°C; ^{1}H NMR identical with that of the uncrystallized sample.

Anal. Calcd for $C_{31}H_{46}N_4O_2$: C, 73.48; H, 9.15; N, 11.06. Found: C, 73.27; H, 9.02; N, 11.01.

Cycloadduct of Acrylic Acid and 9,10-Bis(((2-(dimethylamino)-ethyl)methylamino)methyl)anthracene: 21. The direct cycloaddition of acrylic acid to anthracene **16** was unsuccessful, so we prepared this adduct from the corresponding ester. A solution of ester **20** (408 mg, 0.8 mmol) in concentrated HCl (50 mL) was heated to reflux for 20 h. The resulting light-green solution was evaporated to dryness, and the residue was chromatographed on a column of Sephadex CM-25 cation-exchange resin by eluting with a linear gradient of 0-0.9 M NH_4HCO_3. Fractions were monitored at 258 nm, and the major peak was pooled, evaporated several times from H_2O, and finally lyophilized from H_2O to afford a hygroscopic colorless solid (180 mg, 48%): ^{1}H NMR (D_2O) δ 1.7 (dd, 1, C*H*CHCOOH cis to acid), 2.3-3.9 [m, 29, aliphatic H's except that from C*H*CHCOOH cis to acid; notable signals: 2.35 (s, 6, $N(CH_3)_2$), 2.4 (s, 3, NCH_3), 2.5 (s, 3, NCH_3), 2.65 (s, 6, $N(CH_3)_2$)], 7.0-7.5 (m, 8, ArH).

Anal. Calcd for $C_{29}H_{42}N_4O_2 + 1.5H_2O$: C, 68.88; H, 8.97; N, 11.08. Found: C, 69.15; H, 8.61; N, 11.10.

Kinetic Measurements in the Attempted Conversion of Nitrile 18 to Amide 19 (Reaction A, Scheme VI). The attempted conversion of **18** to **19** was monitored conveniently by HPLC as described in the general Experimental Section with the 7:4 solvent system. Typical retention times at a flow rate of 2 mL/min are nitrile **4**, 6 min; amide **5**, 3 min; and acid **11**, 2 min. A solution of Na-HEPES buffer (pH 9.5, 66.67 mM) containing nitrile **4** (1.67 mM) and metal perchlorate (20 mM) was heated at 94 °C in a pressure tube, and aliquots were withdrawn at various times for HPLC analysis. The control reaction was identical, except that there was no added metal ion. The formation of amide was not observed in any of these reactions, though even a few percent conversion would have been detectable.

Attempted Conversions of Amide 19 to Ester 20, Amide 19 to Acid 21, and Ester 20 to Acid 21 (Reactions B, C, and D, Scheme VI). Each attempted conversion was carried out similarly to that described above by using Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}, a variety of pHs between 5 and 9.5, and temperatures as high as 95 °C. The formation of the expected product was not observed in any of these reactions, though even a few percent conversion would have been detectable.

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Mechanistic Studies on the Mode of Reaction of Mitomycin C under Catalytic and Electrochemical Reductive Conditions[1]

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Abstract: The catalytic and electrochemical reduction of select mitosene derivatives and mitomycin C (**1**) are described. Treatment of *trans*- (**11**) and *cis*-1-hydroxy-2,7-diaminomitosene (**13**) with PtO_2 and H_2 led to the formation of the novel carbon-10 methyl products **12** and **14**, respectively. The corresponding 1-methoxy-2,7-diaminomitosene carbon-10 methyl adducts **19** and **20** were obtained from the controlled potential reduction of **1** at mercury (-1.5 V vs. SCE) or platinum (-1.0 or -1.2 V vs. SCE) electrodes in methanol. At a lower applied voltage (mercury electrode, -0.8 V), reduction of **1** in methanol gave predominantly *trans*- (**17**) and *cis*-1-methoxy-2,7-diaminomitosenes (**18**). The product profile of **1** in aqueous buffered solutions (pH 5.0, 6.5, 8.0) has also been elucidated. At pH 5.0, 2,7-diaminomitosene (**30**) and 10-decarbamoyl-2,7-diaminomitosene (**31**) were produced in high yields; while at pH 8.0, the major compounds isolated were *trans*- (**11**) and *cis*-1-hydroxy-2,7-diaminomitosene (**13**) and *cis*-2-acetamido-1-hydroxy-7-aminomitosene (**32**). The electroreduction of mitomycin C and *trans*- (**17**) and *cis*-1-methoxy-2,7-diaminomitosenes (**18**) were also investigated by cyclic voltammetry and ESR spectroscopy. These data were used in conjunction with the observed product profiles to postulate probable pathways in both the methanol and aqueous reduction experiments. Significantly, loss of methanol and aziridine ring-opening in **1** proceeds from the quinone anion radical stage. The isolation of the carbon-10 methyl products (**12**, **14**, **19**, and **20**) in the methanol-based experiments provides evidence that reaction at this site funnels through an iminium ion.

Mitomycin C (**1**) is a clinically significant antineoplastic antibiotic in which several key functional groups are exquisitely deployed within the drug.[2] Recent studies[3-15] have begun to unmask the chemical role of many of these groups and have contributed to the contention that mitomycin C functions as a bioreductive alkylating agent where both carbons-1 and -10 are likely DNA binding sites. Consideration of these points led Moore to propose the general mechanism in Scheme I.[16]

(1) This paper has been presented in part. See: *Abstracts of Papers*; The 191st National Meeting of the American Chemical Society, New York, NY, April 13-18, 1986; American Chemical Society: Washington, D.C., 1986; Abstr. Orgn. 184.

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(16) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249-280. Please see this article for earlier references.

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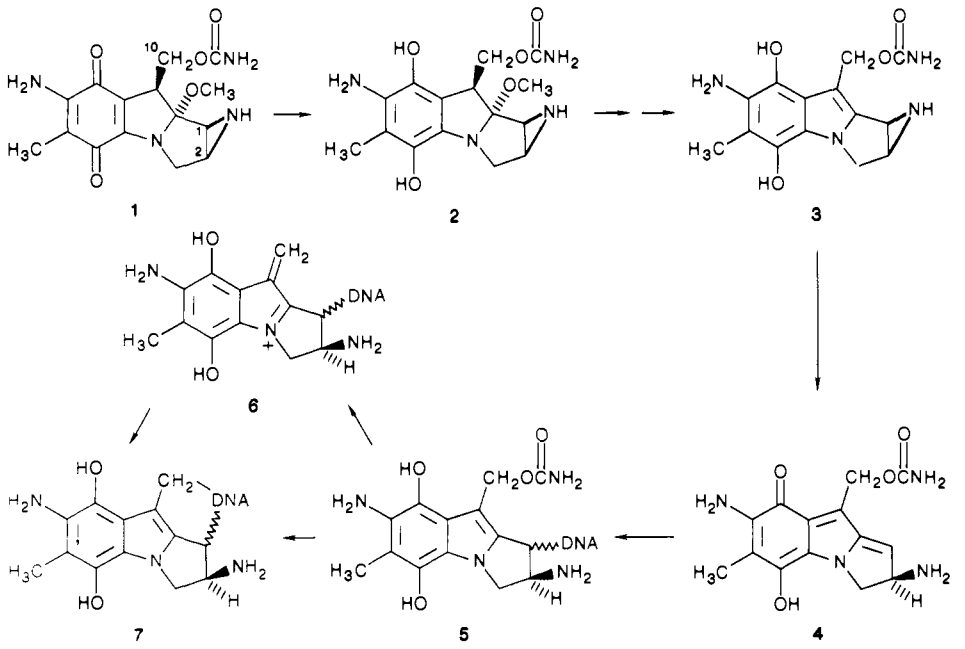
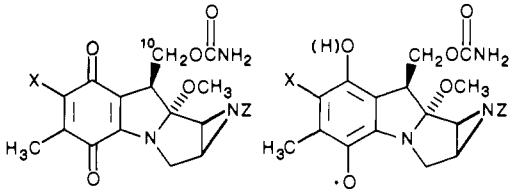
Scheme I



