Studies on the Reactivity of Reductively Activated Mitomycin C

Pascal Schiltz and Harold Kohn*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77204-5641

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Abstract: Mitomycin C (**1a**), a clinically significant antineoplastic antiobiotic, is considered to be the prototype of bioreductive alkylating agents. It has been reported that, in the absence of DNA, reductive activation of **1a** furnished both solvolytic C(1) electrophilic (2,7-diaminomitosene (**7**)) and C(1) nucleophilic (*trans*-(**8**) and *cis*-1-hydroxy-2,7-diaminomitosene (**9**)) products. The detection of **7** as well as **8** and **9** suggested that the aziridine ring-opened quinone methide **4** served as a precursor to both sets of products. Sodium dithionite-mediated reduction of mitomycin C under conditions furnishing near complete **1a** consumption revealed that proton capture to give **7** was the dominant process (77.2–87.8%) between pH 5.5 and 8.5. Earlier observations that **8** and **9** were generated in mildly basic solutions have now been largely attributed to secondary transformations proceeding from 7-aminoaziridinomitosene (**21**). The propensity of reductively activated mitomycin C to undergo C(1) electrophilic substitution processes was further assessed by incorporating aniline in the reaction mixture. In moderately basic solutions, C(1) electrophilic transformations predominated, whereas in mild acid, appreciable amounts of C(1) nucleophilic adducts were detected. The observed results are discussed in terms of both the *in vivo* mitomycin C reductive process and the requirements for the efficient cross-linking of complementary strands of DNA by **1a**.

Mitomycin C (**1a**) is considered the protypical bioreductive alkylating agent.[1] Drug function has been proposed by others to proceed by initial enzymatic reduction of the quinone ring followed by loss of methanol and aziridine ring opening to give quinone methide **4** (Scheme I).[2] Attack by a nucleophilic site on DNA at C(1) in **4** generates the mitomycin C–DNA monoadduct **5**. Subsequent displacement of the C(10) carbamate group in **5** by a base on the complementary DNA strand produces the cross-linked DNA product **6**.[2a,3] The antitumor activity of the drug has been attributed to its ability to inhibit DNA replication and subsequent cell division, presumably through **6**. Attempts to model the biological reductive process in the absence of DNA have provided information concerning this mechanistic scenario.[4-17]

Use of a variety of reductants (enzymatic,[4b,5,8a,10] catalytic,[4b,9d,13a] chemical,[5,6b,c] electrochemical,[7,8b,13c] and pulse radiolytic[11,14]) have shown that mitomycin C reduction leads first to the modification of the C(1) site. Characteristic of these investigations was the marked dependence of pH on the C(1) product profile, the diminished reactivity of the C(10) site versus the C(1) position,[6b,c,9a,13c-g,18] and the incomplete consumption of **1a**. Under slightly acidic conditions, the electrophilic product **7** dominated, whereas in moderate base, *trans*- (**8**) and *cis*-1-hydroxy-2,7-diaminomitosene (**9**) were generated in high yields.[4b,8a,10,13i] Quinone methide **4**[1c] has been suggested to be the central precursor to **7** and to **8** and **9** (Scheme II).[4b] Significantly, the finding that the C(1) hydroxy adducts **8** and **9** were the principal products at near neutral pH values has been cited as evidence that reductively activated mitomycin C is prone to nucleophilic substitution transformations.[4b,8a,10,13i] Recently, the importance of 2,7-diaminomitosene (**7**)[4b,13a,h] in these processes has been emphasized by the demonstration of **7** as the principal metabolite produced after administration of **1a** to HT-29 cytosol, purified HT-29 human colon carcinoma cells, and rat hepatic DT-diaphorase.[18]

We report here that the proton-transfer process (**1** ⟶⟶**4** ⟶ **7**) is the *major* pathway for reductively activated mitomycin C at all operational pH values (pH 5.5–8.5) in the absence of external

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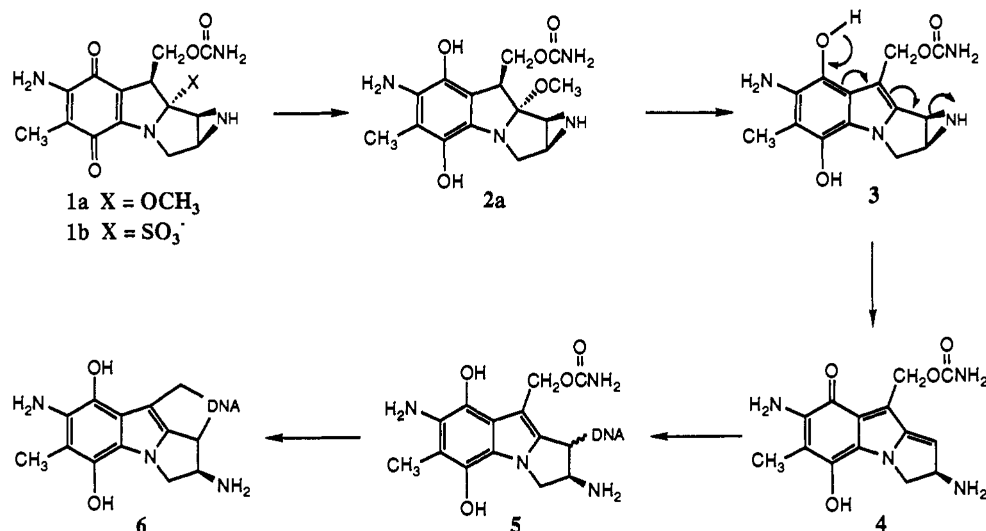
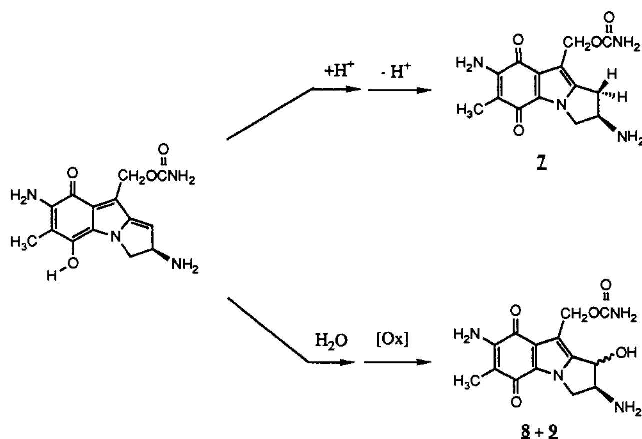
Scheme I. Proposed Mechanism for the Mode of Action of Mitomycin C



