The supernatant was removed, and the pellet was resuspended in 70% aqueous ethanol (1 mL), centrifuged at 14000 rpm for 10 min, and dried under vacuum. For alkali-treated samples, the dried pellet was dissolved in 1 M piperidine (100 μL) and heated to 90 °C for 30 min. After addition of 0.5 M sodium acetate in 1 M acetic acid (200 μL), the DNA was precipitated with absolute ethanol (750 μL) at −70 °C for 10 min, followed by centrifugation (14000 rpm) for 10 min. The pellet was resuspended, repelleted, and dried as described above.

Gel Electrophoresis of DNA Reaction Samples. The DNA pellet (untreated or $NaBH_4$- or alkali-treated) was dissolved in 95% formamide (4 μL) containing 10 mM sodium EDTA and 0.1% bromophenol blue (pH 9.1). After being heated at 90 °C for 1 min, the sample was immediately cooled on ice and loaded onto a 20% polyacrylamide slab gel (30:1 acrylamide/*N,N'*-methylenebis(acrylamide)) containing 7 M urea. The gel was subjected to electrophoresis (35 mA, 2700 V) in a buffer (pH 8.3) containing 100 mM Tris, 100 mM boric acid, and 2 mM sodium EDTA at 50 °C until the bromophenol blue dye reached the bottom of the gel (~3.5 h). The gel was dried on Whatman 3MM chromatography paper at 75 °C under vacuum for 45 min on a slab gel dryer. The dried gel was exposed to Kodak X-OMAT AR film for 40 h.

Results and Discussion

Analysis of Cleavage by Esp A and C of 5'-^{32}P-End-Labeled DNA. Our experience has been that the analysis of short DNA fragments by high-resolution gel electrophoresis is most informative for dissecting out specific cleavage chemistries.[11-13] We have, therefore, applied these methods to an analysis of the esperamicins. The analysis by high-resolution gel electrophoresis of the reaction of esp A and esp C with a the 5'-^{32}P-labeled *Hin*dIII–*Eco*RI restriction fragment (29 bp) of pBR322 is shown in Figure 2. DNA cleavage by esp A afforded exclusively fragments that were found to comigrate with Maxam–Gilbert fragments[10] (not shown) consistent with the formation of 3'-phosphate termini (B, lanes 1 and 2; see lesion at A_{19} and G_{22}, for example). Treatment with piperidine (lane 2), in an attempt to reveal any alkaline-labile sites, did not result in the appearance of additional lesions or in a difference in the relative intensities of the lesions observed under neutral conditions (lane 1). For esp C, in addition to 3'-phosphate termini (B), faster migrating bands diagnostic for 3'-phosphoglycolate termini (C) were also observed under neutral conditions (Figure 2, lane 3). At a number of the sites (see A_{19} and T_{15}, for example), slower moving bands (A) were also seen that decomposed upon treatment with piperidine to 3'-phosphate termini (B, lane 4). An increase in the amount of 3'-phosphate relative to 3'-phosphoglycolate upon piperidine treatment clearly assigns this conversion.

The results with 5'-^{32}P-end-labeled DNA suggest a single chemistry of cleavage for esp A. The complete formation of 3'-phosphate termini under neutral conditions is best explained by exclusive 5'-hydrogen abstraction, a point further investigated below. In contrast, a more complex chemistry was observed for esp C suggesting at least one additional mode of DNA cleavage. The formation of 3'-phosphoglycolate termini (C) and alkaline-

(11) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107 and references therein.
(12) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396.
(13) Frank, B. L.; Worth, L., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J.; Kappen, L. S.; Goldberg, I. H. *J. Am. Chem. Soc.* **1991**, *113*, 2271. Kappen, L. S.; Goldberg, I. H.; Frank, B. L.; Worth, L., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1991**, *30*, 2034.

