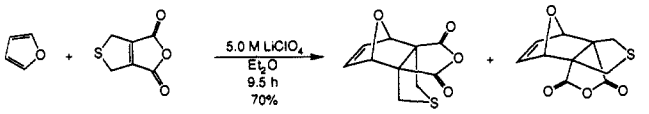
yield of cycloadduct, whereas the same reaction in benzene[9] requires 72 h at 60 °C in order to realize a 74% yield of product.

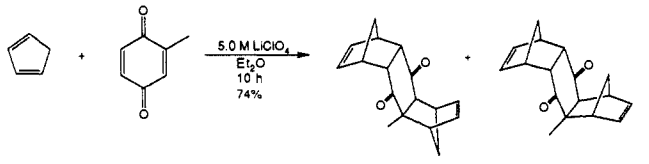
In order to fully define the scope of 5.0 M lithium perchlorate in diethyl ether as a medium for effecting Diels–Alder reactions, we set out to examine the reaction of furan with 2,5-dihydrothiophene-3,4-dicarboxylic anhydride (**1**). Furan is a poor Diels–Alder diene due to its aromaticity and generally requires pressures in the range of 10–20 kbar to effect cycloaddition.[10] High temperatures are not compatible with furan Diels–Alder chemistry since the cycloaddition products derived from furan undergo cycloreversion at high temperatures. In his classic synthesis of cantharidin,[11] Dauben found that the reaction of furan with dienophile **1**[12] in methylene chloride required 6 h under 15 kbar of pressure in order to realize an 85:15 mixture of cycloadducts **2** and **3**. In sharp contrast, the Diels–Alder reaction



1 2 3

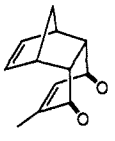
between furan and dienophile **1** in 5.0 M lithium perchlorate in diethyl ether proceeded at ambient temperature and pressure, giving rise (70% yield) after 9.5 h to cycloadducts **2** and **3** in an 85:15 ratio. A systematic examination of this reaction confirmed a direct correlation between reaction rate and molarity, with the rate increasing on going from 1.0 to 5.0 M lithium perchlorate in diethyl ether (Table II).

Equally remarkable was the observation that exposure of methylbenzoquinone to cyclopentadiene in 5.0 M lithium perchlorate in diethyl ether for 10 h at room temperature and atmospheric pressure afforded in 74% yield bis adducts **4** and **5** in a 6:1 ratio.



4 5

Admixture of cyclopentadiene and methylbenzoquinone in diethyl ether without lithium perchlorate gives rise in excellent yield to the 1:1 Diels–Alder adduct **6** with no evidence for the formation of bis adducts **4** and **5**. It is of interest to note that the formation of bis adducts **4** and **5** has been reported to occur at high pressure.[13] For example, heating a toluene solution of cyclopentadiene (large excess required due to competing diene dimerization) and methylbenzoquinone at 75 °C under 7895 atm overnight affords **4** and **5** in 60% yield.



6

The ease with which these normally demanding cycloadditions proceed points to the operation of factors that are not adequately explained in the current literature. The ability of 5.0 M $LiClO_4$–Et_2O, a unique ionic medium, to confine solute movement and hence retain solvent ordering may well be responsible for the observed rate accelerations by compressing the reactants in much the same manner as the "macroscopic" application of external pressure.[15] As the Diels–Alder reaction is known to possess a large negative volume of activation, this action would serve to raise the ground-state energy of the reactants relative to the transition state, thereby lowering the activation energy.

In conclusion, the utilization of 5.0 M lithium perchlorate in diethyl ether to promote intermolecular [4 + 2] cycloaddition at ambient temperature and pressure has been established, thus permitting the use of water-sensitive substrates and obviating the necessity of effecting these chemical reactions at high temperatures and ultrahigh pressures.

Acknowledgment. This investigation was supported by a grant from the National Science Foundation. We thank Professor Joseph J. Gajewski for helpful discussions.

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(15) The possibility that lithium perchlorate is stabilizing a polar transition state has not been ruled out; however, the observed rate accelerations cannot be fully accounted for by this mechanism alone.

Observations on the Activation of Mitomycin C. Requirements for C-10 Functionalization

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Mitomycin C (**1**), a proven antineoplastic agent of clinical significance,[1] is believed to require reductive activation prior to bonding to DNA.[2] Despite 25 years of intense research our understanding of the reductive process is limited. Uncertainty exists in the specific reduction and ionization states in **1** necessary for the C-1 and C-10 drug bonding steps.[2] In this communication, we report on the first use of transition-metal ions for the activation of mitomycin C. Employment of $Cr(ClO_4)_2$ as a one-electron reductant[3] has dramatically altered the reactivity pattern of the two DNA-bonding sites within **1**.[2e] Moreover, analysis of the data permitted us to propose a detailed description of the molecular events necessary for complete drug function.