Scheme II. Previously Proposed Pathway for the Generation of Mitosenes **7–9**



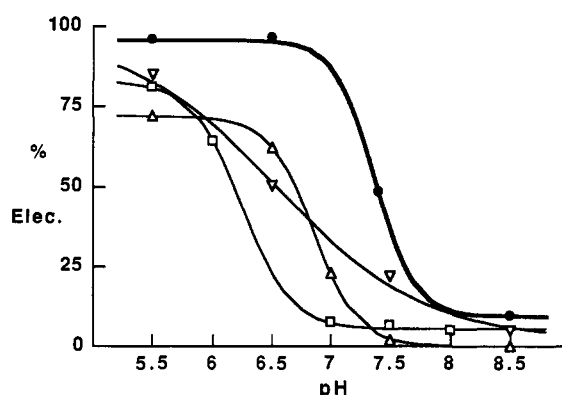


Figure 1. Percent of C(1) electrophilic mitosene products generated as a function of pH using various reductive protocols. Reductants: $Na_2S_2O_4$ (0.2 equiv)[21] (●), *cis*-10-(decarbamoyloxy)-1,10-dimethoxy-2,7-diaminomitosene–$Cr(ClO_4)_2$ complex[13i] (□), (4-methoxyphenyl)hydrazine[13g,h] (▲), PtO_2/H_2[24b] (▽).

nucleophiles.[19] Previous reports on the generation of the solvolytic C(1) hydroxy adducts **8** and **9** at pHs above neutrality[4b,8a,10,13i] are now attributed to secondary transformations that are not considered characteristic of the inherent reactivity of reductively activated mitomycin C. Informaiton is presented on the effect of an added nucleophile on the mitomycin C(1) product profile. We discuss these findings in light of previous results for mitomycin C–DNA cross-linked adduct **6** generation as a function of pH.[20]

Results

We first observed that the activation of aqueous solutions of mitomycin C at pH 7.4 with $Na_2S_2O_4$ provided higher levels of drug activation and increased percentages of C(1) electrophilic products than did other reductants (Figure 1).[21] Moreover, further enhancement in the relative amounts of C(1) electrophilic versus C(1) nucleophilic products was noted when 1 equiv of $Na_2S_2O_4$ was added in a single shot rather than in five 0.2-equiv incremental additions. These findings prompted us to revisit these transformations. Two different $Na_2S_2O_4$-based reductive procedures were established. In the first one, excess amounts of $Na_2S_2O_4$ (1.2–2.0 equiv) were used to obtain near complete drug consumption. The $Na_2S_2O_4$ was added in a single shot. In the second procedure, we used limiting amounts of $Na_2S_2O_4$ (0.2 equiv) to ensure a low percentage of drug activation. In both procedures, the concentration of mitomycin C was 0.3 mM.[22] A parallel set of experiments were conducted in the presence of aniline.

a. Use of Excess Amounts of $Na_2S_2O_4$. Deaerated, aqueous buffered solutions (pH 5.5, 6.5, 7.4, and 8.5) of mitomycin C were treated with an excess amount of $Na_2S_2O_4$. The amount of reductant needed to ensure high levels of drug modification depended upon the pH of the reaction. The optimal conditions were determined by performing the reactions under varying amounts of $Na_2S_2O_4$ and analyzing the product mixtures by HPLC. These preliminary studies indicated that as the pH of the solution was raised the reductant had to be increased to obtain levels of **1a** activation that exceeded 89%. This trend has been attributed in part to the increased difficulty to reduce quinones with increasing pH. Previous studies have demonstrated that the potential of electrochemical reductions of quinones depends upon the effective hydrogen ion concentration of the solution and that the potentials shift negatively by 60 mV per each 10-fold decrease in hydrogen ion concentration.[23] Accordingly, at pH 5.5 and 6.5, 1.2 equiv of $Na_2S_2O_4$ was used, whereas at pH 7.4 and 8.5, 1.5 and 2.0 equiv were used, respectively. The reaction

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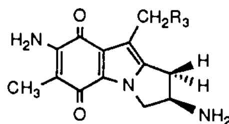
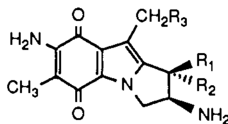
was stirred at room temperature (6 min), quenched by introducing O_2 to the solution (30 s), and analyzed by HPLC (programs 1 and 2).

These HPLC chromatograms revealed only trace amounts of unreacted **1a** and mitosenes **7–15**,[4b,8a,10,13a,h,i,21] and that the major





7 $R_3 = OC(O)NH_2$
16 $R_3 = SO_3^-$
17 $R_3 = OH$
18 $R_3 = H$
24 $R_3 = NHPh$

8 $R_1 = H$, $R_2 = OH$, $R_3 = OC(O)NH_2$
9 $R_1 = OH$, $R_2 = H$, $R_3 = OC(O)NH_2$
10 $R_1 = H$, $R_2 = SO_3^-$, $R_3 = OC(O)NH_2$
11 $R_1 = SO_3^-$, $R_2 = H$, $R_3 = OC(O)NH_2$
12 $R_1 = H$, $R_2 = OH$, $R_3 = SO_3^-$
13 $R_1 = OH$, $R_2 = H$, $R_3 = SO_3^-$
14 $R_1 = H$, $R_2 = SO_3^-$, $R_3 = SO_3^-$
15 $R_1 = SO_3^-$, $R_2 = H$, $R_3 = SO_3^-$
19 $R_1 = H$, $R_2 = OH$, $R_3 = H$
20 $R_1 = OH$, $R_2 = H$, $R_3 = H$
22 $R_1 = H$, $R_2 = NHPh$, $R_3 = OC(O)NH_2$
23 $R_1 = NHPh$, $R_2 = H$, $R_3 = OC(O)NH_2$
25 $R_1 = H$, $R_2 = NHPh$, $R_3 = NHPh$
26 $R_1 = H$, $R_2 = NHPh$, $R_3 = SO_3^-$
27 $R_1 = NHPh$, $R_2 = H$, $R_3 = SO_3^-$
29 $R_1 = H$, $R_2 = OCH_3$, $R_3 = OC(O)NH_2$
30 $R_1 = OCH_3$, $R_2 = OH$, $R_3 = OC(O)NH_2$

product of each reaction was 10-(decarbamoyloxy)-2,7-diaminomitosene-10-sulfonate (**16**).[21] The identities of mitosenes **7–16** were established by coinjection of authentic samples with the reaction solutions under the two sets of HPLC conditions.[21] As seen on HPLC chromatograms, using program 1, the excess amounts of $Na_2S_2O_4$ employed in this protocol produced a broad, undefined pattern of products located between 17.5 and 23.5 min (approximately 10–15% of the reaction mixture) similar to that described for the addition of 1 equiv of $Na_2S_2O_4$.[21] This undefined pattern of peaks was not observed with program 2. Two new peaks were detected using both HPLC programs. The percentage of these two adducts never exceeded 9.3% of the reaction mixture. Attempts to identify these two products were unsuccessful. Coinjections of authentic samples of the C(1) electrophilic products **17**[13h,i] and **18**[13c] and of the C(1) nucleophilic adducts **19** and **20**[13b] with the reaction mixtures ruled out the possibility that the unidentified peaks corresponded to these compounds. Consequently, the peaks were not included in the calculations of the percent of C(1) and C(1),C(10) mitosene modifications and the percent of C(1) and C(10) nucleophilic adducts.

Table I lists the average percentage yields for the observed products corresponding to each reaction obtained using HPLC program 2.[24] For each analysis, we have also reported the percentage of **1a** remaining, the percentage of the total reaction mixture in which modification of **1a** occurred at only the C(1) or both the C(1) and the C(10) sites, the percentage of the modified products in which nucleophilic processes occurred at the C(1) and C(10) positions, respectively, and the percentage of the C(1) nucleophilic products where nucleophilic sulfonation processes had occurred.