Evidence has been amassed indicating that carbon-1 is more reactive than carbon-10 toward nucleophiles at neutral pH in the presence of a reducing agent[3-14] and in dilute acid.[17-21] The isolation of both carbon-1 diastereomeric products in many of these reactions has suggested that ring opening of the aziridine moiety precedes attack by the nucleophile (i.e., Scheme I, **3** → **4** → **5**). It has been generally accepted that carbon-1 mediated processes proceed at the hydroquinone stage (i.e., **2**) under reductive conditions.[2,16] Recently, several reports have appeared which challenge this notion.[1,14,15] Reduction of mitomycin C (**1**) or *N*-methylmitomycin A (**8**) in polar *aprotic* solvents (dimethylformamide, dimethyl sulfoxide, pyridine) demonstrated that the initial loss of methanol[14,15] and the subsequent aziridine ring-opening step[14] in the drug proceeded from the corresponding semiquinone species (i.e., **9**, **10**).



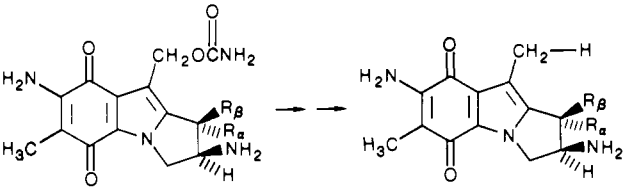
1: X = NH_2, Z = H
8: X = OCH_3, Z = CH_3 **9**: X = NH_2, Z = H
10: X = OCH_3, Z = CH_3

In contrast to our understanding of the mode of reaction at carbon-1, less is known about the corresponding mechanism for carbon-10 alkylation, a situation partly due to the diminished reactivity and achirality of this site. Several possibilities can be envisioned for carbon-10 alkylation. Two of the more attractive routes are the following: (1) S_N2 substitution of the carbamate group in **5** by the genetic material to give **7**; and (2) initial loss of the carbamate moiety in **5** to yield iminium ion **6**, followed by nucleophilic attack by DNA to produce the disubstituted adduct **7**.

In this paper, we provide evidence a *one*-electron reduction of the quinone ring in mitomycin C in the polar *protic* solvent methanol leads to loss of methanol followed by ring-opening of the aziridine moiety. Moreover, new information is presented for the requirements needed for the activation of the carbon-10 site in **1**. Significantly, our results suggest that the nitrogen-4 atom in mitomycin C plays a significant role in the carbon-10 mediated process both under catalytic and electrochemical reductive conditions.

Results and Discussion

1. Catalytic Reductions.[22] Treatment of a 0.2 mM methanol solution of 1,2-*trans*-1-hydroxy-2,7-diaminomitosene[9,23] (**11**) with PtO_2 and H_2 (27 °C, 7 min) led to the isolation of the carbon-10 methyl adduct **12** (80–90% yield) after oxidative workup. No other significant products were detected by HPLC analyses. A comparable result was obtained for the reduction of **13** in ethanol to give **14** (70–80% yield). In both cases, the new carbon-10



11: R_β = H, R_α = OH
13: R_β = OH, R_α = H **12**: R_β = H, R_α = OH
14: R_β = OH, R_α = H

methyl products **12** and **14** were readily identified by their spectroscopic properties. Of particular note was the absence of the carbamate absorption at 1708 cm^{-1} in the infrared spectrum of **12** and the appearance of a singlet at ca. δ 2.25 in the ^1H NMR spectra of **12** and **14** for the carbon-10 methyl group.

Information concerning the origin of these carbon-10 methyl adducts was ascertained by rerunning each of these reductions under slightly modified conditions. First, D_2 was substituted for H_2 (Table I, entries 1 and 3), while in a second experiment the O-deuteriated alcohol (ROD) was used in place of the corresponding protonated solvent (ROH) (Table I, entries 2 and 4). Deuterium incorporation (86–91%) at carbon-10 in **12** or **14** was observed by ^1H NMR spectroscopy only in the second experiment, establishing the solvent as the source of deuterium. Monodeuteriation at carbon-10 led to an appearance of a 1:1:1 multiplet

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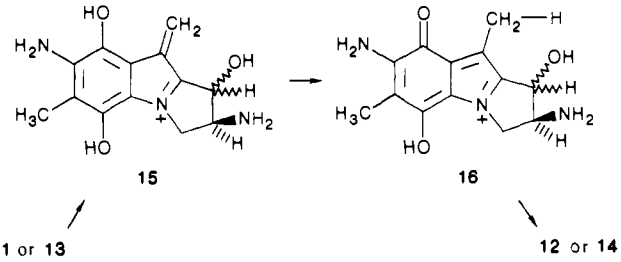
Scheme II

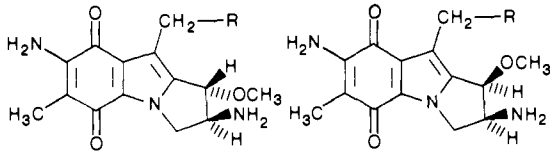


($J_{HD} \sim 2.07$ Hz) upfield ($\sim$0.02 ppm) from the singlet normally associated with the carbon-10 methyl group. Both the upfield shift and the observed coupling constant are diagnostic of deuterium incorporation at this site.[24]

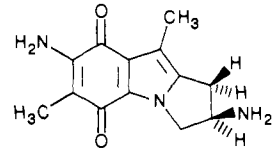
These results are in accord with a pathway depicted in Scheme II in which product formation proceeds through the intermediacy of iminium ion **15**. A comparable result has been obtained in an earlier study concerning the mechanism of carbon-1 ring opening in mitosenes.[8,9]

2. Electrochemical Reductions. The detection of **12** and **14** under catalytic reductive conditions[22] prompted our investigation of the products produced from **1** under bulk electrolysis conditions. Accordingly, 5-mL samples containing mitomycin C (1.5 mM) were reduced at room temperature with use of both Hg and Pt working electrodes. The observed product profiles were dependent upon the solvent and the applied potential utilized.

a. Methanolic Solutions. Controlled potential reduction of **1** was carried out at a Hg electrode in methanol at −0.8 V and led to the appearance of a 1:1 mixture of **17** and **18**, along with trace amounts of unreacted mitomycin C (**1**), **19**, **20**, and an unidentified compound. A comparable product profile was obtained when the effective "pH" of the solution was adjusted to 10.8 by the addition of sodium methoxide prior to reduction.



