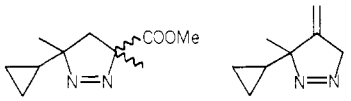
the basis of $R = 0.020$. Because thermolysis of **3** at 205 °C yielded exclusively **4**, the absence of **3** in the thermolysis of CPDBO is not surprising. As seen in Scheme I, it is possible that cyclopropylcarbinyl rearrangement occurs not in **1** but in diazenyl radical **7**, meaning that the 0.9-ns estimated singlet lifetime and the 28-ns triplet lifetime apply to **7**.

Whatever the detailed mechanism might be, the present results are unique, for Sanjiki and Ohta[15] were unable to observe cyclopropylcarbinyl rearrangement products from **9** or **10** under any

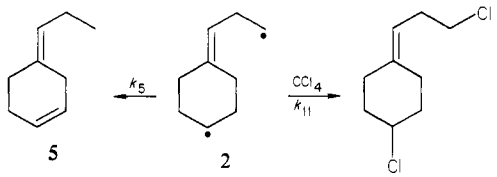


9 10

conditions tried. Thermolysis of two related azoalkanes[16,17] gave only small amounts of such products. On the other hand, cyclopropylcarbinyl rearrangement are common in the photochemistry of ketones[18] where the biradicals live for 30-100 ns.

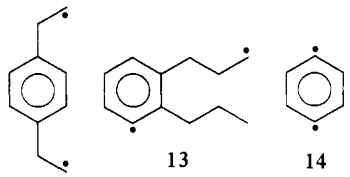
The formation of **5** from biradical **2** is a fascinating reaction because it represents disproportionation over a distance of at least 3 Å. The closest analogy of which we are aware is intramolecular hydrogen transfer over 2.25 Å.[19] Although we entertained the possibility that dienes **5** and **6** formed intermolecularly, this mechanism ought to produce them in more nearly equal amounts, rather than a predominance of **5**. An intermolecular pathway remains appealing in the case of **6** for obvious geometric reasons.

The observation that **2** undergoes such an unfavorable-looking reaction suggested to us that its lifetime might be relatively long. When the benzophenone-sensitized irradiation of CPDBO was carried out in CCl_4, the GC trace revealed about a dozen products, one of which was three times larger than the second most abundant product. Mass spectral analysis showed that it was a dichlorinated hydrocarbon, whose structure (**11**) is strongly supported by



5 2 11

NMR.[20] Although some **5** was formed in Ph_2CO/CCl_4, the absence of **6** in this radical scavenging solvent verifies the above suggestion that **6** is an intermolecular product. Because CCl_4 is not a very efficient radical trapping agent, it seems that **2** is indeed long lived. The trapping rate[21] is $k_{11}[CCl_4] = 1.3 \times 10^3 \times 10.4 = 1.4 \times 10^4\ s^{-1}$. The ratio **11**/**5** was 6.32, which allows us to calculate from the above mechanism that k_5 is $2.2 \times 10^3\ s^{-1}$ and that τ in the absence of a trapping agent is 0.45 ms. It must be emphasized that this very long lifetime is correct only if **11** derives exclusively from **2**; in fact, other possible sources of **11** are under consideration. The closest literature analogies for the trapping of **2** by CCl_4 are the reaction of **12** with dienes,[22] hydrogen donation from 1,4-cyclohexadiene to **13**,[23] and formation of *p*-dichlorobenzene from **14** and CCl_4.[24] Only a few other examples of biradical trapping have appeared.[11,18,25]



12 13 14

In summary, we can rationalize our data if azoalkane CPDBO decomposes to biradical **1** or **7**, which upon cyclopropylcarbinyl rearrangement, ultimately gives **2**. The latter biradical undergoes predominant intramolecular disproportionation over the extraordinarily long distance of 3 Å. Triplet-sensitized photolysis of CPDBO in CCl_4 allows trapping of biradical **2** as the dichloroalkene **11**.

Acknowledgment. We thank the National Science Foundation and the Robert A. Welch Foundation for financial support. Valuable criticism from Professor Peter B. Dervan is gratefully acknowledged.