Examination of the results obtained in Table I indicated that the use of excess amounts of $Na_2S_2O_4$ permitted the near complete consumption of **1a** (% unreacted **1a** = 3.1–11.0). Furthermore, C(1) electrophilic adducts were the major products at all pH values (% **7** + **16**: pH 5.50, 87.8; pH 6.50, 89.9; pH 7.40, 83.4; pH 8.50, 77.2) (Figure 2). This finding was in contrast with previously reported results, which showed that C(1) electrophilic adducts were the dominant compounds produced below neutrality, while C(1) nucleophilic adducts were generated in high yields above pH 7.[4b,8a,10,13i]

(24) The small amount of **1a** remaining at the conclusion of the reaction permitted us to determine the relative amounts of **7** and **9** using this HPLC program.

Table I. HPLC Product Profile Observed for Reduction of Mitomycin C in H_2O with Excess Amounts of $Na_2S_2O_4$[a]

compd no.	pH 5.5	pH 6.5	pH 7.4	pH 8.5
7	1.2 [1.3]	4.3 [4.5]	6.6 [7.0]	11.0 [12.0]
16	81.2 [83.8]	73.2 [76.7]	63.3 [68.3]	50.9 [57.3]
8 + **9**[b]	2.7 [2.7]	2.9 [3.0]	3.7 [4.0]	8.8 [9.7]
10 + **11**[c]	0.6 [0.7]			
12 + **13**[b]	4.1 [4.3]	2.1 [2.2]	7.0 [7.6]	5.2 [5.9]
14 + **15**[c]	4.1 [4.2]	3.7 [3.9]	3.2 [3.5]	4.3 [4.8]
unknowns[d]	3.0 [3.0]	9.3 [9.7]	9.0 [9.6]	8.8 [10.3]
1a	3.1	4.5	7.2	11.0
% C(1) mod[e]	4.8	8.4	12.3	24.7
% C(1),C(10) mod[f]	95.2	91.6	87.7	75.3
% C(1) nucl[g]	12.2	10.1	16.6	22.8
% C(10) nucl[h]	100	100	100	100
% C(1) SO_3^- nucl[i]	40.9	42.5	23.0	23.5

[a] Reductive activation of a deaerated, aqueous buffered solution of **1a** was initiated by the addition of a freshly prepared, deaerated, aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$. The reaction was stirred for 6 min (room temperature), quenched by the administration of O_2 (30 s), and analyzed by HPLC (UV detection). All the data in this table were computed from the HPLC profiles obtained using program 2 conditions. The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of the product after exclusion of unreacted **1a**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] Significantly greater amounts of the trans adduct versus the cis isomer were detected. [d] Two peaks (HPLC t_R: program 1 17.1, 19.6 min; program 2 17.3, 22.7 min) were not identified. [e] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. The unidentified compounds were not included in this calculation. [f] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. The unidentified compounds were not included in this calculation. [g] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) sulfonato adducts. Unreacted **1a** and unknown compounds were not included in this calculation. [h] MC C(10) nucleophilic compounds corresponded to C(10) sulfonato adducts. The percentage does not include unreacted **1a**, unidentified products, and only C(1)-modified MC compounds. [i] The percent of MC C(1) sulfonato products over all C(1) nucleophilic products.

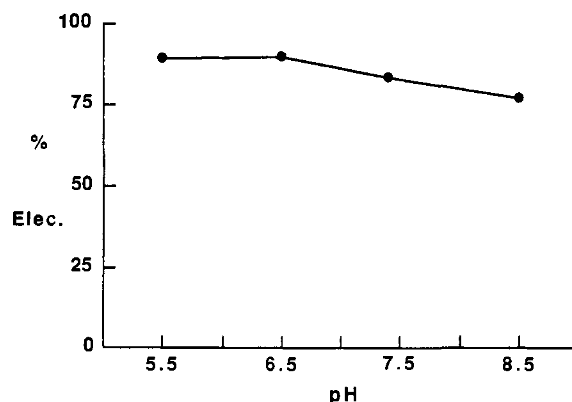


Figure 2. Percent of C(1) electrophilic mitosene products generated as a function of pH with excess amounts of $Na_2S_2O_4$.

b. Use of Limiting Amounts of $Na_2S_2O_4$. A comparable procedure was employed for the reductive activation of **1a** with substoichiometric amounts of $Na_2S_2O_4$. Deaerated aqueous buffered solutions of mitomycin C were treated with 0.2 equiv of $Na_2S_2O_4$ at pH 5.5, 6.5, 7.4, and 8.5. The reactions were stirred at room temperature (6 min), quenched by introduction of air to the solution (30 s), and analyzed by HPLC (programs 1 and 2). Inspection of the HPLC chromatograms from program 1 conditions for the pH 5.5, 6.5, and 7.4 reactions revealed that (1) large amounts of unreacted mitomycin C were present at the conclusion of each reaction (76.0–79.7%), (2) mitosenes **7–9** were the major products of the reactions (21.2–33.2%), (3) the mitosene

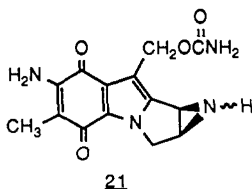
Table II. HPLC Product Profile Observed for Reduction of Mitomycin C in H_2O with Limiting Amounts of $Na_2S_2O_4$[a]

compd no.	pH 5.5[b]	pH 6.5[b]	pH 7.4[b]	pH 8.5[b]	pH 8.5[c]
7	20.9 [87.0]	15.0 [73.9]	6.6 [32.5]	0.0 [0.0]	0.0 [0.0]
16	0.9 [3.7]	2.8 [13.9]	2.2 [11.1]	2.4 [6.9]	2.6 [6.3]
8 + **9**[d]	0.3 [1.2]	0.2 [0.9]	9.2 [45.2]	22.0 [62.4]	33.2 [81.2]
10 + **11**[e]				0.5 [1.6]	0.5 [1.3]
12 + **13**[d]	0.6 [2.5]	0.4 [2.0]	0.3 [1.4]		
unknowns[f]					2.9 [7.1]
21				8.8 [24.9]	
1b	1.3 [5.6]	1.9 [9.3]	2.0 [9.8]	1.5 [4.2]	1.7 [4.1]
1a	76.0	79.7	79.7	64.8	59.1
% C(1) mod[g]	93.5 (88.3)	82.6 (74.9)	86.3 (77.8)	90.4 (85.2)	92.7 (88.5)
% C(1),C(10) mod[h]	6.6 (6.3)	17.4 (15.8)	13.7 (12.3)	9.6 (9.1)	7.3 (6.9)
% C(1) nucl[i]	4.0	3.3	51.9	90.4	92.7
% C(10) nucl[j]	100	100	100	100	100
% C(1) SO_3^- nucl[k]	0.0	0.0	0.0	2.2	1.5