The product profiles observed from the reduction of **1** in unbuffered methanol were monitored as a function of time by HPLC analysis with use of an applied potential of −1.5 V at a Hg electrode or an applied potential of −1.0 or −1.2 V at a Pt electrode. The product profiles were independent of electrode and were initially the same as those noted after reduction at −0.8 V at the Hg electrode. Reduction at the more negative voltages led to the rapid consumption of **1** and the formation of an approximate 1:1 mixture of **17** and **18**, along with small amounts of **19**, **20**, and an unidentified compound. However, continued electrolysis led to a decrease in **17** and **18** and a concomitant increase in **19** and **20** as well as the production of an adduct which has been tentatively identified as **21**. Under conditions where oxygen was



21

rigorously excluded, conversion of **1** to **19** and **20** was rapid (<20 min). At the conclusion of the reaction the effective "pH" of the methanolic solution was 10.8. Select variations of this experiment were also conducted. First, reduction of **1** at −2 to −4 °C led to a product profile which was similar in all respects to the room temperature electrolysis except the yields of **19** and **20** were slightly diminished. Second, addition of water (10% v/v) to the methanolic solution prior to reduction of **1** led to the formation of the corresponding *trans*- (**11**) and *cis*- (**13**) amino alcohols along with **17**–**20**. Third, reduction (Hg electrode, −1.5 V; Pt electrode, −1.2 V) of a purified sample of *cis*-1-methoxy-2-aminomitosene (**18**) in methanol gave predominantly the carbon-10 methyl adduct **20** (HPLC analysis).

Table I. Percent Deuterium Incorporation Observed in the Conversion of **11** (**13**) to **12** (**14**)

entry	substrate	gas utilized	solvent utilized	% D incorporation at carbon-10 in **12** or **14**[a]
1	**11**	D_2	CH_3OH	0
2	**11**	H_2	CH_3OD	91
3	**13**	D_2	C_2H_5OH	0
4	**13**	H_2	C_2H_5OD	86

[a] Percent deuterium incorporation was determined by ^1H NMR. Accuracy of the measurement is ±5%.

The identities of mitosene adducts **17**–**20** were confirmed by performing the bulk electrolysis experiments on a semipreparative scale. Five methanolic solutions (15 mL) of **1** (1.5 mM) were sequentially reduced at the desired voltage with a mercury electrode and the products pooled for analysis. Approximately 85% of the mitomycin C was converted to products (chromatographic analyses). The samples were combined, purified, and analyzed. Key resonances in the ^1H and ^{13}C NMR spectra for these compounds are listed in Tables II and III, respectively. The signal for the carbon-1 methine hydrogen for **17**–**20** appeared in the ^1H NMR spectra between δ 4.27 and 4.43 (Table II), while the corresponding peak in the ^{13}C NMR spectra was observed between 73.96 and 81.61 ppm (Table III). In agreement with previously reported trends,[6,25] the ^{13}C NMR signals for carbons-1 and -2 in the trans adducts (**17** and **19**) appeared downfield from the corresponding signals in the isomeric cis compounds (**18** and **20**).

Several detailed scenarios for the formation of **17**–**20** in the methanolic reduction experiments can be envisioned. One attractive hypothesis is depicted in Scheme III. An initial reduction of the quinone moiety in **1** to the quinone anion radical species **9** permits the rapid loss of methanol to give **22**. Subsequent aziridine ring-opening (**22** → **23**) yields the carbon-1 mitosene adducts **24** (R_α or R_β = OCH_3) after nucleophilic attack by methanol. The carbon-1 methoxy adducts can either be reoxidized to give **17** and **18** or they can undergo further reduction and loss of the carbamate group to produce the corresponding carbon-10 methyl adducts **19** and **20** by a pathway similar to that described in the catalytic reduction of **11** and **13**.

Formation of **17** and **18** vs. **19** and **20** will depend upon the applied potential. At less negative voltages (i.e., Hg electrode, −0.8 V) reoxidation of **24** to **17** and **18** occurs preferentially, while at higher voltages (i.e., Pt electrode, −1.0 to −1.2 V; Hg electrode, −1.5 V) conversion of **24** to **19** and **20** proceeds rapidly.

Support for this mechanistic scenario comes from cyclic voltammetric data of **1**, **17**, and **18** and a profile analysis of the products carried out during reduction at different potentials. Cyclic voltammograms of mitomycin C at a Hg electrode in methanol or in methanol/sodium methoxide mixtures show the presence of two major electroreduction processes between −0.60 and −0.90 V vs. SCE. These processes are labeled as peaks I and II in Figure 1a. Both peaks I and II are irreversible in methanol and occur at E_p = −0.66 and −0.80 V for a scan rate of 0.1 V/s. Peak II also appears to involve an overlapping adsorption process. The stepwise addition of sodium methoxide to solutions of mitomycin C does not result in a shift of potentials for these two peaks but rather seems to decrease the adsorption currents for peak II while splitting the single sharp peak into two closely overlapping reduction peaks.

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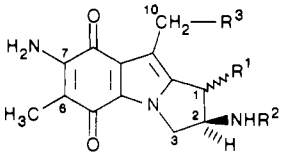
Table II. ^{1}H NMR Assignments of Mitosene Derivatives[a]



compd no.	R^1	R^2	R^3	C_1H_α	C_1H_β(s)	C_2H	C_3H_α	C_3H_β	C_6-CH_3	$C_{10}H_2$	$C_{10}H_3$
11[b]	OH	H	$OCONH_2$		4.79; d; 2.4	3.82–3.84; m	4.45; d, d; 5.9, 13.1	3.93; d, d; 3.2, 13.1	1.79; s	5.16; d; ABq; 12.6 5.23; d; ABq; 12.6	
12[b]	OH	H	H		4.68; d; 2.8	3.71–3.78; m	4.44; d, d; 6.1, 13.0	3.86; d, d; 3.8, 13.0	1.70; s		2.28; s
17[c]	OCH_3	H	$OCONH_2$		4.36; s	3.89; s	4.19; d, d; 4.9, 12.5	3.85; br d; 12.5	1.73; s	5.04; s	
19[c]	OCH_3	H	H		4.27; s	3.87; br s	4.19; d, d; 5.3, 13.1	3.82; br d; 13.1	1.73; s		2.24; s
13[c]	OH	H	$OCONH_2$	4.65; d; 5.1		3.71–3.75; m	4.30; d, d; 7.3, 12.1	3.55; d, d; 8.3, 12.1	1.72; s	5.00; d, ABq; 12.4 5.05; d, ABq; 12.4	
14[b]	OH	H	H	4.72; d; 5.3		3.77–3.86; m	4.44; d, d; 7.2, 12.2	3.68; d, d; 8.5, 12.2	1.75; s		2.27; s
32[c]	OH	$COCH_3$	$OCONH_2$	4.89; d; 5.5		4.64–4.68; m	4.40; d, d; 7.6, 12.4	3.80; d, d; 8.5, 12.4	1.73; s	5.01; d, ABq; 12.6 5.06; d, ABq; 12.6	
18[b]	OCH_3	H	$OCONH_2$	4.42; d; 4.9		3.73–3.87; m	4.36–4.41; d, d; 9.0, 12.0	3.60; d, d; 7.3, 12.0	1.69; s	5.14; s	
20[c]	OCH_3	H	H	4.31; d; 5.2		3.76–3.87; m	4.32; d, d; 7.4, 12.0	3.51; d, d; 8.9, 12.0	1.73; s		2.28; s
30[c]	H	H	$OCONH_2$	2.95; d, d; 7.0, 16.2	2.44; d, d; 5.0, 16.2	3.98–4.01; m	4.19; d, d; 6.5, 12.4	3.69; d, d; 5.04, 12.4	1.70; s	4.97; s	
33[c,d]	D	H	$OCONH_2$		2.41–2.47; m	4.00–4.02;[e] m	4.13–4.24;[e] m	3.66–3.73;[e] m	1.70; s	4.98; s	
31[c]	H	H	OH	2.95; d, d; 6.9, 16.1	2.46; d, d; 5.0, 16.1	3.95–4.02; m	4.16; d, d; 6.5, 12.5	3.68; d, d; 4.7, 12.5	1.70; s	4.52; s	
34[c,d]	D	H	OH		2.46–2.51; m	4.00–4.02;[e] m	4.13–4.24;[e] m	3.66–3.73;[e] m	1.71; s	4.53; s	
21[c]	H	H	H	2.88; d, d; 6.8, 15.8	2.35; d, d; 4.7, 15.8	4.00–4.14; m	4.19; d, d; 6.3, 12.4	3.70; d, d; 5.0, 12.4	1.71; s		2.12; s

