

the vapor to 670 K oven temperature.

The PE spectroscopic assignment is easily accomplished by either the Koopmans' correlation $IE_n = -\epsilon_j^{MNDO}$ with MNDO eigenvalues—the MO sequence (Figure 1) starts with the lone pair n_s and 2π orbitals—or by M^+ state comparison with the iso (valence) electronic acrolein, the PE spectrum⁸ of which (Figure 1) displays a similar ionization pattern shifted to higher energy due to the increased effective nuclear charge of oxygen relative to sulfur.

Thus, by thermal retrodiene splitting of the Diels–Alder dimer mixtures, which can be isolated and steam-distilled, thioacrolein becomes available for preparative use⁶ and further spectroscopic investigation.

Acknowledgment. This work was supported by Land Hessen and by grants from the Deutsche Forschungsgemeinschaft and the Fond der Chemischen Industrie.

Registry No. Thioacrolein, 53439-64-8; diallylsulfide, 592-88-1; 2-vinyl-4*H*-1,3-dithiine, 80028-57-5; 3-vinyl-4*H*-1,2-dithiine, 62488-53-3.

(7) Anal. Calcd for $(C_3H_4S)_2$: C, 49.96; H, 5.59; S, 44.45; M_r , 144.25. Found: C, 49.60; H, 5.80; S, 45.0. The structure of the dimers and their relative concentrations have been established by 1H NMR⁴ spectroscopy: 90% 2-vinyl-4*H*-1,3-dithiine and 10% 3-vinyl-4*H*-1,2-dithiine. MNDO calculations predict $\Delta H_f^\circ = 111$ kJ/mol for the 1,3 isomer and 126 kJ/mol for the 1,2 isomer with a disulfide link, in good agreement with the product ratio found.

(8) Cf., e.g., D. W. Turner, C. Baker, A. D. Baker, and C. R. Brundle, "Molecular Photoelectron Spectroscopy", Wiley-Interscience, London, 1970; A. Katrib and J. W. Rabalais, *J. Phys. Chem.*, **77**, 2358 (1973). The vibrational fine structures in our record amount to $\nu^+ = 1200 \pm 100$ cm^{-1} for IE_1 to $\nu^+ = 1450 \pm 100$ cm^{-1} for IE_2 , further confirming the assignment ($\nu_{C=S} \sim 1200$ cm^{-1} and $\nu_{C=C} \sim 1650$ cm^{-1}).

Cleavage of Double Helical DNA by (Methidiumpropyl-EDTA)iron(II)

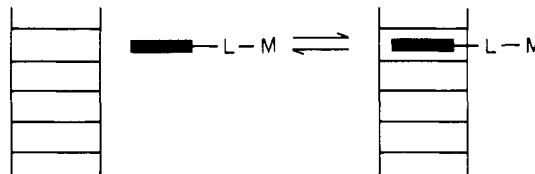
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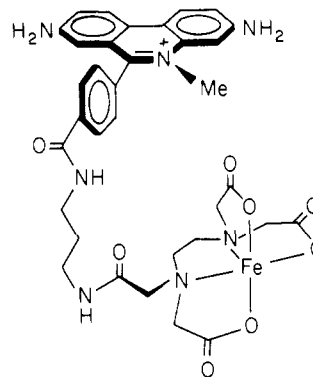
Metal ions have been implicated as cofactors in the strand scission of DNA for a number of antitumor antibiotics. Bleomycin, a glycopeptide antibiotic, binds to and cleaves DNA in a reaction that depends on ferrous ion and molecular oxygen.²⁻⁴ The antitumor agent streptonigrin is capable of causing single-strand breaks in DNA by using oxygen and cuprous ions.⁵ Recently, the 1,10 phenanthroline–cuprous complex has been shown to cleave DNA in the presence of oxygen.^{6,7} These examples involve the concept of using a DNA-binding molecule to deliver a metal ion to the site of the DNA helix where activation of molecular oxygen results in cleavage of the DNA.