[a] Reductive activation of a deaerated, aqueous buffered solution of **1a** was initiated by the addition of a freshly prepared, deaerated, aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$ (0.2 equiv). The solution was stirred for 6 min (room temperature), quenched by administration of air (30 s), and analyzed by HPLC (UV detection). The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of the product after exclusion of unreacted **1a**. [b] The data were computed from the HPLC profiles obtained using program 1 conditions. [c] The data in this column were derived from the HPLC profiles obtained using program 2 conditions. [d] Appreciable quantities of both cis and trans adducts were detected. [e] Significantly greater amounts of the trans adduct versus the cis isomer were detected. [f] Two peaks (HPLC t_R: program 2 17.8, 18.4 min) were not identified. [g] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. The value in parentheses included **1b** in the denominator. [h] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. The value in parentheses included **1b** in the denominator. [i] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) sulfonato adducts. Unreacted **1a**,**b** and **21** were not included in this calculation. [j] MC C(10) nucleophilic compounds corresponded to C(10) sulfonato adducts. The percentage does not include unreacted **1a**,**b**, **21**, and only C(1)-modified MC compounds. [k] The percent of MC C(1) sulfonato products over all C(1) nucleophilic products.

sulfonato adducts **10**–**13** and **16** represented only a minor amount of the reaction mixtures (1.5–3.2%), and (4) small amounts of the C(9a) sulfonate **1b**[6a,21] were detected (1.3–2.0%). The identities of **1b**, **7**–**13**, and **16** were established by coinjection of authentic samples with the reaction solutions under two sets of HPLC conditions. Analysis of the HPLC product composition for these three reactions using program 2 gave nearly identical results.

Examination of the corresponding pH 8.5 reaction using program 1 conditions showed that two additional mitosene peaks (retention times = 18.6 and 20.2 min) were present (8.8%) in a 1.6:1 ratio, along with mitosenes **8** and **9** (22.0%), traces of **10**, **11**, **16**, and **1b**, and substantial amounts of unreacted **1a** (64.8%). The 18.6- and 20.2-min peaks were absent in the program 1 HPLC analysis of the reaction solution after 1 h. Other than **1a**, compounds **8**–**11**, **16**, and **1b** were the only observed mitomycin adducts, and no further consumption of **1a** was noted. A somewhat different product profile was observed for the pH 8.5 transformation using the HPLC program 2. The two new mitosene peaks were absent after 6 min of reaction, and there was a concomitant increase in the relative amounts of **8** and **9** (33.2%). This increase was approximately equal to the percentage of the two unknown peaks observed with HPLC program 1. These findings were consistent with the notion that the two species detected on HPLC program 1 were converted to **8** and **9** under program 2 conditions. One likely set of candidates for these species were *syn*- and *anti*-7-aminoaziridinomitosene (**21**).[25] These adducts were first



reported by Plambeck and co-workers[7] and were recently prepared on a semipreparative scale by Han and Kohn.[25]

In order to test this hypothesis, 7-aminoaziridinomitosene (**21**) was synthesized.[25] Program 1 HPLC analysis of a DMSO solution of **21** revealed the presence of two peaks eluting at 18.6 and 20.2 min in a ratio of 1.6:1 (% **21** = 44.6) in addition to **8** and **9** (% **8** + **9** = 55.4). This result agrees with the ^1H NMR spectrum (DMSO-d_6) of **21** that indicated that the *syn* and *anti* conformers of 7-aminoaziridinomitosene were present in a 1.7:1 ratio.[25] Coinjection of a DMSO solution containing **21** with the pH 8.5 reaction solution revealed the coelution of the two unknown peaks with the two peaks corresponding to **21** (program 1). Program 2 HPLC analysis of the DMSO solution of **21** identified peaks **8** and **9** after coinjection with authentic samples.

Conclusive identification of the key peaks observed in the HPLC chromatograms at each pH for the $Na_2S_2O_4$ reduction of mitomycin C in H_2O permitted us to quantitatively monitor the course of the reaction. Table II lists the average percentage yields for the observed products corresponding to each reaction using HPLC program 1. For the pH 8.5 reaction, we have included the data obtained with HPLC program 2 as well. For each analysis, we have also summarized the extent and type of modification that proceeded at the C(1) and C(10) sites in **1a**.

Inspection of the results in Table II indicated that using limiting amounts of $Na_2S_2O_4$ (0.2 equiv) led to substantial levels of unreacted **1a** (% unreacted **1a** = 79.7–64.8). Significantly, we noted a marked shift in the percentage of C(1) nucleophilic adducts in the product mixture as the pH of the solution was increased from pH 5.5 to 8.5. In moderately acidic solutions, the C(1) nucleophilic adducts accounted only for a small portion of the products generated (≤4%), while **1a** activation in base furnished high amounts of these products (>90%). This pH dependence on the product profile was consistent with previous reports[4b,8a,10,13i] and differed from those observed under conditions where drug utilization was near complete (Table I).

c. Use of Excess Amounts of $Na_2S_2O_4$ in the Presence of Aniline. The proclivity of reductively activated mitomycin C to furnish C(1) electrophilic adducts between pH 5.5 and 8.5 prompted us to determine the effect of an added nucleophile on the pH–product profile. Accordingly, near complete reductive activation of a deaerated, aqueous buffered solution containing **1a** and aniline (4 equiv) was accomplished with an excess amount of $Na_2S_2O_4$. The reaction was performed at pH 5.5, 6.5, 7.4, and 8.5. The same amounts of reductant previously employed in the absence of aniline (Table I) were utilized in these investigations, and the

(25) (a) Han, I.; Kohn, H. *J. Org. Chem.* **1991**, *56*, 4648–4653. (b) Han, I. Ph.D. Dissertation, University of Houston, Houston, TX, 1991.

reactions were performed and analyzed using the conditions previously described.

Inspection of HPLC chromatograms (programs 1 and 2) obtained under these conditions indicated the presence of mitosenes **7–9**, **12**, **13**, **16**, **18**, and **22–25**[26] and a small amount of unreacted **1a**. Two new mitosenes **26** and **27** were also detected in these reactions. Once again, the program 1 HPLC chromatograms revealed a broad, undefined pattern of products that was not observed using HPLC program 2. An authentic sample of **26** was prepared by treatment of an aqueous solution containing **22** and K_2SO_3 (20 equiv) with $Na_2S_2O_4$ (0.5 equiv). The identities of mitosenes **7–9**, **12**, **13**, **16**, **18**, and **22–26** were established by coinjection of authentic samples with the reaction solutions under the two HPLC programs. Assignment of compound **27** was tentative and was based on supporting HPLC data. The $Na_2S_2O_4$ reduction (0.5 equiv, 10 min) of an aqueous solution containing **22** and **23** revealed the presence of two peaks eluting at the same retention times as compounds **26** and **27** (HPLC analysis). Coinjection in the HPLC (programs 1 and 2) of this solution with the reaction mixture containing **26** and **27** led to a proportional increase in the signals attributed to **26** and **27**. Finally, two peaks (retention times = 17.1 and 19.6 min) could not be identified in the pH 7.4 and 8.5 reactions. These two peaks were listed as unknown compounds and were not included in the calculation of the percent of C(1) and C(1),C(10) mitosene modification and the C(1) and C(10) nucleophilic adducts. The percentage yield of these two compounds never exceeded 7.6%.