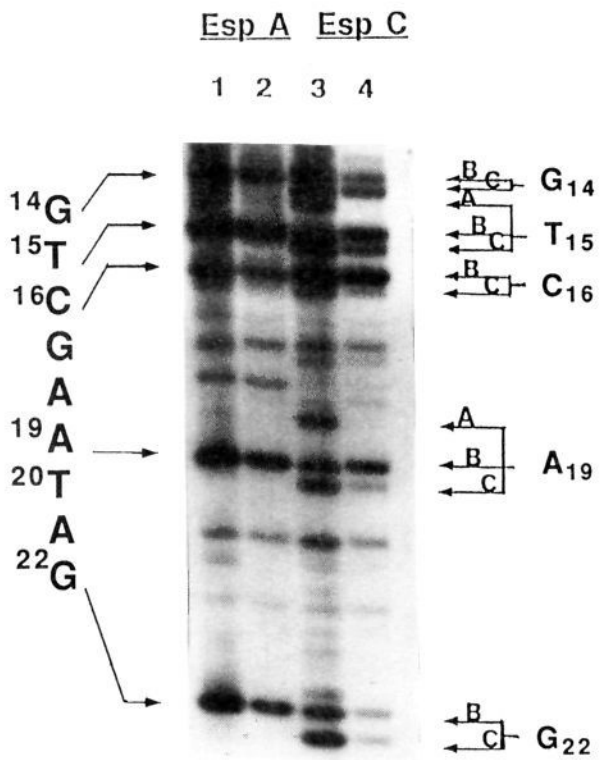


Figure 2. Autoradiogram of the reactions of esp A and esp C with 5′-^{32}P-end-labeled DNA. Reactions (25 μL) contained labeled DNA (~150 000 cpm; minus strand of *Hin*dIII–*Eco*RI fragment of pBR322) treated with 2 μM esp A or esp C. Samples were analyzed under neutral conditions (lanes 1 and 3) or treated with piperidine prior to analysis (lanes 2 and 4). Sample workup, polyacrylamide gel electrophoresis, and autoradiography were performed by established methods (see Experimental Section). Cleavage by esp A (lanes 1 and 2) revealed only 3′-phosphate ends (B) consistent with 5′-hydrogen abstraction. Cleavage by esp C (lanes 3 and 4) gave, in addition to 3′-phosphate ends (B), 3′-phosphoglycolate ends (C) and alkaline-labile moieties (A).

labile moieties (A) that convert to 3′-phosphate termini (B) is reminiscent of our extensive studies with Fe·bleomycin[11,12] and with neocarzinostatin.[13] In those cases 4′-hydrogen abstraction has been unambiguously established as the common source of both lesions. The lack of observation of 4′-chemistry in earlier work is best explained by the use of low-resolution gel electrophoresis and the relatively large fragment analyzed which minimized the separation of the two fragments, B and C.[1] Esp C, therefore, appears to effect both 5′- and 4′-hydrogen abstraction (Scheme II). Aside from this mechanistic difference, both esp A and esp C had essentially identical sequence specificities. Esp D and esp E afforded results comparable to those of esp C with the exception that higher concentrations of the two were required and the sequence specificities were slightly broadened (data not shown).

Analysis of Cleavage by Esp A and C of 3′-^{32}P-End-Labeled DNA. The abstraction of the 5′-hydrogen will result in the generation of fragments terminating in a 5′-nucleoside aldehyde moiety, in addition to the 3′-phosphate termini (Scheme II). The aldehyde-containing fragments have been extensively studied with neocarzinostatin and are diagnostic for 5′-hydrogen abstraction.[14] In order to investigate this point, a cleavage analysis of the 3′-^{32}P-labeled DNA fragment was performed (Figure 3). Stabilization of 5′-aldehyde-terminating fragments was effected by $NaBH_4$ reduction to the corresponding nucleoside. This results in the formation of fragments with considerably slower mobility (~2 bp) than the 5′-phosphate fragments generated by Maxam–Gilbert procedures.[10]

(14) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. Similar arguments have been made for 5′-hydrogen abstraction by calicheamicin (Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198).