Registry No. **1**, 83615-84-3; **2**, 83615-85-4; **3**, 83615-86-5; **4**, 83615-87-6; **5**, 83615-88-7; **6**, 83632-67-1; **7**, 83615-89-8; **11**, 83615-90-1; CCl_4, 56-23-5.

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(20) **11**: *m/e* (rel abundance) 41 (52.9), 79 (100.0), 107 (55.0), 143 (4.24), 145 (1.36), 192 (1.45), 194 (0.79), 196 (0.22); NMR (90 MHz) δ 1.64-2.72 (m, 10 H), 3.49 (t, 2 H), 4.04-4.36 (m, 1 H), 5.16 (t, 1 H). The 500-MHz ^1H NMR spectrum with decoupling experiments also supports the structure of **11**. We thank Professor Peter B. Dervan for carrying out this work at the Southern California Regional NMR facility.
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Design and Synthesis of a Sequence-Specific DNA Cleaving Molecule. (Distamycin-EDTA)iron(II)

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The sequence-dependent recognition of nucleic acids by proteins and small molecules is important in the regulation of many biological processes. A large class of these molecules are bifunctional in nature, combining a chemically reactive moiety with a DNA binding unit. One such molecule is the naturally occurring antitumor antibiotic bleomycin, which binds to and cleaves DNA sequence specifically in a reaction that depends on Fe(II) and oxygen.[4] Recently we reported the synthesis of a DNA binding-DNA cleaving molecule, methidiumpropyl-EDTA (MPE).[5] This bifunctional molecule has the DNA intercalator, methidium, tethered to a metal chelator, EDTA. MPE·Fe(II) cleaves double helical DNA in the presence of dithiothreitol (DTT) with efficiencies comparable to those of bleomycin·Fe(II)/DTT. Unlike bleomycin·Fe(II), MPE·Fe(II) cleaves DNA non-sequence-specifically,[6] consistent with solution studies demonstrating that the intercalator methidium has no overall base composition specificity.[7]

(1) National Science Foundation Predoctoral Fellow, 1979-1982.
(2) Supported by Fellowship DRG-526 of the Damon Runyon-Walter Winchell Cancer Fund.
(3) Camille and Henry Dreyfus Teacher Scholar, 1978-1983.
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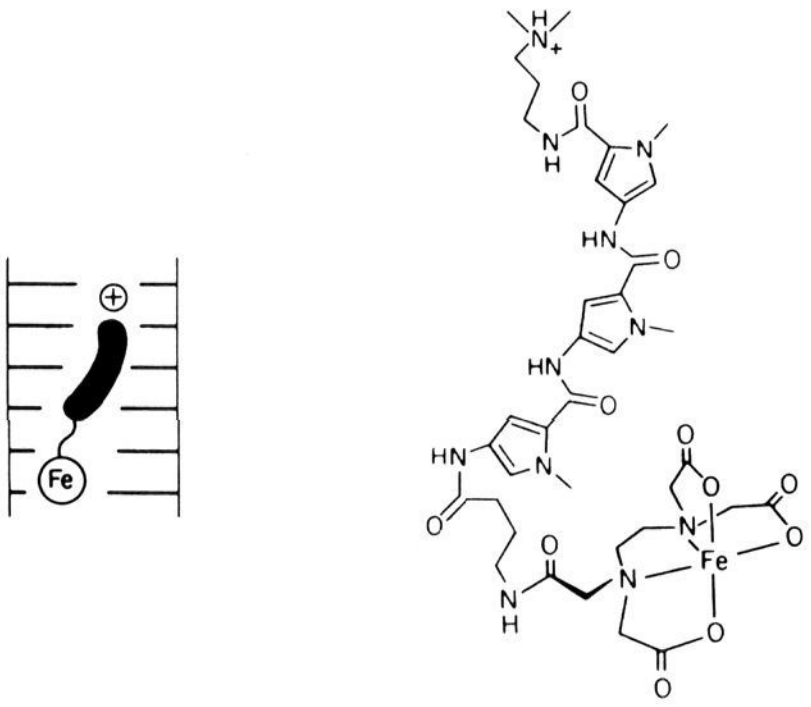
Table I. Cleavage of pBR-322 Plasmid in the Presence of DTT[a]

reagent	concn, M	form, % I	II	III
EDTA·Fe(II)	10^{-6}	94	6	0
DE–Fe(II)	10^{-6}	31	69	0
MPE·Fe(II)	10^{-7}	0	98	2
belomycin·Fe(II)	10^{-7}	0	43	57