Table III lists the average percentage yields for the observed products corresponding to each reaction using HPLC program 2, along with a summary of the reaction processes that occurred at both C(1) and C(10) in **1a**. Under these conditions, near complete consumption of mitomycin C was observed. Moreover, substantial amounts of the C(1) anilino nucleophilic products **22**, **23**, and **25–27** were only detected at low pH values (pH 5.5, 74.8%), while at high pH values the C(1) electrophilic adduct **16** predominated (pH 8.5, 70.8%).

d. Use of Limiting Amounts of $Na_2S_2O_4$ in the Presence of Aniline. Our initial studies concluded by the treatment of deaerated, buffered solutions (pH 5.5–8.5) of mitomycin C and aniline (4 equiv) with substoichiometric amounts of $Na_2S_2O_4$ (0.2 equiv) under the same conditions previously described. The HPLC chromatograms (programs 1 and 2) indicated the major compounds were the anilino-substituted mitosenes **22–25**. In addition, we observed **7–9**, **16**, traces of **1b**, and appreciable amounts of **1a**.

Table IV lists the average percentage yields for the observed products corresponding to each reaction, along with a compilation summarizing the extent of modification that proceeded at the C(1) and C(10) sites in **1a** and the type of C(1) adducts. Examination of these results indicated that although only limiting amounts of $Na_2S_2O_4$ (0.2 equiv) were used substantial amounts of mitomycin C consumption occurred (% **1a** consumption: pH 5.5, 89.0; pH 6.5, 58.7; pH 7.4, 43.2; pH 8.5, 33.4). Furthermore, under acidic conditions, >50% of **1a** was modified at *both* the C(1) and C(10) sites. Finally, we noted that the C(1) nucleophilic adducts accounted for a major portion of the C(1)-activated products (% C(1) nucl: pH 5.5, 72.3; pH 6.5, 59.2; pH 7.4, 52.1; pH 8.5, 89.3). The high yields of C(1) nucleophilic adducts at pH 5.5 and 6.5 were in contrast to the near exclusive production of C(1) electrophilic compounds when aniline was omitted from these reactions (Table II).

Discussion

Previous investigations have shown that the percentage of C(1) electrophilic products generated in mitomycin C reductive activation processes had a marked dependence on pH level[4b,8a,10,13g,h,i,25b] (Figure 1). In these transformations, substantial amounts of **1a** remained at the conclusion of the reaction. The origin of this pH dependence has been discovered by monitoring the product profiles for $Na_2S_2O_4$-mediated **1a** reductive processes under conditions that mitomycin C consumption was near complete and conditions that it was not.

Use of excess amounts of $Na_2S_2O_4$ (1.2–2.0 equiv) permitted the near complete activation of **1a** (≥89%). Under these conditions, C(1) electrophilic transformations arising from proton-transfer processes dominated (≥77.2%) (Figure 2), and a large percentage (≥75.3%) of the products were modified at both the C(1) and C(10) sites at all pH values examined. Substoichiometric amounts of $Na_2S_2O_4$ (0.2 equiv) only partially consumed **1a** (≤40.9%). HPLC analysis of the pH 8.5 reaction revealed that *syn*- and *anti*-7-aminoaziridinomitosene (**21**) were present along with **7**, **8**, and **9**. Little C(10) activation in **1a** was observed (≤17.4%).

The detection of significant amounts of **21** at pH 8.5 with 0.2 equiv of $Na_2S_2O_4$ permitted us to suggest that formation of *trans*-(**8**) and *cis*-1-hydroxy-2,7-diaminomitosene (**9**) in these processes arose from solvolysis of 7-aminoaziridinomitosene (**21**) and not from the reductively activated species **3** or **4**. We propose, at high pH values when reduction of **1a** is incomplete, the acid-promoted aziridine ring opening of **3** is sufficiently slow to permit the reoxidation of this species to **21**. Generation of **21** could have

Table III. HPLC Product Profile Observed for Reduction of Mitomycin C in H_2O with Excess Amounts of $Na_2S_2O_4$ in the Presence of Excess Aniline[a]

compd no.	pH 5.5	pH 6.5	pH 7.4	pH 8.5
7	3.5 [3.5]	2.1 [2.2]	3.2 [3.4]	2.8 [3.1]
16	17.5 [18.0]	35.6 [36.6]	60.5 [63.9]	70.8 [78.1]
18	1.7 [1.8]	5.3 [5.4]		
24			5.4 [5.7]	0.8 [0.9]
8 + **9**[b]				2.4 [2.6]
12 + **13**[b]			6.9 [7.3]	2.5 [2.8]
26 + **27**[b]	42.9 [44.0]	40.9 [42.2]	6.2 [6.5]	3.8 [4.1]
22 + **23**[b]	4.9 [5.1]	2.5 [2.6]	0.3 [0.4]	
25	27.0 [27.6]	10.7 [11.0]	10.1 [10.8]	
unknowns[c]			2.0 [2.0]	7.6 [8.4]
1a	2.5	2.9	5.4	9.3
% C(1) mod[d]	8.6	4.7	3.8	6.3
% C(1),C(10) mod[e]	91.4	95.3	96.2	93.7
% C(1) nucl[f]	76.8	55.7	25.5	10.5
% C(10) nucl[g]	98.1	94.3	100	100
% C(1) aniline nucl[h]	100	100	70.6	43.7
% C(10) aniline nucl[i]	30.9	12.3	17.4	1.0

[a] Reductive activation of a deaerated, aqueous buffered solution of **1a** in the presence of aniline (4 equiv) was conducted by the addition of a freshly prepared, deaerated, aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$. The reaction mixture was stirred for 6 min (room temperature), quenched by the administration of O_2 (30 s), and analyzed by HPLC (UV detection). All the data in this table were computed from the HPLC profiles obtained with program 2 conditions. The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of the product after exclusion of unreacted **1a**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] Two peaks (HPLC t_R: program 1 17.1, 19.6 min; program 2 17.3, 22.7 min) were not identified at pH 7.4 and 8.5. [d] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. The unidentified compounds were not included in this calculation. [e] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. The unidentified compounds were not included in this calculation. [f] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) anilino adducts. Unreacted **1a** and unknown compounds were not included in this calculation. [g] MC C(10) nucleophilic compounds corresponded to C(10) anilino and C(10) sulfonato adducts. The percentage does not include unreacted **1a** and only C(1)-modified MC compounds. [h] The percent of MC C(1) anilino products over all C(1) nucleophilic products. [i] The percent of MC C(10) anilino products over all C(10) nucleophilic products.

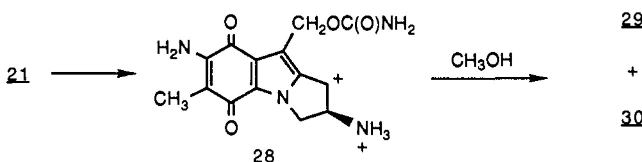
(26) (a) Kohn, H.; Hong, Y. P. *J. Org. Chem.* **1991**, *56*, 6479–6482. (b) Hong, Y. P. Ph.D. Dissertation, University of Houston, Houston, TX, 1991.