Two different $Cr(ClO_4)_2$-mediated reductive techniques were developed. In the first procedure, $Cr(ClO_4)_2$ (1–2 equiv) was directly added to **1** at various pH values (Table I, entries 1–7). Important observations included the following: (1) Consumption of **1** was rapid and generated both *trans*- and *cis*-10-decarbamoyl-1-hydroxy-2,7-diaminomitosene[4] (**5**) as the major products. (2) The reaction efficiency increased at lower pH values. (3) Between pH 6.0 and 7.0, the difunctionalized mitosene adducts **3** and **5** accounted for nearly half of the product profile even though noticeable amounts of unreacted **1** remained. (4) Significant amounts of C-1 electrophilic products (i.e., **2** and **3**) were

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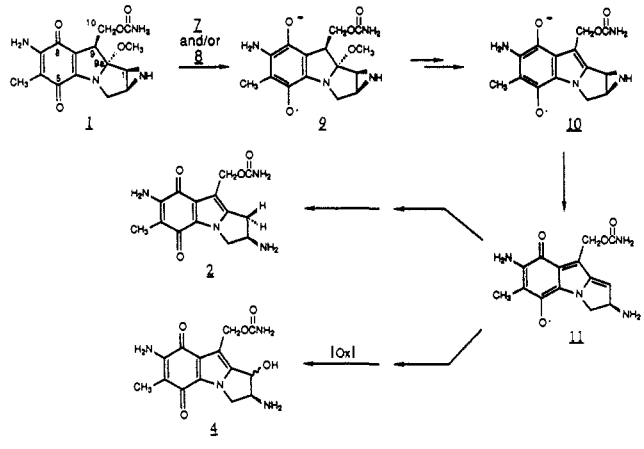
Table I. Product Profiles from $Cr(ClO_4)_2$-Mediated Reductions of Mitomycin C (**1**)[a]

entry	pH[b]	2	3	4	5	1
1[c]	5.10	5.1	3.3	9.0	82.6	
2[c]	5.00[d]	1.8	11.3		86.9	
3[c]	6.04	26.6	6.3	18.8	40.6	7.7
4[c]	6.00[d]	2.2	13.3		84.5	
5[c]	6.94	11.5	11.4	21.2	30.6	25.3
6[c]	7.00[d]	1.6	24.2	6.6	67.6	
7[c]	8.00	2.2		51.1	4.7	42.0
8[e]	4.98	33.7	1.5	9.6	8.7	46.5
9[e]	5.98	30.6		17.0		52.5
10[e]	7.01	5.5		69.5		25.1
11[e]	8.03	4.5		87.7		7.9

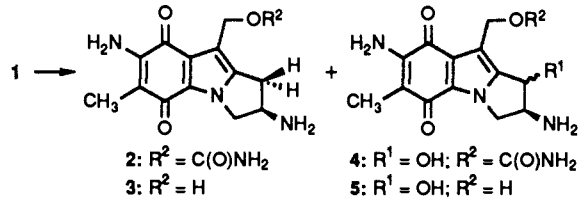
[a] The reactions were monitored by HPLC (see ref 8) and all products were identified by co-injection (co-spotting) of an authentic sample with the reaction mixture in the HPLC (TLC). The reactions were run in duplicate and averaged. [b] Bis·Tris·HCl (0.2 M) was employed at pH 5 and 6, and Tris·HCl (0.2 M) was used at pH 7 and 8. [c] Reaction was initiated by the addition of an aqueous solution of $Cr(ClO_4)_2$ (1 equiv unless otherwise stated) to a deaerated, aqueous buffered solution of **1** (final concentration 1.2 mM). The reaction was maintained at room temperature (30 min), exposed to air, and analyzed. [d] $Cr(ClO_4)_2$ (2 equiv) was added. [e] **6** (2 equiv, final concentration 1.3 mM) was dissolved in an aqueous buffered solution (2 mL) that was then deaerated, and then an aqueous $Cr(ClO_4)_2$ (1 equiv) solution was added and with stirring at room temperature (5 min). A deaerated aqueous solution (0.5 mL) of **1** (1 equiv) was added and the reaction was maintained at room temperature (30 min) and exposed to air and the solution was analyzed.