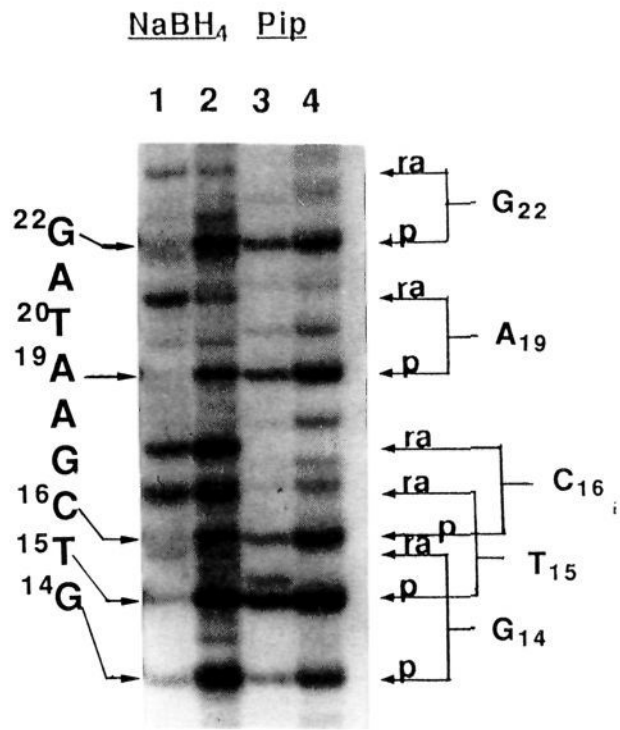


Figure 3. Autoradiogram of the reactions of esp A and esp C with 3′-^{32}P-end-labeled DNA. $NaBH_4$ treatment was performed on lanes 1 and 2 and piperidine treatment on lanes 3 and 4. Under $NaBH_4$ treatment esp A (lane 1) showed the reduced 5′-nucleoside aldehyde (ra) and esp C (lane 2) showed both the reduced 5′-nucleoside aldehyde and 5′-phosphate (p). After piperidine treatment, only 5′-phosphate ends (p) were evident for both drugs (lanes 3 and 4).

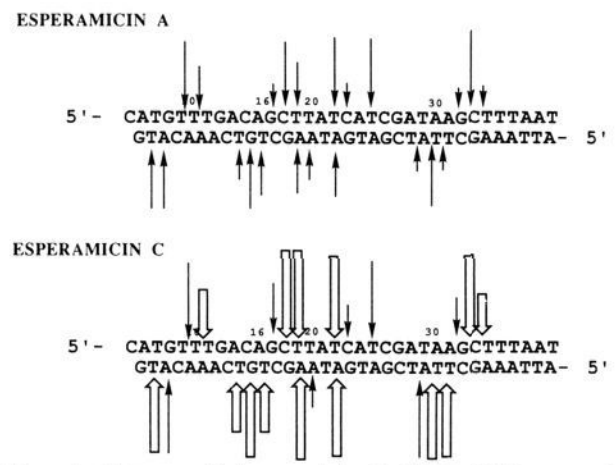
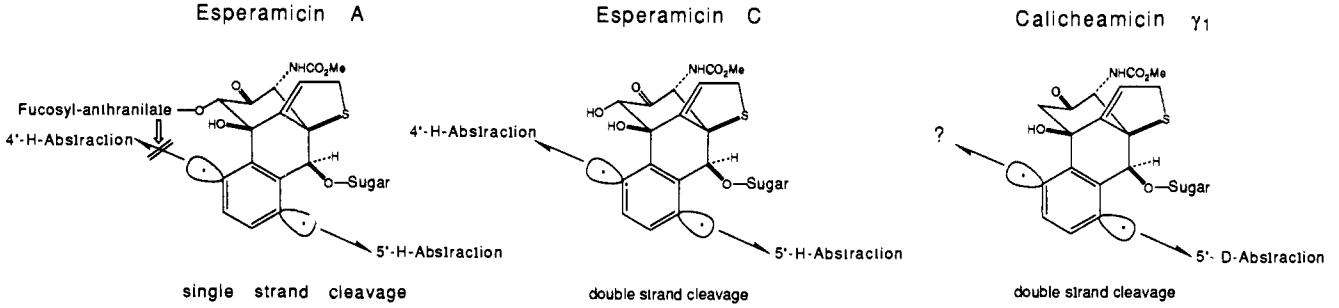