Table IV. HPLC Product Profile Observed for Reduction of Mitomycin C in H_2O with Limiting Amounts of $Na_2S_2O_4$ in the Presence of Excess Aniline[a]

compd no.	pH 5.5	pH 6.5	pH 7.4	pH 8.5
7	13.7 [15.5]	13.0 [22.3]	7.0 [16.2]	2.9 [9.4]
16		0.2 [0.4]	0.4 [0.9]	
24	10.0 [11.4]	9.3 [15.8]	12.4 [28.6]	1.3 [3.7]
8 + **9**[b]			4.1 [9.6]	6.8 [20.9]
22 + **23**[b]	12.6 [14.2]	14.2 [24.6]	14.5 [33.6]	15.1 [44.6]
25	49.4 [55.3]	18.5 [30.9]	2.9 [6.9]	3.1 [8.7]
unknowns[c]	2.1 [2.3]			3.6 [10.9]
1b	1.2 [1.3]	3.5 [6.0]	1.9 [4.2]	0.6 [1.8]
1a	11.0	41.3	56.8	66.6
% C(1) mod[d]	30.7 (30.3)	49.3 (46.3)	62.0 (59.3)	88.5 (86.6)
% C(1),C(10) mod[e]	69.3 (68.4)	50.7 (47.7)	38.0 (36.3)	11.5 (11.2)
% C(1) nucl[f]	72.3	59.2	52.1	89.3
% C(1) anil nucl[g]	100	100	80.9	75.5

[a] Reductive activation of a deaerated, aqueous buffered solution of **1a** in the presence of aniline (4 equiv) was initiated by the addition of a freshly prepared, deaerated, aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$ (0.2 equiv). The reaction was conducted at pH 5.5, 6.5, 7.4, and 8.5. The solution was stirred for 6 min (room temperature), quenched by administration of O_2 (30 s), and analyzed by HPLC (UV detection). The reactions were run in duplicate and averaged. All the data in this table were computed from the HPLC profiles obtained with program 2 conditions. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of the product after exclusion of unreacted **1a**. [b] Greater amounts of the trans adducts versus the cis isomers were detected. [c] At pH 5.5, a peak (HPLC t_R: program 1 20.8 min; program 2 21.6 min) was not identified, while at pH 8.5, two signals were unidentified (HPLC t_R: program 1 19.6, 21.8 min; program 2 17.9, 22.1 min). [d] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. The value in parentheses includes **1b** in the denominator. The unidentified compound(s) were not included in this calculation. [e] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. The value in parentheses includes **1b** in the denominator. The unidentified compound(s) were not included in this calculation. [f] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) anilino adducts. Unreacted **1a,b** and the unknown compounds were not included in this calculation. [g] The percent of MC C(1) anilino products overall C(1) nucleophilic products.

occurred by a redox reaction with **1a**.[10,11] Compound **21** has



been shown to undergo ring opening by an acid-catalyzed S_N1-type process via **28**.[25] In buffered methanolic solutions, **21** rapidly furnished near equal amounts of *trans*- (**29**) and *cis*-1-methoxy-2,7-diaminomitosene (**30**). The $t_{1/2}$ value for methanolysis was 3 min at "pH" 7.0 and 228 min at "pH" 8.5. Consistent with this scenario were the high amounts of C(1) electrophilic products generated with excess $Na_2S_2O_4$. Under these conditions, redox transformations proceeding from **3** were minimized, thereby permitting **3** sufficient time to undergo ring opening to give quinone methide **4** and then **7**. This hypothesis argues that **7** is characteristic of the inherent reactivity of reductively activated **1a** at pH 8.5 with limiting amounts of $Na_2S_2O_4$, whereas **8** and **9** are manifestations of secondary transformations arising from **21**.

We have speculated that, at higher pH values when conversion of **1a** to **2** was incomplete, the reductive activation cascade terminated in part at **3**. Additional experiments were performed to test this hypothesis. Deaerated, aqueous buffered pH 8.5 solutions of mitomycin C were treated with 0.2 equiv of $Na_2S_2O_4$. In the first two reactions, the solutions were permitted to remain at room temperature for 6 min. The reactions were then analyzed using different workup procedures. In the first experiment (protocol A), a deaerated aqueous solution of aniline (10 equiv) was added to the solution. HPLC analysis was performed before aniline was added and again after the reaction was completed. In the second reaction (protocol B), the solution was first quenched with O_2. Aliquots for HPLC analysis were then obtained, both before and after the addition of an aqueous solution of aniline (10 equiv). Protocol C, a variant of protocol B, was also conducted. In this experiment, the time of reaction was reduced from 6 min to 20 s. The reaction was then quenched with O_2, and then aniline (10 equiv) was added. Protocol C HPLC analyses were conducted only at the conclusion of the reaction.

Inspection of the HPLC chromatograms indicated that the anilino-modified mitosenes **22**–**24** were present in addition to **8**, **9**, and **21** and traces of **1b**, **7**, **10**, **12**, and **16**. Analysis of the data set (Table V) indicated that nearly the same product profiles were observed with protocols A and B. This observation agrees with the notion that the same species, presumably **21**, was present in both reactions prior to the addition of aniline. The absence of appreciable amounts of C(1),C(10)-modified adducts in protocol A provided further evidence that reoxidation of **3** had taken place before O_2 was added. Consistent with this proposed scenario was the observed increase in the percentage of C(1) anilino mitosene products in the total C(1) nucleophilic pool when aniline was added after only 20 s. Under these conditions, the relative percentage of C(1) anilino products increased from 26.3% to 73.6% (Table V). Moreover, we noted a drop in the percent of **1a** consumption, in agreement with the notion that the decreased reaction time curtailed redox transformations. Finally, repetition of the experiment performed using protocol C (pH 7.4 vs 8.5) led to only **7**–**9** and the absence of C(1) anilino adducts (data not shown), demonstrating that the increased acid permitted aziridine ring opening (**3** → **4**) to occur within the time allotted for reaction.

An important finding was the high percentage of mitomycin C(1), C(10) difunctionalized products that were generated when excess $Na_2S_2O_4$ was used (Table I). These adducts accounted for greater than 75% of the products, and in all cases, modification of the C(10) site proceeded by nucleophilic displacement of the carbamate group by HSO_3^- ($S_2O_4^{2-}$[27]). Previous studies have shown that both nucleophilic and electrophilic processes can take place at this position.[6b,c,9a,13d–g] By comparison, use of limiting amounts of $Na_2S_2O_4$ produced low levels of C(1),C(10) modification (6.6–17.4%) where nucleophilic substitution by HSO_3^- ($S_2O_4^{2-}$) had occurred. These results indicated that, under conditions where substantial amounts of **1a** and C(1) mitosene products were reduced, sufficient time existed for nucleophilic displacement of the C(10) carbamate group by HSO_3^- ($S_2O_4^{2-}$). Correspondingly, under conditions where only fractional amounts of **1a** were reduced, oxidation of C(1)-modified mitosenes occurred more rapidly than C(10) functionalization.