We report the synthesis of a simple bifunctional molecule, methidiumpropyl-EDTA (MPE) (1), which contains the DNA intercalator methidium⁸ covalently bound by a short hydrocarbon

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- (8) Previous studies⁹ have shown that substitution of a methyl for an ethyl group or addition of a *p*-carboxyl group to ethidium bromide¹⁰ has little effect on the unwinding angle.



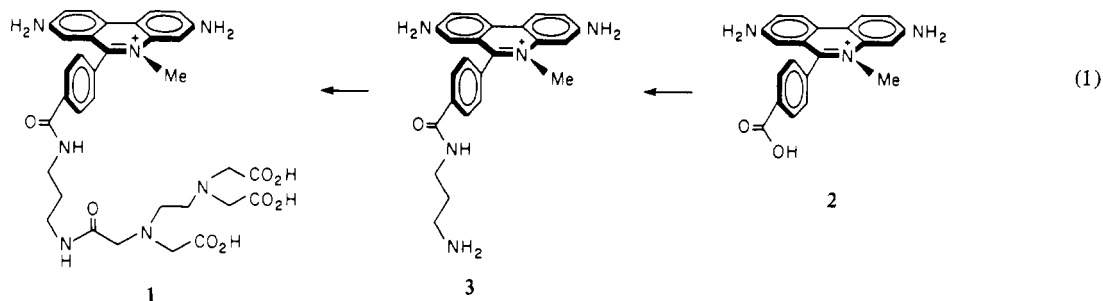
tether to the metal chelator EDTA.¹¹ In the presence of ferrous ion and oxygen this reagent *efficiently* produces single-strand breaks and some double-strand breaks in double helical DNA.



The acylimidazole ester of *p*-carboxymethidium chloride (2)^{12,13} was allowed to react with an excess of 1,3-diaminopropane in Me_2SO at 25 °C, affording a maroon solid product, methidiumpropylamine (3) (eq 1). Condensation of 3 with excess EDTA in dry DMF at 120 °C yielded (methidiumpropyl-EDTA) (MPE), in an overall yield of 59% after chromatography on silica gel 60 (230–400 mesh ASTM). MPE was rendered metal free by treatment of an acidic aqueous solution with Na_2EDTA followed by purification on Amberlite XAD-2 polystyrene resin.¹⁴

The cleavage of DNA was followed by monitoring the conversion of supercoiled (form I) pBR-322 plasmid DNA, 10^{-5} M in base pairs (bp), to open circular and linear forms (forms II and III, respectively). The introduction of one single-strand break converts form I to form II. EDTA- Fe^{II} at $>10^{-4}$ M concentrations will cleave plasmid DNA; however, at concentrations $\leq 10^{-4}$ M little or no cleavage takes place. The addition of intercalator ethidium bromide (EB) to Fe^{II} or EDTA- Fe^{II} does not promote the cleavage reaction. We find that MPE- Fe^{II} at two orders of magnitude lower concentration (10^{-6} M) cleaves plasmid DNA (Table I). MPE alone or MPE- Fe^{III} is inactive at these con-

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- (14) MPE was $\geq 99\%$ pure by HPLC in two solvent systems (ALTEX Ultrasphere ODS; 86:14 H_2O-CH_3CN , retention time 8.4 min, and 70:30 $H_2O-MeOH$, retention time 19.2 min). The NMR and IR spectra data were consistent with the assigned structure. MPE was isolated from methanol/water as the hexahydrate. Anal. Calcd for $C_{34}H_{51}N_7O_{14}$: C, 52.23; H, 6.57; N, 12.54. Found: C, 52.48; H, 6.12; N, 12.50.
- (15) pBR-322 plasmid was 95% form I and 5% form II. The data in Tables I, II, and III are corrected for the 5% form II and the decreased stainability of form I.¹⁶
- (16) The correction factor for form I pBR-322 DNA was determined to be 1.22 by the method of Haidle et al.¹⁷
- (17) Haidle, C. W.; Lloyd, R. S.; Roberson, D. L. In "Bleomycin: Chemical Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer Verlag: New York, 1979; p 222.
- (18) The average number of single-strand scissions per DNA molecule, S , was calculated from the Poisson distribution, $P_n = [S^n/n!]e^{-S}$ where P_n is the fraction of molecules that have n nicks each.¹⁹ This equation assumes that the nicks are distributed at random among the DNA population.²⁰ When only forms I and II of the DNA are present, this simplifies to $S = -\ln f_1$ where f_1 is the fraction of form I molecules. When all three forms of DNA are present, S can be calculated from the following equation:²¹ $f_1 + f_{11} = [1 - S(2h + 1)/2L]^{S/2}$ where h is the distance between nicks on opposite strands needed to produce a linear molecule (16 bp)²¹ and L is the total number of bp's in pBR-322 (4361).