Scheme I. Proposed Pathway for Mitomycin C-1 Mediated Processes[a]

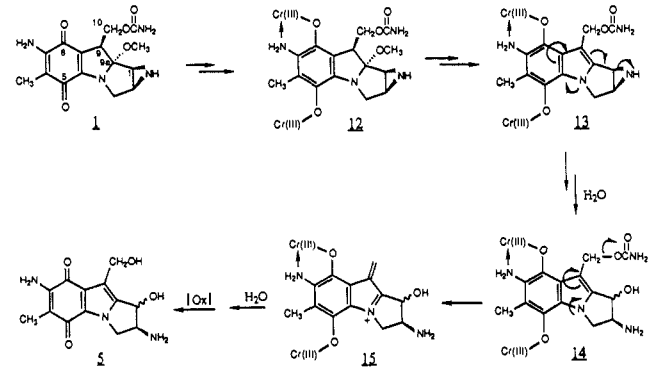


[a] The proposed process is depicted to occur at the semiquinone reduction level in **1**. A comparable pathway can be drawn for the hydroquinone form of **1**. See refs 2, 4–7, 9–11, 15, and 16 for additional details.

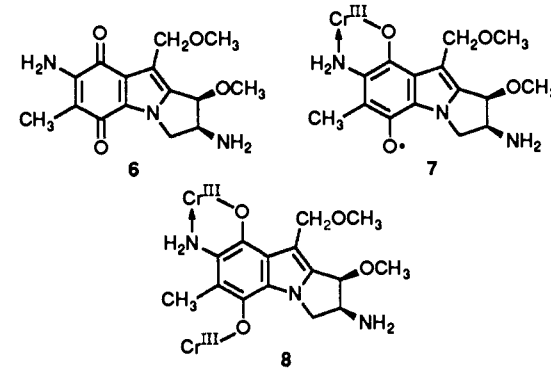
not observed under acidic conditions, while no C-10 electrophilic adducts were detected at pH 8.00. The last two observations were *opposite* to that reported for the activation of **1** with use of other reductive methods.[5-11]



Scheme II. Predominant Pathway for the Reductive Activation of Mitomycin C by $Cr(ClO_4)_2$ in Water



The products observed with the second $Cr(ClO_4)_2$-mediated reductive technique (Table I, entries 8–11) were markedly different. Activation of mitomycin C was accomplished by the prior addition of $Cr(ClO_4)_2$ to excess *cis*-10-decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene[12] (**6**) to generate the putative monochromate **7** and dichromate **8** species[13] in situ, followed by the addition of **1** (1 equiv per $Cr(ClO_4)_2$). The product distribution obtained in these experiments was similar to those previously reported.[4-11] Specifically, the percentage of C-1 electrophilic adducts (i.e., **2**, **3**) generated was dependent upon pH, and the reactions gave predominantly C-1 monosubstituted products (i.e., **2** and **4**).[14]



What factors are responsible for this divergent chemistry? We propose that the indirect reductive procedure proceeds by a mechanism similar to that previously proposed (Scheme I).[5,15,16] Reductive activation of **1** occurs by an outer-sphere electron-transfer process from **7** and/or **8** to give the *uncomplexed* mitomycin C semiquinone anion **9** or the corresponding hydroquinone intermediate. Subsequent expulsion of the C-9a methoxy group followed by the loss of the acidic C-9 proton permits aziridine ring-opening to give the extended quinone methide **11**. This species can then react with solvent to furnish **2** (**3**) and **4** (**5**). In contrast,

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(12) Compound **6**: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.71 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 3 H), 3.64 (dd, J = 9.1, 12.2 Hz, 1 H), 3.80–3.87 (m, 1 H), 4.33 (d, J = 5.0 Hz, 1 H), 4.44 (dd, J = 7.4, 12.2 Hz, 1 H), 4.99 ($^1/_2$ AB$_q$, J = 12.6 Hz, 1 H), 4.58 ($^1/_2$ Abq, J = 12.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) 8.43, 51.80, 57.04, 58.78, 58.84, 66.58; 75.05, 107.06, 117.40, 120.83, 129.60, 138.66, 146.66, 177.94, 178.95 ppm; M_r (EI) 305.1370 (calcd for $C_{15}H_{19}N_3O_4$ 305.1376).
(13) For a discussion of ligand substitution reactions of Cr^{II} and Cr^{III} ions, see: Douglas, B. E.; McDaniel, D. H.; Alexander, J. J. *Concepts and Models of Inorganic Chemistry*, 2nd ed.; Wiley: New York, 1983; pp 334–339.
(14) Comparable results were observed when excess **1** (2 equiv) was added.
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we suggest that the direct addition of $Cr(ClO_4)_2$ to **1** leads to the rapid two one-electron reduction of the quinone ring to give the bis-Cr^{III}-bound complex **12** (Scheme II).[17] Loss of methanol then yields **13**, which allows the C-1 and C-10 *nucleophilic substitution processes to proceed by an indole-assisted pathway.*[18] The corresponding electrophilic transformations should be inhibited by the prior complexation of the phenolic-type oxygens at C-5 and C-8 in **12** and **13** by the chromium ion. The high yields of the C-1, C-10 disubstituted adducts **3** and **5** have been attributed to the full two-electron reduction of **1** to species **12** (**13** and **14**). We suspect that the corresponding indole-assisted expulsion of the C-10 carbamate group at the semiquinone stage would not proceed rapidly due to the electron deficiency of this species.