The proclivity of quinone methide **4** to undergo C(1) electrophilic transformations (**1** → **4** → **7**) prompted us to explore whether this species could be intercepted by nucleophiles. Quinone methide **4** has been suspected to be the penultimate intermediate that binds to select regions in DNA prior to covalent bonding to give mitomycin–DNA monoadducts.[2b–e] In these experiments aniline (4 equiv) was the added nucleophile. Under conditions when an excess amount of $Na_2S_2O_4$ was employed along with the aniline (Table III), near complete drug consumption (≥90.7%) was observed, leading to the almost exclusive generation of C(1),C(10)-modified products (≥91.4%). By comparison, with limiting amounts of $Na_2S_2O_4$ (Table IV), drug utilization was modest at pH 7.4 and above (33.4–43.2%) and rose substantially as the pH was decreased (pH 5.5, 89.0%). The percentage of the mitosene adducts in the product pool in which the reaction had proceeded at both C(1) and C(10) increased from 11.2% (pH 8.5) to 68.4%

(27) Guadiano, G.; Frigerio, M.; Sangsurasak, C.; Bravo, P.; Koch, T. H. *J. Am. Chem. Soc.* **1992**, *114*, 5546–5553.

Table V. HPLC Product Composition Observed for Reduction of Mitomycin C in H_2O (pH 8.5) with Limiting Amounts of $Na_2S_2O_4$ (0.2 equiv) Followed by the Addition of Aniline[a]

	protocol A[b]		protocol B[c]		protocol C[d]
compd no.	6 min[e]	anil quench[f]	6-min O_2 quench[g]	O_2/anil quench[f]	20-s O_2/anil quench[f]
7		2.4 [7.1]			
16	tr[h] [0.1]	1.8 [4.7]	0.4 [1.4]	0.2 [0.4]	0.1 [0.4]
24		0.3 [0.9]		0.6 [1.7]	0.6 [3.5]
8 + **9**[i]	24.5 [72.7]	21.4 [61.1]	24.3 [69.0]	26.1 [71.9]	3.8 [22.6]
22 + **23**[j]		7.8 [21.7]		7.0 [19.3]	10.9 [64.6]
10	0.3 [1.0]	0.5 [1.4]	0.3 [0.8]	0.7 [2.0]	0.1 [0.5]
12	tr[h] [0.1]		tr[h] [0.1]		
21	8.1 [23.0]		9.0 [25.1]		
1b	1.1 [3.1]	1.1 [3.1]	1.3 [3.6]	1.7 [4.7]	1.4 [8.4]
1a	66.0	64.7	64.7	63.7	83.1
% C(1) mod[k]	99.8 (95.8)	93.9 (90.9)	98.4 (93.5)	97.7 (93.1)	95.5 (87.6)
% C(1),C(10) mod[l]	0.2 (0.2)	6.1 (5.9)	1.6 (1.5)	2.3 (2.2)	4.5 (4.1)
% C(1) nucl[m]	99.9	86.8	98.4	97.7	95.5
% C(10) nucl[n]	100	100	100	100	100
% C(1) anil nucl[o]		26.3		20.7	73.6
% C(10) anil nucl[p]		14.3		75.0	85.7

[a] Reductive activation of a deaerated (Ar), aqueous buffered (pH 8.5) solution of **1a** was conducted by the addition of a freshly prepared, deaerated (Ar), aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$ (0.2 equiv). The reactions were monitored using HPLC (UV detection). The reactions were run in duplicate and averaged. The data were computed from HPLC profiles obtained using program 1 conditions. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of the product after exclusion of unreacted **1a**. [b] Protocol A: the reaction was stirred for 6 min (room temperature), prior to the administration of a deaerated (Ar), aqueous solution of aniline (10 equiv), and then stirred for 40 min. [c] Protocol B: the reaction mixture was stirred for 6 min (room temperature) and quenched by the administration of O_2 (30 s), and then a solution of aniline (10 equiv) was added and the reaction was stirred for 40 min. [d] Protocol C: the reaction mixture was stirred for 20 s (room temperature) and quenched by the administration of O_2 (30 s), and then a solution of aniline (10 equiv) was added and the reaction was stirred for 40 min. [e] HPLC aliquot obtained after 6 min of reaction prior to the addition of aniline. [f] HPLC aliquot obtained at the conclusion of the reaction. [g] HPLC aliquot obtained after 6 min of reaction and O_2 administration but prior to the addition of aniline. [h] tr = trace. [i] Appreciable quantities of both cis and trans adducts were detected. [j] Significantly greater amounts of the trans adduct versus the cis isomer were detected. [k] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. The value in parentheses includes **1b** in the denominator. Compound **21** was not included in this calculation. [l] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. The value in parentheses includes **1b** in the denominator. Compound **21** was not included in this calculation. [m] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy, C(1) anilino, or C(1) sulfonato adducts. Unreacted **1a**, **1b**, and **21** were not taken into account in this calculation. [n] MC C(10) nucleophilic compounds corresponded to C(10) anilino or C(10) sulfonato adducts. The percentage does not include unreacted **1a**, **1b**, **21**, and only C(1)-modified MC compounds. [o] The percent of MC C(1) anilino products over all C(1) nucleophilic products. [p] The percent of MC C(10) anilino products over all C(10) nucleophilic products.

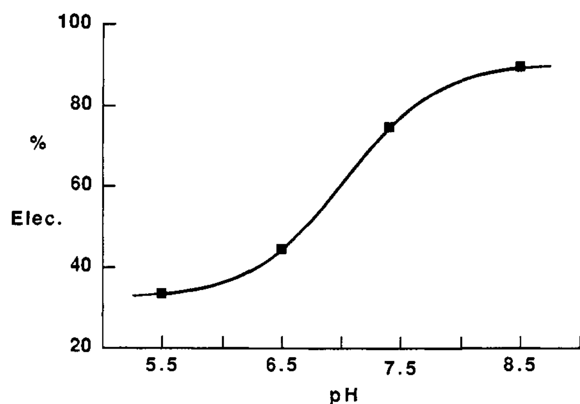
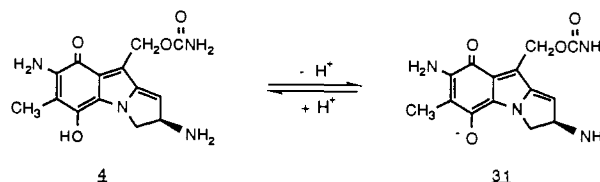


Figure 3. Percent of C(1) electrophilic mitosene products generated as a function of pH with excess amounts of $Na_2S_2O_4$ in the presence of excess aniline.