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant(s) in Hz. All spectra were recorded at 300.1 MHz. [b] The solvent used was CD_3OD–100%. [c] The solvent used was Me_2SO-d_6–100%. [d] The NMR sample consisted of a mixture of **33** and **34**. [e] The presence of overlapping peaks did not permit the determination of the coupling constants for this signal.

The electrochemical reduction of quinones in nonaqueous media is known to involve the initial formation of an anion radical, prior to formation of the semiquinone and hydroquinone species.[26] The potential for anion radical formation depends upon the specific type of quinone and nonaqueous solvent system employed. The overall two-electron reduction of a quinone to a hydroquinone or the one-electron reduction of a quinone to a semiquinone intermediate will involve the consumption of protons.[26] In both of these reactions the potential of electrochemical reduction will depend upon the effective hydrogen ion concentration of the solution, and potentials will shift negatively by 60 mV per each 10-fold decrease in hydrogen ion concentration. This pattern was reported for the reduction of mitomycin C in aqueous solution[27] and is classical for the quinone hydroquinone system in aqueous media.[28] In contrast, the reduction of mitomycin C in methanol occurs at potentials which are independent of the solution pH. This suggests that the first process for the reduction of mitomycin C in methanol

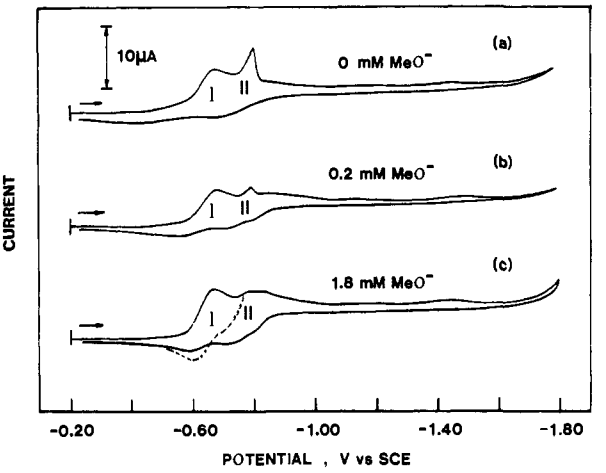


Figure 1. Cyclic voltammograms of 1.5 mM mitomycin C (**1**) in MeOH/0.1 M TBAP containing MeONa at the Hg(Au) electrode. Potential scan rate: 100 mV/s. Concentration of MeONa: (a) 0 M; (b) 0.2 mM; and (c) 1.8 mM.

(26) Ryun, C.-H.; Park, S.-M. *J. Electrochem. Soc.* **1985**, *132*, 2426–2428. Eggins, B. R.; Chambers, J. Q. *Ibid.* **1970**, *117*, 186–192. Wawzonek, S.; Berkey, R.; Blaha, E. W.; Runner, M. E. *Ibid.* **1956**, *103*, 456–459. Jaworski, J. S.; Lesniewska, E.; Kalinowski, M. K. *J. Electroanal. Chem.* **1979**, *105*, 329–334. Kamel, L. A.; Shams El Din, A. M. *Ibid.* **1970**, *24*, 230–233 and references therein.

(27) Rao, G. M.; Begleiter, A.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1977**, *124*, 199–202.

(28) Bates, R. G. In *Treatise on Analytical Chemistry*; Kolthoff, I. M., Elving, P. J., Eds.; Wiley: New York, 1978; Vol. 1, pp 805–807.

Scheme III

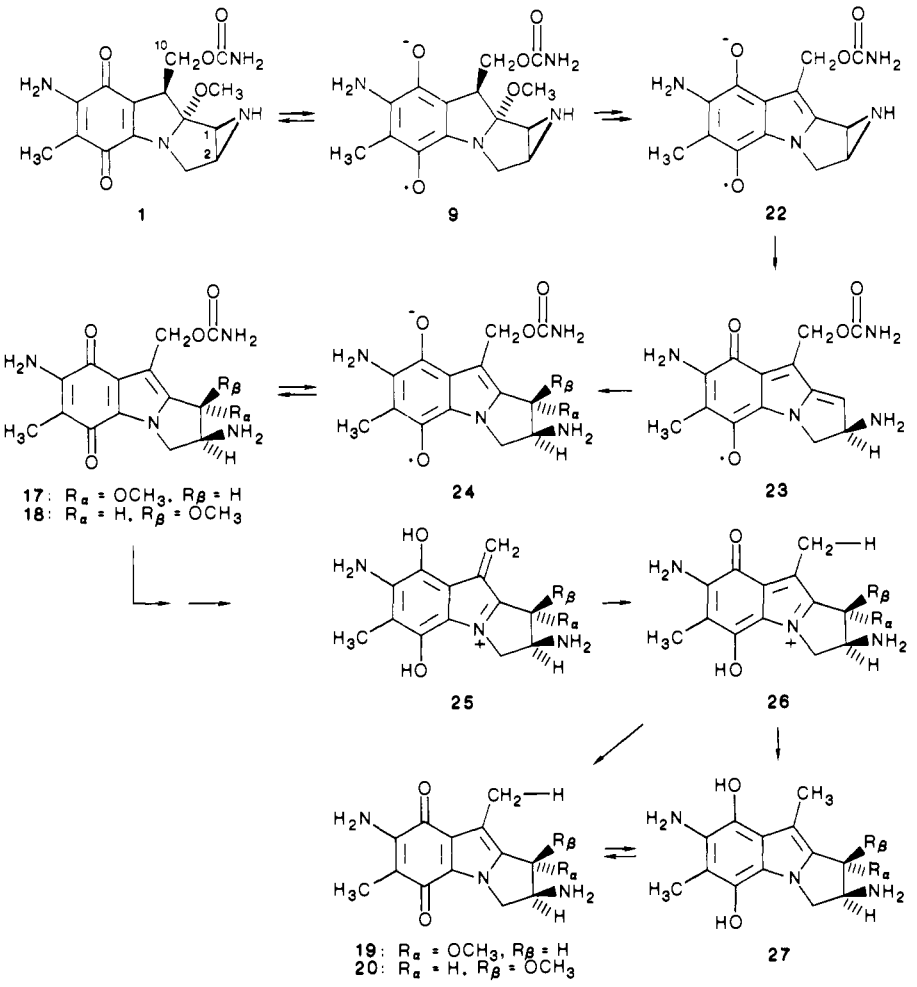
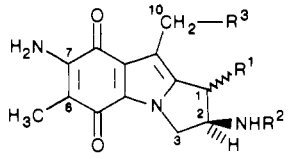