Table I. Cleavage of pBR-322 Plasmid^a

reagent	concn, M	% form			S ^b
		I	II	III	
Fe(II)	10 ⁻⁴	92	8	0	0.08
EDTA-Fe ^{II} ^c	10 ⁻⁴	94	6	0	0.06
EDTA-Fe ^{II} ^c	5 × 10 ⁻⁴	38	62	0	0.97
MPE-Fe ^{II}	10 ⁻⁶	72	28	0	0.33
MPE-Fe ^{II}	5 × 10 ⁻⁶	40	60	0	0.92
bleomycin-Fe ^{II}	10 ⁻⁷	65	29	6	
bleomycin-Fe ^{II}	10 ⁻⁶	0	49	51	

^a Form I pBR-322 (10⁻⁵ M bp), reagent and buffer (40 mM Tris-HCl, 5 mM NaOAc, pH 7.8) were allowed to react at 37 °C for 60 min. Forms I, II, and III were analyzed with agarose gel electrophoresis and quantitated by ethidium bromide staining and densitometry.¹⁵ ^b Calculated average number of strand scissions per DNA molecule.¹⁸ These values cannot be calculated for bleomycin because of a nonrandom accumulation of single-strand breaks. ^c Values are the same for EDTA-Fe^{II} in the presence of 10⁻⁵ M EB.

Table II. Cleavage of pBR-322 Plasmid in the Presence of DTT^a

reagent	concn, M	% form			S
		I	II	III	
MPE-Fe ^{II}	10 ⁻⁸	82	18	0	0.20
	10 ⁻⁷	43	57	0	0.84
	10 ⁻⁶	0	85	15	9.2
bleomycin-Fe ^{II}	10 ⁻⁸	67	29	4	
	10 ⁻⁷	0	79	21	
	10 ⁻⁶	0	54	46	
Fe(II)	10 ⁻⁶	90	10	0	0.11

^a All reactions contain 1 mM DTT. Reaction conditions and analyses are as in Table I.

centrations. In the presence of dithiothreitol (DTT), MPE-Fe^{II} at 10⁻⁸ M concentration cleaves plasmid DNA comparable to efficiencies found with bleomycin²² (Table II). Presumably, DTT acts as a reducing agent and regenerates Fe(II) from Fe(III) to produce a continuous source of active metal ion.

Inhibition studies reveal the data shown in Table III. Addition of Fe(II) to MPE in the presence of strong iron chelators such as desferrioxamine (Des)^{23,24} or EDTA shows no DNA cleavage. Addition of Fe(II) to MPE-Ni^{II} or MPE-Zn^{II} shows no DNA cleavage. Ni(II) and Zn(II) are known to form stable complexes with EDTA²⁵ and presumably compete with Fe(II) for the chelation site on MPE.

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(20) MPE has been shown to produce random breaks in a 3'-end labeled DNA fragment consisting of a 375-bp segment of pBR-322 (Van Dyke, M., California Institute of Technology, unpublished work).

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(23) The log *K* for the binding of Fe(III) to Des is 30.60. Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York, 1977; Vol. 3, p 303.

(24) Des was a generous gift of Ciba-Geigy, Summit, NJ.