[a] Form I pBR-322 (10^{-5} M bp), reagent and buffer (10 mM Tris·HCl, 50 mM NaCl, pH 7.4), and dithiothreitol (1 mM) were allowed to react at 25 °C for 30 min. Forms I–III were analyzed with agarose gel electrophoresis and quantitated after ethidium bromide staining by densitometry.[15]

In principle, attachment of EDTA·Fe(II) to a sequence-specific DNA binding molecule could create a *sequence-specific DNA cleaving molecule*. The natural product antibiotic distamycin is an oligopeptide containing three *N*-methylpyrrole carboxamides that binds in the minor groove of double helical DNA with a strong preference for A + T rich regions.[8] The sequence specificity of distamycin binding presumably results from hydrogen bonding between the amide N–H's of the antibiotic and the O(2) of thymine and the N(3) of adenine.[9] We report here the synthesis of a sequence-specific DNA cleaving molecule, distamycin–EDTA (DE, **1**). This bifunctional molecule has three pyrrolecarboxamide residues tethered to the iron chelator, EDTA. DE·Fe(II) binds heterogeneous double helical DNA and in the presence of O_2 and DTT cleaves the DNA backbone adjacent to a four base pair A + T recognition site.



DE·Fe(II)

The *N*-hydroxybenzotriazole ester[10] of the known nitro acid **2** (Scheme I), available in eight steps by known methodology from *N*-methylpyrrole-2-carboxylic acid,[11] was condensed with 3-(dimethylamino)propylamine. Subsequent reduction of the nitro group (hydrogen over 10% Pd/C, DMF) afforded diamine **3**. This was condensed with the imidazolide[12] of the triethyl ester **5**,

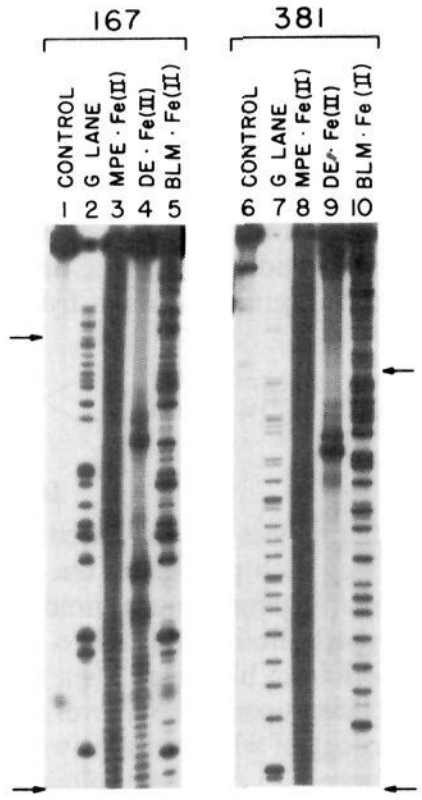


Figure 1. DNA cleaving agent was allowed to react with the 167 and 381 base pair fragments for 20 min at room temperature, frozen, lyophilized, and suspended in formamide. The 3′ end-labeled DNA products were electrophoresed on a 0.4 mm 8% polyacrylamide/50% urea gel capable of resolving DNA fragments differing in length by one nucleotide: lane 1, intact 167 bp end-labeled fragment; lane 2, G lane; lane 3, MPE·Fe(II) 2.5×10^{-6} M; lane 4, DE·Fe(II), 2×10^{-5} M; lane 5, bleomycin·Fe(II), 10^{-6} M; lane 6, intact 381 bp end-labeled fragment; lane 7, G lane; lane 8, MPE·Fe(II), 2.5×10^{-6} M; lane 9, DE·Fe(II), 2×10^{-5} M; lane 10, bleomycin·Fe(II), 10^{-6} M. All reactions are made up to 10^{-4} M bp DNA with sonicated calf thymus DNA. Arrows indicate sequence in Figure 2.