This study documents the advantages accrued by the direct use of $Cr(ClO_4)_2$ for the reductive activation of mitomycin C. Reactions were rapid and permitted the functionalization of both DNA bonding sites within **1**. Our attribution of the high percentage of nucleophilic products in these transformations to the complexation of the C-5 and C-8 phenolic-type oxygens in **12** by the metal raises the intriguing suggestion that a similar process (i.e., protonation, hydrogen-bonding, chelation) may be necessary for the full expression of drug function of reduced **1** in in vivo transformations.

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(17) Analysis of the HPLC profiles indicated that a minor fraction of the products under acidic conditions may arise from an autocatalytic pathway in which a chromium bound mitomycin C species serves as a chemical reductant for **1**.

(18) Addition of $Cr(ClO_4)_2$ to buffered *methanolic* solutions of **1** led to high yields of *cis*- and *trans*-10-decarbamoyl-1,10-dimethoxymitosene. Analogously, addition of $Cr(ClO_4)_2$ (1 equiv) to aqueous buffered (pH 6.00) solutions of **2** and **4** gave principally **3** and **5**, respectively.[19]

(19) Hong, Y. P.; Kohn, H. Unpublished results.

Formation of Monolayer Pits of Controlled Nanometer Size on Highly Oriented Pyrolytic Graphite by Gasification Reactions As Studied by Scanning Tunneling Microscopy

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We report here that nanometer-size pits with monolayer depth are reproducibly formed on the basal (0001) plane of highly oriented pyrolytic graphite (HOPG) by gasification reactions at elevated temperature in air. These are clearly imaged and easily studied by scanning tunneling microscopy (STM).

Gas–carbon reactions are important in studies of combustion, water gas production, and the gasification of solid fuels.[1] Progress in this area has largely depended upon the application of microscopic techniques, from optical microscopy to the more powerful etch-decoration transmission electron microscopy (ED-TEM)[2-4] or scanning electron microscopy (ED-SEM).[5] In ED-TEM a graphite sample etched by reaction with O_2, CO_2, H_2O, H_2, or Cl_2 is decorated with evaporated gold which nucleates at the etched edges, allowing surface changes to be imaged by electron microscopy. Etch decoration with gold is needed because clear images of unmodified carbon surfaces are not obtained by TEM. Studies by ED-TEM[2-4] on natural graphite led to a model in which monolayer pits form during gasification reactions. These were proposed to start at existing defects (e.g., atomic vacancies) and grow in a shape determined by the higher reactivity of carbon atoms at edge sites. However, it was not possible to study by EM the initial stages of this process on unmodified graphite or to show that these etch pits are one atomic layer deep.[2a]

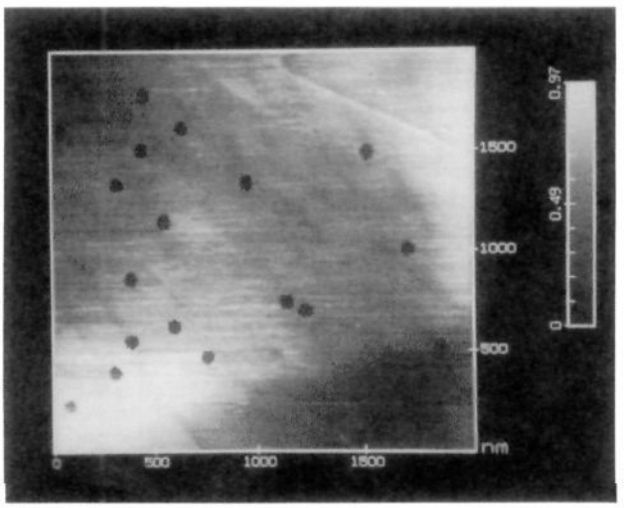

Figure 1. STM image of an HOPG sample treated at 650 °C in air for 15 min.

However, STM[6] allows surface imaging with atomic resolution on graphite surfaces. We felt that STM could be used to image etch pits, without the requirement of gold decoration, from the initial stages of their formation. We are interested in these pits not only because they provide information about the mechanism of carbon oxidation but also because pits of a controlled and uniform size could have interesting applications as templates and markers. In this work HOPG,[7] rather than the related natural graphite crystals used in earlier investigations,[2a] was studied. The oxidation of HOPG has not been investigated by high-resolution electron microscopy.[8]

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