Table III. ^{13}C NMR Upfield Assignments of Mitomycin C Derivatives[a]



compd no.	C-1	C-2	C-3	C-10	C_1-OCH_3	C_6-CH_3
11[b]	74.78	64.33	54.43	58.77		8.15
12[c]	73.26	63.79	53.19	9.88		8.47
17[c]	81.61	60.57	54.06	56.89[d]	56.01[d]	8.48
19[c]	81.27	60.47	53.90[e]	10.46	53.94[e]	8.41
13[c]	65.14	57.85	51.78	56.67		8.43
18[b]	75.98	59.23[f]	52.68	59.57[f]	57.20	8.19
20[c]	73.96	58.22	51.78	10.61	56.19	8.38
30[c]	33.05	55.39[g]	55.21[g]	57.02		8.45
31[c]	33.48	54.99[h]	54.86[h]	55.41[h]		8.40

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. All spectra were recorded at 75 MHz. [b] The solvent used was MeOD-d_4. [c] The solvent used was Me_2SO-d_6. [d-h] The assignments are tentative and may be interchanged within each class.

involves formation of a quinone anion radical.

The addition of one electron in the initial reduction of **1** is also suggested by the shape of cyclic voltammograms in methanol/sodium methoxide mixtures (Figure 1c). Reversal of the negative potential sweep at −0.78 V (dashed line) leads to a well defined current voltage curve indicative of a reversible one-electron transfer ($|E_{pc} - E_{pc}| = 60 \pm 5$ mV). A one-electron addition is also indicated by ESR spectroscopy. Controlled potential electrolysis with ESR monitoring of the products was carried out on the rising portion of peak I and gave a strong free radical signal at $g = 2.01$.[29] These data indicate the formation of either a quinone anion radical or a semiquinone as the first reduction product but combined with the lack of "pH" dependence on the reduction process in methanol/sodium methoxide mixtures suggests that the former assignment is the correct one. Moreover, these data are consistent with the overall results obtained in the electrochemical reduction (cyclic voltammetry and preparative flow cell electrolysis) of mitomycin C in both dimethylformamide and dimethyl sulfoxide.[14]

Rao, Begleiter, Lown, and Plambeck have investigated the reduction of mitomycin C in buffered aqueous solutions.[27] Two successive electrochemical reduction waves were observed at −0.368 and −0.468 V and were attributed to the mitomycin C (**1**) quinone–hydroquinone couple and the ring-opened 1-hydroxy-2,7-diaminomitosene (**11**, **13**) quinone–hydroquinone couple, respectively. Significantly, the reduced 1-hydroxy-2,7-diaminomitosene hydroquinones underwent reoxidation to the corresponding quinones **11** and **13** at the applied potential utilized to reduce mitomycin C (**1**). Moreover, aziridine ring-opening was postulated to proceed at the hydroquinone level under these conditions. The quinone anion radical intermediate was not detected as is generally the case in aqueous media.

Our electrochemical results suggest that ring-opening proceeds at the quinone anion radical stage and that **24** can be reoxidized to **17** or **18** upon its formation at the electrode surface. This was

(29) For other ESR studies of **1**, see: Lown, J. W.; Sim, S.-K.; Chen, H.-H. *Can. J. Biochem.* **1978**, *56*, 1042–1047. Pan, S.-S.; Andrews, P. A.; Glover, C. J.; Bachur, N. R. *J. Biol. Chem.* **1984**, *259*, 959–966. Kalyanaraman, B.; Perez-Reyes, E.; Mason, R. P. *Biochim. Biophys. Acta* **1980**, *630*, 119–130 and references therein.

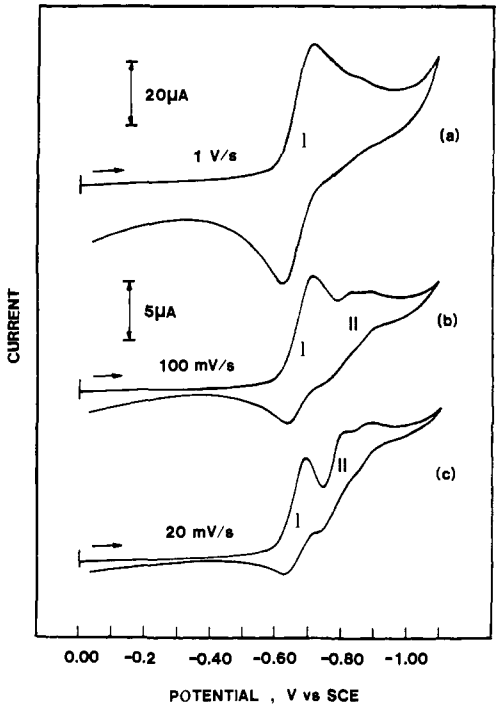


Figure 2. Cyclic voltammograms of 2.1 mM mitomycin C (**1**) in MeOH/0.1 M TBAP, 0.02 M MeONa at the Pt electrode. Potential scan rate: (a) 1 V/s; (b) 100 mV/s; and (c) 20 mV/s.

ascertained by monitoring the voltammograms as a function of scan rate and by studying genuine samples of **17** and **18**. At a scan rate of 1.0 V/s a single reversible one-electron transfer is observed for **1** $\rightleftarrows$ **9** (peak I, Figure 2a). However, as the scan rate is decreased to 100 mV/s a chemical reaction occurs (**9** $\rightarrow$ **24** $\rightarrow$ **17** + **18**) and two additional processes appear (Figure 2b). Finally, at 20 mV/s these latter reduction peaks become well defined (Figure 2c).

Controlled-potential thin-layer electrolysis was also carried out at a potential between I and II (−0.70 V) and the coulometric value of n determined in the first reduction. If the reoxidation of **24** to **17** and **18** occurred, values of n should be less than 1. The complete electroreduction gave only 0.3 to 0.4 electron and resulted in the total disappearance of the process associated with peak I. The low coulometric value of n is consistent with the ring opening and the reoxidation process shown in Scheme III (**1** $\rightarrow$ **24** $\rightarrow$ **17** + **18**). The data further suggest that the reductions at E_p = −0.80 and −0.89 V are associated with the stepwise reduction of compounds **17** and **18** and this assignment was confirmed by cyclic voltammograms for genuine samples of **17** and **18**. Pure samples of **17** and **18** have two reduction peaks which are identical in shape and potential with peaks at E_p = −0.80 and −0.89 found for the reduction of mitomycin C. This is shown in Figure 3b for the reduction of **18**. The implication of the data in Figure 3 is that controlled-potential reductions at −0.8 V (Hg electrode) allow the reaction **1** $\rightarrow$ **17** and **18** to proceed but controlled potential reductions at more negative potentials (−1.0 or 1.5 V vs. SCE) will lead to **19**, **20**, and **21**.