available in four steps from EDTA (**4**).[13] Hydrolysis (0.5 M aqueous LiOH) and acidification gave **1**. DE (**1**) was purified by chromatography on silica gel (70–230 mesh ATM) with ammonia/ethanol. DE was rendered metal free by supporting it on an Amberlite XAD-2 column and washing with an acidic aqueous solution of Na_2EDTA and distilled water and eluting with acidic methanol.[14]

DNA cleavage by DE·Fe(II) was followed by monitoring the conversion of supercoiled (form I) pBR-322 plasmid DNA (10^{-5} M in base pairs) to open circular and linear forms (forms II and III, respectively).[15] The introduction of one single strand break converts form I to form II. We find that at 10^{-6} M concentrations DE·Fe(II) in the presence of O_2 and DTT cleaves DNA, although less efficiently than MPE·Fe(II) and bleomycin·Fe(II)[16] (Table I).

The sequence-specific cleavage of double helical DNA by DE·Fe(II)/DTT was tested on DNA restriction fragments, 167 and 381 nucleotides in length, prepared by the usual methods from bacterial plasmid pBR322[18] and 3′ end-labeled with ^{32}P.[18,19] The

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(14) The NMR, IR, and UV spectral data are consistent with the assigned structure.

(15) pBR322 plasmid was 98% form I and 2% form II. The data in Table I are corrected for the 2% form II and the decreased stainability of form I.[5]

(16) We are grateful to Bristol Laboratories, Syracuse, NY, for their generous gift of bleomycin, supplied as the clinical mixture Blenoxane.

(17) The 167 base pair fragment results from cleavage at the EcoRI-RsaI sites, (nucleotides –2–165). The 381 base pair fragment results from cleavage at the EcoRI-BamHI sites (nucleotides –2–379). These were 3′ end-labeled with ^{32}P by the Klenow fragment of polymerase I and [^{32}P]dATP at EcoRI and BamHI sites, respectively.

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(20) The fragments were fully sequenced and agree with the published sequence.[18a] Only the G lane is shown for clarity.

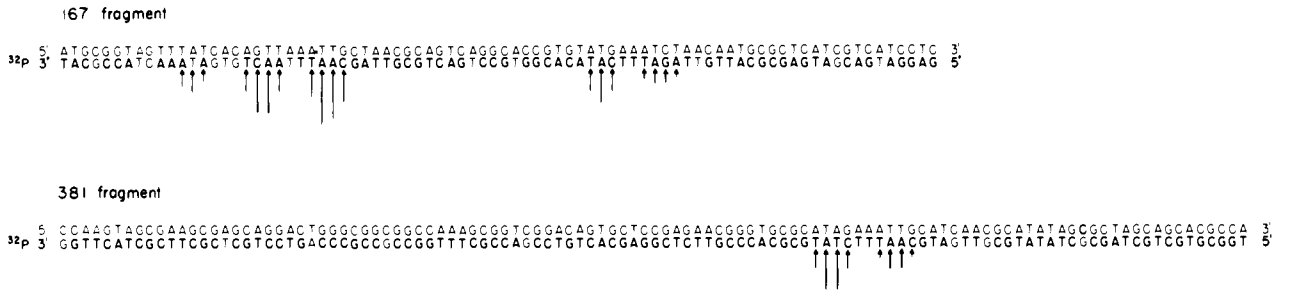
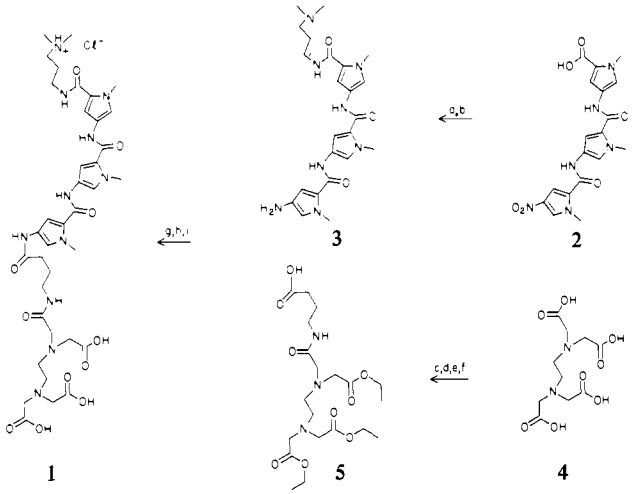


Figure 2. Arrows indicate cleavage sites on *one* strand of DNA restriction fragments by DE·Fe(II).