(25) The log *K* for binding Ni(II) and Zn(II) to EDTA is 18.62 and 16.50, respectively. Bell, C. F. "Metal Chelation, Principles and Applications"; Clarendon Press: Oxford, 1977; p 78.

Table III. Inhibition Studies^a

inhibitor	concn, M	% form			
		I	II	III	S
none		38	62	0	0.97
EDTA	10 ⁻²	86	14	0	0.15
EDTA	5 × 10 ⁻²	94	6	0	0.06
Des	10 ⁻²	97	3	0	0.03
Des	5 × 10 ⁻²	100	0	0	0.0
Ni(II)	10 ⁻⁴	98	2	0	0.02
Zn(II)	10 ⁻⁴	88	12	0	0.13
superoxide dismutase	100 ^b	81	19	0	0.21
catalase	100 ^b	96	4	0	0.04

^a pBR-322 plasmid DNA (10⁻⁵ M bp), MPE (10⁻⁵ M), and inhibitor were combined in buffer and then Fe(II) (10⁻⁵ M) was added. Analysis was carried out as in Table I. ^b Concentration in μg/mL.

The reactions of MPE-Fe^{II} and plasmid DNA with and without DTT were repeated in the absence of oxygen, and no strand scission was observed. The nature of the activated oxygen species in the MPE-Fe^{II} reaction which cleaves the DNA is not yet known. Two classes of intermediates that might be considered as the ultimate DNA-cleaving species are free oxygen radicals or an iron-bound oxygen species.²⁶ Superoxide ion (O₂⁻) has been shown to be involved in the single-strand scission of DNA in the presence of trace metal ions.²⁷ Superoxide has also been implicated as an intermediate in the reaction mechanisms of bleomycin,²⁻⁴ streptonigrin,⁵ and copper phenanthroline.^{6,7} In these systems, hydroxyl radicals are suggested to be at least one ultimate species which degrades DNA. Hydrogen peroxide has been implicated as an intermediate in hydroxyl radical generation by known Fenton-type chemistry.²⁻⁷

The enzyme superoxide dismutase (SOD) converts superoxide to hydrogen peroxide and oxygen,²⁸ thus depleting the system of "free" superoxide. The observation that SOD inhibits the MPE-Fe^{II} cleavage of DNA indicates the importance of O₂⁻. Catalase, which converts hydrogen peroxide to water and oxygen,²⁹ also inhibits the MPE-Fe^{II} DNA cleavage reaction, indicating the apparent importance of "free" hydrogen peroxide as an intermediate in strand scission (Table III).

With regard to iron-bound oxygen as the ultimate DNA-cleaving species, we find that direct oxidation of MPE-Fe^{III} (10⁻⁵ M) with iodosylbenzene (PhIO) (10⁻⁵ M) enhances cleavage of plasmid DNA. Whether this involves a ferryl species MPE-(Fe^V=O) will be the subject of future work. Controls show that PhIO and EDTA-Fe^{III} (10⁻⁵ M) or PhIO (10⁻⁵ M) alone do not cleave DNA.

In summary, MPE cleaves plasmid DNA in a reaction that is dependent on Fe(II) and O₂ at concentrations of two orders of magnitude lower than EDTA-Fe^{II}. In the presence of DTT, concentrations of MPE-Fe^{II} as low as 10⁻⁸ M cleave DNA comparable to efficiencies found with the antibiotic bleomycin. An

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interpretation consistent with the data suggests that the intercalator portion of MPE "delivers" the iron/oxygen chemistry to the DNA helix.

Acknowledgment. We are grateful to the National Institutes of Health for grant support (GM27681) and a National Research Service Award (GM 07616) to R.P.H.

Registry No. 1, 80082-09-3; 2, 66442-94-2; 3, 80082-10-6; MPE-Fe(II), 80105-72-2; MPE-Ni(II), 80105-73-3; MPE-Zn(II), 80105-74-4; EDTA-Fe(II), 15651-72-6; Fe, 7439-89-6; 1,3-diaminopropane, 109-76-2; EDTA, 60-00-4.