The exact nature of the intermediates in the reduction of **17** and **18** has not been elucidated, but the two peaks in process II may be tentatively assigned as the stepwise formation of the semiquinone and hydroquinone species.[26] Furthermore, the mechanism for the formation of the carbon-10 methyl adducts **19** and **20** in the methanol bulk electrolysis experiments has not been determined. Information which suggests that the pathway in the electrolysis experiments is similar to that described in the catalytic reductions was obtained by examining the electrolytic behavior of benzyl carbamate (**28**) in methanol. Electrolysis of **28** (Pt electrode, −1.2 V) for 1 h led to no apparent loss of starting material (HPLC analysis). The inability to observe toluene (**29**) in this experiment suggested that electrochemical reduction of the carbamate group in **24** and **28** is not an efficient process under these conditions.

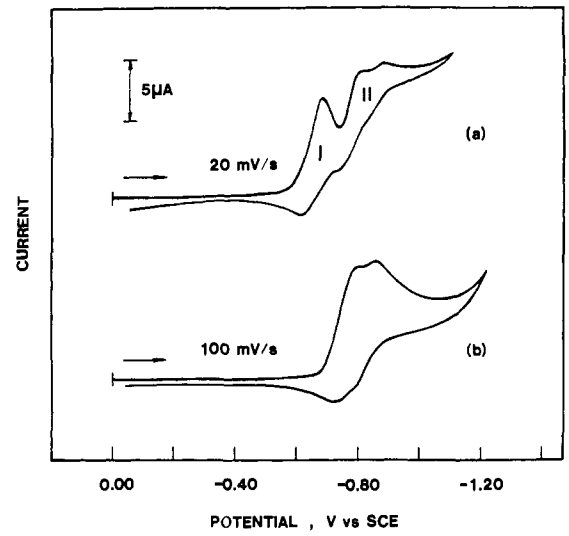
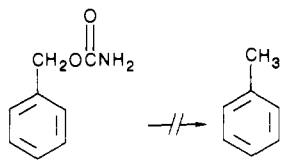


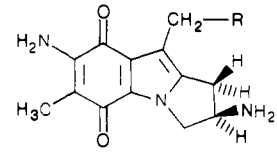
Figure 3. Cyclic voltammograms: (a) mitomycin C (**1**) in MeOH/0.1 M TBAP, 0.02 MeONa at 20 mV/s; and (b) *cis*-1-methoxy-2,7-diaminomitosene (**18**) in MeOH/0.1 M TBAP at 100 mV/s at a Pt electrode.



28 **29**

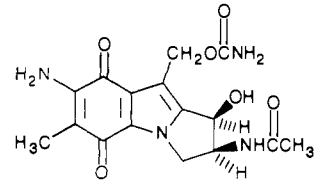
b. Aqueous Solutions. Five-milliliter samples of mitomycin C (**1**) (1.5 mM) were reduced at room temperature for 7 min with either a Hg (−1.2 V) or a Pt (−1.0 V) working electrode in three different buffered (tris acetate) solutions (pH values: 5.0, 6.5, 8.0). The Hg and Pt electrode mediated reductions gave nearly identical results. HPLC analyses of the reactions at the conclusion of the experiments indicated that only small amounts of **1** remained. Extension of the reaction time led to a rapid loss of products.

Variation of the solution pH led to important differences in the product profiles. At pH 5.0, the major products produced in near equal amounts were 2,7-diaminomitosene[8,9] (**30**) and the corresponding decarbamoyl adduct **31**. Only small amounts of *trans*- (**11**) and *cis*-1-hydroxy-2,7-diaminomitosenes[9] (**13**) were observed.



30: R = OC(O)NH$_2$
31: R = OH

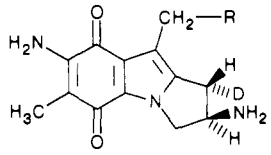
At pH 6.5, **11**, **13**, **30**, **32**, and an unidentified compound were produced in approximately equivalent amounts. The decarbamoyl derivative **31** was not detected in the product mixtures. At pH 8.0, the HPLC profile simplified. Compounds **11**, **13**, and **32** were produced in an approximate 1:1:1 ratio.



32

Repetition of these experiments under semipreparative conditions (15 mL) gave comparable results and permitted the NMR spectral characterization (see Tables II and III) of **11**, **13**, and **30–32**. Under these conditions, approximately 60% of the mitomycin C was converted to products (chromatographic analyses).

Information concerning the stereochemical pathway for the formation of **30** and **31** was obtained by rerunning these experiments (Hg electrode, −1.2 V; Pt electrode, −1.0 V) in deuteriated buffer solutions (pD 5.0). Analysis of the ^{1}H NMR spectra for the two isolated compounds **33** and **34** indicated that D-incorporation proceeded preferentially from one face of the molecule. A comparable result was previously observed under catalytic (PtO_2, H_2) reductive conditions.[8] The stereochemical assignment[8] in **33** and **34** is tentative.



33: R = $OC(O)NH_2$
34: R = OH

A pathway for the production of **11**, **13**, and **30–32** similar to Scheme III may exist in the aqueous bulk electrolysis experiments. On the basis of the earlier study of Rao, Plambeck, and coworkers,[27] reduction of **1** in water may have proceeded to the hydroquinone stage.

The absence of detectable amounts of the carbon-10 methyl adducts **12** and **14** under aqueous conditions was conspicuous. The experimental parameters (i.e., solvent, basicity, reaction times) differed in the two (water, methanol) sets of reductions. In this regard, we noted significant loss of products (i.e., precipitation) upon prolonged (>7 min) reduction in water. This undesirable factor may have prevented the buildup of the carbon-10 methyl adducts **12** and **14**. The variations noted in the product profiles for the aqueous electrolytic reductions of **1** as a function of pH were expected. Previous investigations have shown that catalytic and enzymatic reduction of **1** in H_2O (pH 5.1–9.0) leads to the production of **11**, **13**, and **30** along with other adducts.[9] Moreover, the proportion of **30** in the product mixture was noted to increase with decreasing pH values. The pathway leading to the decarbamoylated adduct **31** at this pH value has not been elucidated. This product was also reported in the electrochemical reduction of **1** in *N*,*N*-dimethylformamide at −1.450 V.[14]

Conclusions

Important new information concerning both the carbon-1 and the carbon-10 mediated processes in mitomycin C has been secured. The electrochemical studies of **1** in methanol have demonstrated that the initial reduction step, subsequent loss of methanol, and the aziridine ring-opening step all proceed at the quinone anion radical stage. These findings in conjunction with those of Andrews, Pan, Glover, and Bachur[14] and Danishefsky and Egbertson[15] provide conclusive evidence which dispels the concept that the hydroquinone species[2,16] is the obligatory intermediate in the reductive activation cascade. Moreover, our studies conducted in the protic polar solvent methanol suggest that a similar process may occur under physiological conditions. The observation of carbon-10 methyl adducts (i.e., **12**, **14**, **19**, and **20**) in both the catalytic and electrolytic reduction of mitomycin C based compounds in alcoholic solvents provide evidence that reaction at carbon-10 in **1** in vitro proceeds through the intermediacy of an iminium ion. This mechanism is similar in nature to the pathway employed for substitution at carbon-1.