Figure 4. Histogram of cleavage of the *Eco*RI–*Hin*dIII fragment of pBR322 by esp A and esp C. Filled arrows indicate sites where only 5′-hydrogen abstraction occurs; open arrows indicate *both 4′- and 5′-abstraction.* Length of arrow approximates the relative extent of damage at each site.

For esp A, reduction afforded the expected 5′-nucleoside termini (Figure 3, lane 1; see ra for T_{15}, C_{16}, and A_{19}). Under these conditions, virtually no 5′-phosphate termini were detected (lane 1; see p). Treatment of an identical sample with piperidine instead of $NaBH_4$ resulted in the clean conversion of the 5′-aldehydes to the corresponding 5′-phosphate termini (lane 3; see p for T_{15}, C_{16}, and A_{19}). This conversion is consistent with a β-elimination from the reactive aldehyde liberating the 5′-phosphate (Scheme II). The findings strongly support the hypothesis that 5′-hydrogen abstraction is the major, and perhaps the exclusive, chemistry effected by esp A.

For esp C a more complex chemistry was again observed. Reduction with $NaBH_4$ afforded both 5′-nucleoside and 5′-

Scheme III. Proposed Regiochemistry of Hydrogen Abstraction by Esp A and C[a]



[a] Results for 5'-^{2}H transfer to calicheamicin have been reported.[18]

phosphate termini (Figure 3, lane 2; see ra and p for T_{15}, C_{16}, and A_{19}). The formation of 5'-phosphate termini under neutral conditions is known to be complementary to the production of 3'-phosphoglycolate termini generated by 4'-hydrogen abstraction (Scheme II). Treatment with piperidine converted, as expected, the 5'-aldehyde termini to 5'-phosphate termini (lane 4). These data corroborate the occurrence of both 5'- and 4'-hydrogen abstraction for esp C. Again, esp D and esp E gave results similar to those of esp C (data not shown).

Sequence-Specific Isotope Effects on the Cleavage of DNA by Esp A and Esp C. The isotope effects on 5'- and 4'-hydrogen abstraction by esp A and esp C were determined by gel electrophoresis using DNA fragments containing [4'-^{2}H]- or [5',5'-$^2H_2$]thymidine. This technique has proven to be valuable in the mechanistic analysis of the bleomycins[12,15] and of neocarzinostatin.[13] A variety of sequence contexts for reactive thymidine residues were explored using both esp A and esp C. The data (not shown) revealed that within experimental error all isotope effects were not significantly different from 1 (±0.2). In addition, deuteriation at either position had no effect on the relative ratio of 5'- and 4'-chemistry for esp C.

While these findings are subject to multiple interpretations,[15] our previous experience with neocarzinostatin, another enediyne antibiotic, is relevant here. We found a variation in the observed deuterium isotope effects on both 4'-abstraction ($k_H/k_D \sim$ 2.4–5.5) and 5'-abstraction ($k_H/k_D \sim$ 1.0–2.6) from T residues located in GT steps.[13] Moreover, the results were consistent with an internal kinetic partitioning between the two sites of abstraction; that is, suppression of damage due to 4'-hydrogen abstraction by deuteriation at that position resulted in an enhancement of damage due to 5'-hydrogen abstraction. Molecular modeling studies have suggested that the C-6 radical in activated neocarzinostatin is nearly equidistant between the 4'- and 5'-hydrogens at these sites.[16] A recent report has also demonstrated that the relative partitioning is sensitive to the sequence context.[17] Thus, a partitioning between both cleavage pathways by the same drug radical from a common DNA·neocarzinostatin complex is likely.