Scheme I



[a] $H_2N(CH_2)_3N(CH_3)_2$, HOBt, DCC. [b] H_2, Pd/C. [c] H_2SO_4, EtOH. [d] $CuCl_2$, NaOH. [e] DCC, NHS. [f] $H_2N(CH_2)_3CO_2H$, $NaHCO_3$. [g] CDI. [h] LiOH. [i] HCl.

autoradiogram of cleaved DNA fragments on the Maxam–Gilbert gel is shown in Figure 1.[20]

The MPE·Fe(II) lanes show an *even* DNA cleavage pattern, indicative of non-sequence-specific cleavage. In contrast, DE·Fe(II) shows a nonrandom pattern, with cleavage confined to highly localized sites. Comparison with the Maxam–Gilbert G lane or the bleomycin·Fe(II) lane reveals the complementary sites (A + T rich) cleaved by DE·Fe(II). Moreover, DE·Fe(II) cleaves fewer times and has higher specificity than the natural product bleomycin·Fe(II). This could be explained by the differences in the *size* of their sequence specific binding sites. In the case of bleomycin, a *two* base pair 5′–3′ GT or GC sequence is sufficient for binding and scission.[21] Although we cannot describe yet the precise details of DE binding, we believe there is a minimum three and perhaps *four* base pair recognition site composed of A and T bases. We note that predominant cleavage is centered around a 5′-ATTT-3′ site (Figure 2). The several DNA strand scissions flanking each DE·Fe(II) binding site could reflect the "reach" of the flexible tether connecting EDTA·Fe(II) to the A + T-binding pyrrolecarboxamide moieties, multiple binding modes within the preferred site, or generation of a diffusible reactive species.

With regard to future studies, the attachment of EDTA·Fe(II) to other sequence-specific DNA binding molecules such as antibiotics, polypeptides, oligonucleotides, or proteins should provide a new class of "DNA affinity cleaving molecules" and may form a primitive basis for the design and construction of artificial restriction endonucleases with defined target sequences and binding site sizes.

Acknowledgment. We are grateful to the National Institutes of Health for grant support (GM-27681).

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Biosynthesis of Swainsonine in *Rhizoctonia leguminicola*. Epimerization at the Ring Fusion

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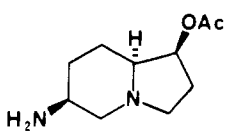
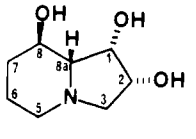
Received July 12, 1982

Slaframine and swainsonine[3] are alkaloidal toxins produced by *Rhizoctonia leguminicola*, a fungus that infests red clover and similar forages.[4] The biosynthesis of slaframine has been studied extensively,[4a,5] but little is known about the pathway leading to swainsonine. A preliminary study showed that, as with slaframine, pipecolic acid (and L-lysine) was incorporated into swainsonine by the fungus.[6] A later study suggested that malonate was the source of C-2 and C-3 and showed that one and two deuterium atoms of acetate-d_3 were incorporated into swainsonine and slaframine, respectively.[5a]



Slaframine



Swainsonine

A further study of the biosynthesis of swainsonine has now been undertaken employing perdeuteriopipecolic acid, which was prepared by α-methylation of pyridine-d_5 [(1) MeLi, Et_2O, room temperature; (2) refluxing benzene], oxidation of the resulting α-picoline-d_4 to the picolinic-d_4 acid·hydrochloride [(1) $KMnO_4$, H_2O, 100 °C; (2) HCl], catalytic reduction (1 atm of D_2, PtO_2, D_2O), and treatment with water to remove exchangeable deuterium atoms, to give the pipecolic-d_9 acid·hydrochloride in 29% overall yield. The mass spectrum of the product confirmed the presence of nine deuterium atoms.

(1) (a) Department of Chemistry; (b) Department of Biochemistry.
(2) (a) NSF Predoctoral Fellow 1977–1980; (b) Eastman Kodak Graduate Fellow 1980-1981.
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