In addition to these insights into the mode of action of the drug, several distinctive experimental features characterized the mitomycin bulk electrolysis studies. The reactions were efficient and the yields of aziridine ring opened mitosene adducts were competitive to those obtained with other reductive techniques. The product profiles and the stereochemical features of the electrochemical experiments in water and methanol also corresponded well with those reported for the reductive activation of mitomycin C with chemical, catalytic, and enzymatic methods. These observations argue that electrochemical activation of mitomycin C should serve as an attractive alternative to the traditional methods now utilized for the activation of the antineoplastic agent. Studies are now in progress aimed at determining the feasibility of using this technique for examining the interaction of mitomycin C with select biological substrates.

Experimental Section

Instrumentation and Solvents. Infrared spectra (FT-IR) were recorded on an IBM FT-IR/32 spectrometer. Electronic absorption spectra were obtained on a Perkin-Elmer 330 spectrophotometer. Proton (^{1}H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Nicolet NT-300 spectrometer. Chemical shifts are expressed in parts per million relative to Me_4Si, and coupling constants (J values) are given in hertz. Field-desorption mass spectra was performed by Dr. Stephen Reynolds at Exxon Research and Engineering Co. HPLC analyses were conducted with the following Waters Associates Units: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The products were eluted using the following linear gradient conditions: condition A; C_{18} μBondapak (SS) column 3.9 mm × 15 cm, from 100% A (3 mM triethylammonium phosphate pH 4.7), 0% B (3 mM triethylamine in acetonitrile) to 50% A, 50% B in 20 min; condition B, C_{18} μBondapak (SS) column 3.9 mm × 30 cm, from 100% A, 0% B to 50% A, 50% B in 25 min. Both columns were fitted with a μBondapak C_{18} guardpak. Under both conditions a flow rate of 1.0 mL/min was used and products were detected at 313 and 254 nm. The organic solvents utilized were HPLC grade and they were filtered (Millipore FH, 0.50 μm) and degassed prior to use. The aqueous buffers were prepared from deionized water (Millipore) and were filtered (Millipore HA, 0.45 μm) and degassed. pH measurements were determined with a Radiometer pHM 26 meter equipped with a Radiometer GK2320C combination glass electrode. The solvents and reactants were of the best commercial grade available and used without further purification unless noted. All H_2O used for the mitomycin C reactions was HPLC grade.

Cyclic voltammetric measurements were made with an EG and G PAR Model 174A polarographic analyzer with a homemade vacuum tight Pyrex cell and a three-electrode system. The utilized working electrode was a platinum button with a surface area of 0.8 mm^2. An aqueous SCE electrode was positioned in a glass frit as the reference electrode. ESR measurements were carried out after controlled potential reduction in a newly designed thin-layer in-situ electrochemical cell with an IBM Bruker ER/100D electron spin resonance spectrometer employing 100-kHz or 12.5-kHz field modulation. This specially constructed cell has a thin-layer chamber with dimensions of 22 × 3.5 × 0.5 mm^3. The cell utilized two auxiliary electrodes and an expanded platinum metal working electrode of dimensions 15 × 3 × 0.3 mm^3. An aqueous SCE reference electrode was positioned in a glass frit. The tip of the frit was a Luggin capillary which was positioned in the thin-layer chamber. Well-defined thin-layer cyclic voltammograms could also be obtained with this in-situ ESR thin-layer electrochemical cell thus indicating good potential control with this electrode.

General Procedures for the Catalytic Reduction of *trans*-1-Hydroxy-2,7-diaminomitosene (11). H_2 (D_2) gas bubbled through a rigorously deaerated methanol (methanol-d_1) solution (50 mL) containing **11** (4.5 mg, 14 μmol) and PtO_2 (3.2 mg) for 7 min at room temperature. The mixture was then exposed to air and filtered. HPLC analysis of the methanol solution indicated the presence of **12**. No other significant products were detected. The filtrate was concentrated in vacuo, dissolved in 99.5% methanol-d_1, and dried in vacuo (2×). The yields obtained for **12** ranged from 80 to 90%.

Compound **12**: HPLC retention time 14.6 min (condition A); FT-IR (KBr) 1607, 1385 cm^{-1}; UV-vis (MeOH) λ_{max} 248, 309, 525 nm; field-desorption mass spectrum, m/z calculated for $C_{13}H_{15}N_3O_3$ 261.111, found 261.113. The ^{1}H and ^{13}C NMR data for **12** are listed in Tables II and III, respectively.

Compound **12**-d_1: HPLC retention time 14.6 min (condition A); ^{1}H NMR (CD_3OD) δ 1.77 (s, 3 H), 2.26 (t, 2 H, J_{HD} = 2.1 Hz), 3.77–3.86 (m, 1 H), 3.87 (dd, 1 H, J = 3.8, 13.0 Hz), 4.45 (dd, 1 H, J = 6.2, 13.0 Hz), 4.69 (d, 1 H, J = 2.9 Hz).

General Procedure for the Catalytic Reduction of *cis*-1-Hydroxy-2,7-diaminomitosene (13). H_2 (D_2) gas was bubbled through a rigorously deaerated ethanol (ethanol-d_1) solution (50 mL) containing **13** (2.5 mg, 7.8 μmol) and PtO_2 (4 mg) until the reaction turned colorless. This reaction was performed at 37–40 °C and the preceding workup procedure was employed. The overall yield for **14** was 70–80%. An NMR sample was prepared by dissolving the product in 99.5% ethanol-d_1 and then concentrating the sample to dryness in vacuo (3×).

Compound **14**: HPLC retention time 16.4 min (condition A); UV–vis (MeOH) λ_{max} 248, 309, 350 (sh), 525 nm. The ^1H NMR data for compound **14** are listed in Table II.

Compound **14**-d_1: HPLC retention time 16.4 min (condition A); ^1H NMR (CD_3OD) δ 1.75 (s, 3 H), 2.25 (t, 2 H, J_{HD} = 2.1 Hz), 3.77–3.86 (m, 1 H), 3.83 (dd, 1 H, J = 7.6, 12.7 Hz), 4.44 (dd, 1 H, J = 7.2, 12.7 Hz), 4.72 (d, 1 H, J = 5.3 Hz).

General Procedure for the Controlled-Potential Reduction of Mitomycin C in Unbuffered Methanol and the Isolation and Characterization of Compounds 17, 18, 19, 20, and 21. Controlled-potential electrolysis of mitomycin C was conducted with a PAR Model 173 potentiostat/galvanostat equipped with a PAR Model 179 digital coulometer. A conventional "H"-type cell (Sargent-Welch Co.) was utilized in a three-electrode configuration. A platinum gauze auxiliary electrode was separated from the mercury pool (5 cm^2 surface area) or platinum gauze (10 cm^2 surface area) working electrode with a sintered glass frit. The reference electrode was a saturated calomel electrode (SCE). When not in use the SCE was stored in an aqueous saturated KCl solution. Deaeration of the methanolic solution was performed by passing high-purity nitrogen through the solution and maintaining a blanket of inert gas over the solution during electrolysis. In several experiments oxygen was more rigorously excluded from the cell by carrying out the experiments in a VAC Model HE-43-2 Single Sided, Single Length Dri-Lab with Model HE-493 Dri-Train. These experiments utilized a platinum electrode for the controlled-potential reduction. No differences in the products were obtained under these conditions. However, the chemical/electrochemical reactions were observed to proceed more rapidly in the drybox.