The absence of isotope effects for esp C does not categorically rule out the possibility of a kinetic partitioning; however, other interpretations remain viable. In particular, the results are consistent with both hydrogen abstractions occurring through distinct binding modes and through distinct radical centers.

Interpretation. Recent observations on the cleavage of supercoiled DNA by esp A and esp C suggest the participation of two distinct radical centers. As mentioned above, esp A is a single-strand cleaver while esp C effects double-strand breaks.[1c,7] Simply interpreted, one radical center in esp A may be inhibited in abstracting a hydrogen from DNA, and the functional radical, therefore, effects 5'-hydrogen abstraction exclusively. Enhancement of double-strand cleavage by removal of the fucosyl–anthranilate moiety giving esp C suggests that this inhibition has been alleviated, revealing the distinctive 4'-chemistry effected by this radical center. If this is correct, then the occurrence of both 5'- and 4'-chemistry at a specific nucleotide is initiated by different drug radicals and requires that the binding of the esperamicins to the minor groove be bidirectional. An analysis of the cleavage on both strands of a DNA fragment by esp A and esp C is consistent with this interpretation (Figure 4). For esp A, clusters of damage sites are observed on both strands displaced 3 bp to the 3'-terminus, supporting the bidirectionality of binding and cleavage of the single-strand cleaver. For esp C the sequence specificity on both strands remains unchanged but double-strand cleavage and 4'-chemistry at most of the cleavage sites are now observed. The bidirectionality of binding and the distinct chemistry of the opposing radical centers merely create an illusion of chemical partitioning at individual cleavage sites.

Recent studies on the regiochemistry of hydrogen abstraction by calicheamicin γ_1[1] permit an assignment of the regiochemistry by esp A and esp C. Using a model oligonucleotide containing [5',5'-$^2H_2$]dC, Townsend, Schreiber, and co-workers have demonstrated that the C-6 position is the exclusive site of deuteriation (Scheme III).[18] In light of the structural similarities of esp C and calicheamicin, 5'-hydrogen abstraction by the esperamicins is most reasonably due to the C-10 radical of the activated drug. No experiments have been thus far reported for the source of the C-3 hydrogen in the quenched drug although Zein and co-workers have implicated the 4'-hydrogen on the basis of modeling studies.[19] The 4'-hydrogen abstraction observed here for esp C–E and the related double-strand cleavage is thus consistent with the unmasking of the C-7 radical, a prediction that should also apply to the hydrogen abstraction effected by C-3 of calicheamicin.

Conclusions. We have demonstrated that 5'- and 4'-hydrogen abstraction are the major chemical events initiated by the esperamicins. The 4'-hydrogen abstraction and bistranded DNA cleavage by the esperamicins are directly affected by the fucosyl–anthranilate group. The mechanism of inhibition of 4'-chemistry by this moiety is unknown but may involve a mispositioning of the radical or an internal quenching of the radical. Experiments to further define this phenomenon and to test the regiochemical predictions implicit in this model are under investigation.

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(15) Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Absalon, M. J.; Stubbe, J.; Kozarich, J. W. Submitted for publication.
(16) Galat, A.; Goldberg, I. H. *Nucleic Acids Res.* **1990**, *18*, 2093.
(17) Sugiyama, H.; Fujiwara, T.; Kawabata, H.; Yoda, N.; Hirayama, N.; Saito, I. *J. Am. Chem. Soc.* **1992**, *114*, 5573.
(18) Townsend, C. A.; DeVoss, J. J.; Ding, W.-D.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669.
(19) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697. A footnote in this reference suggests that 3'-phosphoglycolate ends have been observed for calicheamicin, consistent with 4'-chemistry; however, this point has not been elaborated.