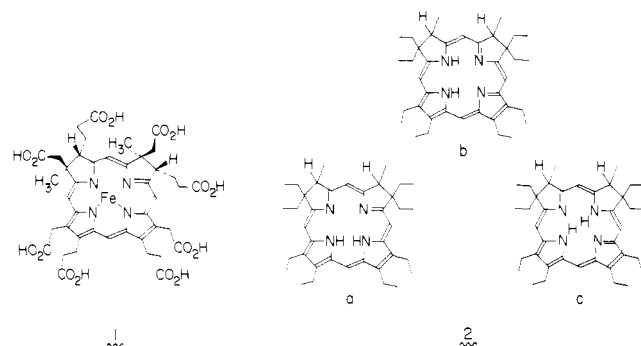
Crystal and Molecular Structure of the Isobacteriochlorin 3,7-Dimethyl-3',7'-dihydro-2,2',8,8',12,13,17,18-octaethylporphyrin. A Model for Sirohydrochlorin and Siroheme

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Received September 18, 1981

In addition to the well-established roles of chlorophylls and bacteriochlorophylls in photosynthesis,² an increasing body of evidence indicates that reduced porphyrins also mediate diverse biological functions such as catalytic decomposition of peroxide³ as well as assimilatory and dissimilatory reductions of nitrites and sulfites.⁴ The prosthetic groups of sulfite and nitrite reductases, enzymes which catalyze the six-electron reductions of sulfite and nitrite to H₂S and NH₃, respectively, have been characterized as iron isobacteriochlorins or sirohemes (1). The demetalated form of siroheme, sirohydrochlorin, has been further identified as a precursor to vitamin B₁₂ and thus may intriguingly bridge the evolutionary roles of the porphyrin and corrin macrocycles.⁵



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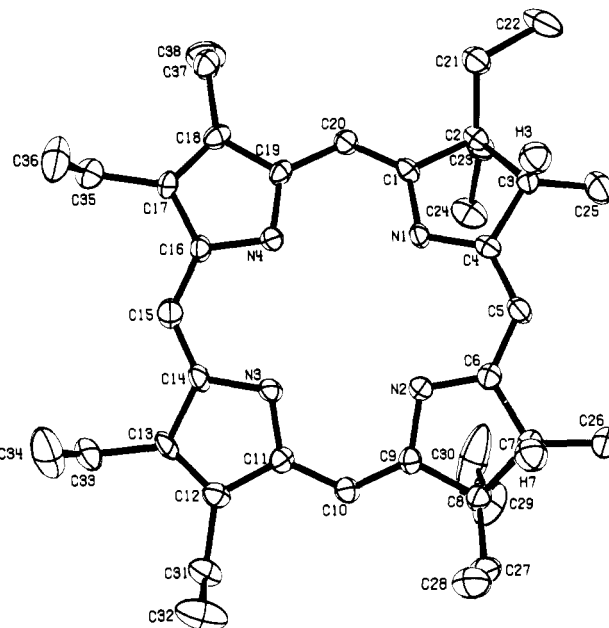


Figure 1. Structure of 2 and atom-numbering system. The hydrogen atoms are numbered according to the carbon atom to which they are bonded. The thermal ellipsoids are drawn to enclose 30% probability. Hydrogen atoms other than those on rings I and II are omitted for clarity.

Considerable attention is now focused on the synthesis of isobacteriochlorins (iBC)⁶⁻¹¹ and their theoretical,¹² structural,^{13,14} and chemical⁶⁻¹⁴ properties as guides to the chemistry of siroheme and sirohydrochlorin in vivo. We present here an X-ray determination of dimethyloctaethylisobacteriochlorin (2), a compound which is spectroscopically similar to sirohydrochlorin and bears a substituent pattern more akin to that found in vivo than other sirohydrochlorin analogues synthesized recently. This pattern was deliberately chosen⁶ to mimic the resistance of siroheme and sirohydrochlorin to oxidative dehydrogenation of the two reduced pyrroles.

The structure of 2, along with atom names, is displayed in Figure 1. The compound crystallizes¹⁵ in a unit cell comprised

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