The solution was agitated with a Teflon coated magnet when the working electrode was a platinum gauze, or by passing a stream of nitrogen through the mercury pool when mercury was the working electrode. The methanol employed was freshly distilled from CaH_2 prior to use. Tetrabutylammonium perchlorate (TBAP) (0.1 M) was used as supporting electrolyte for room temperature reactions while LiCl (0.1 M) was chosen as the supporting electrolyte for experiments at −2 to −4 °C. The TBAP was purified by recrystallization from ethanol–ethyl ether and then dried in vacuo at 40 °C. When platinum was used as the working electrode, the controlled-potential reductions were carried out at either −1.0 or −1.2 V. Controlled-potential reductions at a mercury working electrode were carried out at −0.8 and −1.5 V.

Analytical scale reductions were run with 5.0-mL samples which were 1.5 mM in mitomycin C (2.5 mg, 7.5 μmol). The electorlysis was conducted for times ranging from 2 min up to 1 h. The "best" results in terms of conversion to the final products were usually attained within 20 min of electrolysis under anaerobic conditions. At this time period reduction of mitomycin C was approximately 75–85% complete.

The effective "pH" of the unbuffered methanolic solutions was measured both before and after each controlled-potential reduction and indicated the consumption of protons in the reaction. The electrode was standardized against aqueous buffer solutions. The initial "pH" in methanol was 7.4 but after reduction at −0.8 V this reading changed to 8.6. A shift from 7.4 to an effective "pH" of 10.8 was observed after reduction of mitomycin at either −1.2 V (Pt electrode) or −1.5 V (Hg electrode) vs. SCE. Similar changes in the effective "pH" value were noted in the absence of **1** with use of the same electrochemical conditions. This indicated that the reduction of methanol (to generate methoxide ion and H_2) also occurred during the controlled-potential reduction of mitomycin C.

Analysis of the product mixtures was conducted by HPLC analyses. The retention times (min) for the mitomycin C derived adducts are as follows (condition B): **1** (18.70), **17** (21.38), **18** (21.52), **19** (24.39), **20** (26.49), and **21** (26.02). An unidentified adduct was noted at 22.26 min in the electrochemical reduction conducted at −0.8 and −1.5 V with a mercury electrode and at −1.0 and −1.2 V with a platinum electrode. The relative ratios of the mitosene products reported are approximate and are based on the integrated response from the UV–HPLC detector and the assumption that the extinction coefficient of these adducts at 254 nm in the ultraviolet spectrum are approximately equal.

Isolation and characterization of compounds **17**, **18**, **19**, **20**, and **21** were carried out with 15-mL samples which were 1.5 mM in **1** (7.5 mg, 22.5 μmol). Syntheses of **17** and **18** were accomplished by reduction at a mercury working electrode (−0.8 V) for 12 min, while **19**–**21** were prepared by reduction at a platinum electrode (−1.2 V) for 50 min. The reactions were quenched by disconnecting the applied potential and at the same time admitting air into the reaction system. In order to secure sufficient amounts of purified products for NMR analyses, five samples were reduced sequentially. The samples were pooled, filtered, and concentrated (~1 mL) in vacuo. The residual material was triturated with water (75 mL) after which the aqueous mixture was cooled (~4 °C) and filtered and the filtrate placed on a G25F Sephadex column (5 × 60 cm). The eluent employed in the chromatographic separation was aqueous NH_4HCO_3 (0.02 M) and the flow rate was 30 mL/h. The fractions were then separated, collected, and lyophilized. Prior to NMR spectral analysis, each sample was washed in 99.8% D_2O and lyophilized (2×), after which it was dissolved in 99.5% methanol-d_4 and then dried in vacuo. The observed ^1H and ^{13}C NMR spectral data for these adducts are listed in Tables II and III, respectively. The following UV–vis spectral data in methanol were obtained for the observed products: **17**, λ_{max} 248, 310, 350 (sh), 525 nm; **18**, λ_{max} 248, 310, 350 (sh), 525 nm; **19**, λ_{max} 244, 311, 350 (sh), 525 nm; and **20**, λ_{max} 248, 310, 350 (sh), 525 nm.

General Procedure for the Controlled-Potential Reduction of Mitomycin C in Aqueous Media and for the Isolation and Characterization of Compounds 11, 13, 30, 31, and 32. An experimental setup identical with that described for controlled-potential reductions of **1** in unbuffered methanol was employed for the aqueous experiments. The analytical scale reactions consisted of 1.5 mM aqueous solutions (5 mL) of **1** (2.5 mg, 7.5 μmol). The reductions were carried out with either a mercury (−1.2 V) or a platinum (−1.0 V) working electrode. The reference electrode was a saturated calomel electrode (SCE). The supporting electrolyte was KCl (0.1 M). Buffer solutions were prepared by the addition of glacial acetic acid to an aqueous solution containing Tris (0.1 M) until the desired pH value was attained. Controlled-potential reductions were carried out for 7 min, during which time no significant deviations (±0.1 pH unit) in the pH value of the solution were noted. Electrolysis times longer than 7 min led to precipitation of the products. The reactions were monitored by HPLC. The retention times (min) for the observed products are as follows (condition B): **1** (18.70), **11** (17.51), **13** (20.48), **32** (19.22), **31** (20.10), **30** (21.88). HPLC analyses also indicated the presence of trace amounts of several additional products.

The experimental protocol utilized in the semipreparative methanol reductions was utilized for the isolation and characterization of compounds **11**, **13**, **30**, **31**, and **32** in aqueous media. The reactions were carried out on 15-mL buffered aqueous solutions which were 1.5 mM in **1** (7.5 mg, 22.5 μmol). The applied potentials, supporting electrolyte, buffer concentration, and reduction times were identical with those described above. At the conclusion of the experiment, air was allowed to enter the electrochemical cell.

The observed product mixtures were purified on a G25F Sephadex column (5 × 60 cm). In order to obtain sufficient amounts for NMR spectral characterization, five controlled-potential reductions were run sequentially and the samples combined and filtered. The sample was then applied to the column and eluted with aqueous NH_4HCO_3 (0.02 M) with a flow rate of 30 mL/h. The ^1H and ^{13}C NMR spectral properties for compounds **11**, **13**, and **30**–**32** are listed in Tables II and III, respectively. The following UV–vis spectral data were observed for the hydrolysis products in methanol: **11**, λ_{max} 248, 313, 350 (sh), 525 nm; **13**; λ_{max} 248, 313, 350 (sh), 525 nm; **30**; λ_{max} 243, 311, 350 (sh) nm.

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Registry No. 1, 50-07-7; **11**, 99745-88-7; **12**, 99687-41-9; **12**-d_1, 99687-42-0; **13**, 106471-83-4; **14**, 99745-89-8; **14**-d_1, 99745-90-1; **17**, 93528-47-3; **18**, 106565-34-8; **19**, 106471-84-5; **20**, 106565-35-9; **21**, 106502-73-2; **30**, 92695-32-4; **31**, 102587-27-9; **32**, 54911-22-7; **33**, 106471-85-6; **34**, 106471-86-7.