(pH 5.5). Analysis of these combined data sets indicated that a pronounced dependence of the percent of C(1) electrophilic products in the reaction mixture on pH existed in the excess $Na_2S_2O_4$ experiments when aniline was present (Table III). In moderately basic solutions, C(1) electrophilic adducts predominated (pH 8.5, % C(1) elec = 89.5), while in moderate acid, the reverse was true (pH 5.5, % C(1) nucl = 76.8) (Figure 3). These observations differed from those obtained when aniline was omitted from the experimental protocol (Table I, Figure 2). These reactions were characterized by high levels of electrophilic products. We have attributed the increase in the percentage of nucleophilic products to the trapping of the quinone methide species **4** by the strong nucleophile aniline.[28] We further speculate that the rapid drop in the percentage of C(1) anilino compounds with increasing pH is due to the generation of significant amounts of the conjugated base of quinone methide **4**, **31**. Formation of



31 should promote C(1) electrophilic processes at the expense of C(1) nucleophilic reactions. These results were in agreement with an earlier report that mitomycin C–DNA cross-linking processes were promoted at lower pH values.[20]

Consistent with this mechanistic scenario are the remarkable levels of mitomycin C consumption observed at pH 5.5 and 6.5 with limiting amounts of $Na_2S_2O_4$ and in the presence of aniline (Table IV). We have attributed this phenomenon to the enhanced levels of C(1) nucleophilic products observed in these reactions versus the comparable product profiles when aniline was omitted (Table II). Formation of C(1) nucleophilic adducts in lieu of C(1) electrophilic products should minimize the net consumption of electrons and thereby promote autocatalytic transformations. We also suggest that, in moderately basic solutions where low levels of **1a** modification were observed (Table IV), oxidation of **3** to **21** proceeded. This species is then trapped by a nucleophile (H_2O or aniline) to give C(1) nucleophilic products.[25]

An additional finding was the percentage of C(1) and C(10) anilino products generated as a function of pH when $Na_2S_2O_4$ was employed (Table III). At pH 8.5, we observed modest

(28) Pearson, R. G.; Sobel, H.; Songstad, J. *J. Am. Chem. Soc.* **1968**, *90*, 319–326.

amounts of C(1) anilino products (43.7%) and only traces of the corresponding C(10) anilino adducts (1.0%). The major nucleophilic adducts at this pH were C(1) hydroxy products and C(10) sulfonato adducts. This pattern was consistent with our speculation that C(1) modification occurred by a S_N1-type process through quinone methide **4** and C(10) modification proceeded by S_N2 displacement of the carbamate group.[21] At pH 8.5, a substantial amount of the HSO_3^- in solution should exist as SO_3^{2-} (H_2SO_3: $pK_{a1} = 1.76$, $pK_{a2} = 7.21$[29]). Lowering of the pH led to a rise in the amounts of C(1) anilino and C(10) anilino products. At pH 5.5, all of the C(1) nucleophilic products were C(1) anilino adducts, while C(10) anilino adducts accounted for 30.9% of this product type. The increase in the percentage of C(10) anilino adducts has been attributed in part to the decreased amounts of SO_3^{2-} in solution.

Conclusions

The observed pH–product profiles document that reductively activated mitomycin C in water in the absence of external nucleophiles undergoes principally electrophilic substitution processes. Previous observations[4b,8a,10,13i] reporting the primary formation of *trans*-(**8**) and *cis*-1-hydroxy-2,7-diaminomitosene (**9**) at pH 7 and above are now principally, although not exclusively, attributed to the hydrolysis of the 7-aminoaziridinomitosene (**21**).[25] The sensitivity of reductively activated mitomycin C transformations to both pH and added nucleophile has been demonstrated. We suggest that in **1a** *in vivo* processes DNA bonding will not efficiently proceed unless select interactions between the activated drug and the DNA surface exist,[5,30,31] thereby allowing proper alignment of the activated drug[13i] in the minor groove. Under conditions where this does not occur, self-destruction of the activated drug by primarily C(1) electrophilic transformations proceeded to yield **7**. This process prevents indiscriminate drug–DNA bonding and is expected to contribute to the overall DNA sequence selectivity observed in mitomycin C–DNA transformations. The decreased reactivity of **3** under mildly alkaline conditions raises the intriguing possibility that the effects of the medium or cellular constituents might influence drug activation and bonding by permitting **3** sufficient lifetime to translocate from the site of reduction[32] to the cellular nuclei before DNA bonding.

Experimental Section

Instrumentation and Solvents. The general methods used in this investigation were identical to those employed in the previous investigation.[21] Generous supplies of mitomycin C were kindly provided by Drs. M. Casazza and W. Rose from the Bristol-Myers Squibb Laboratories (Wallingford, CT).

Procedure for Reduction of Dilute Solutions of Mitomycin C Using Excess Amounts of $Na_2S_2O_4$ in the Absence and Presence of Aniline. To a deaerated (Ar), aqueous buffered solution (10 mL) of **1a** (1 mg, 2.99 μmol, 0.3 mM) either without or with aniline (10 μL of a stock solution prepared by the addition of 2.67 μL of aniline to 1 mL of H_2O, 12 μmol) was added in a single step a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.06 M). The reactions were conducted at four different pH values. The buffer used for the pH 5.5 and 6.5 reactions was 0.2 M bis·Tris·HCl, while 0.2 M Tris·HCl was utilized at pH 7.4 and 8.5. The amount of reductant required for the generation of high levels of drug consumption was dependent upon the pH of the reaction. At pH 5.5 and 6.5, 1.2 equiv of $Na_2S_2O_4$ was used, at pH 7.4, 1.5 equiv was added, and 2.0 equiv was utilized at pH 8.5. The reaction mixture was stirred at room temperature (6 min), quenched by the administration of O_2 (30 s), and analyzed by HPLC (UV detection). The pH of the solution at the conclusion of the reaction did not change by more than 0.1 pH unit. The integrated areas of the product peaks and **1a** in the HPLC chromatogram at 313 nm were adjusted to account for the differences in the absorption coefficients of **1a** and the mitosene products[4b,33] and normalized to 100%. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, and the data were averaged. The observed experimental results are listed in Tables I (without aniline) and III (with aniline).

Procedure for Reduction of Dilute Solutions of Mitomycin C Using Limiting Amounts of $Na_2S_2O_4$ in the Absence and Presence of Aniline. To a deaerated (Ar), aqueous buffered solution (10 mL) of **1a** (1 mg, 2.99 μmol, 0.3 mM) either without or with aniline (10 μL of a stock solution prepared by the addition of 2.67 μL of aniline to 1 mL of H_2O, 12 μmol) was added a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.2 equiv). The reactions were conducted at four different pH values. The buffer used for the pH 5.5 and 6.5 reactions was 0.2 M bis·Tris·HCl, while 0.2 M Tris·HCl was utilized at pH 7.4 and 8.5. The solution was stirred at room temperature (6 min), quenched by administration of air (30 s) in the absence of aniline and O_2 (30 s) in the presence of aniline, and analyzed by HPLC (UV detection). The pH of the solution at the conclusion of the reaction did not change by more than 0.1 pH unit. The integrated areas of the product peaks and **1a** in the HPLC chromatogram at 313 nm were adjusted to account for the differences in the absorption coefficients of **1a,b** and the mitosene products[4b,33] and normalized to 100%. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, and the data were averaged. The observed experimental results are listed in Tables II (without aniline) and IV (with aniline).

Preparation of 7-Aminoaziridinomitosene (21).[25] Mitomycin C (20 mg, 59.9 μmol) was dissolved in a 0.1 M $NaClO_4$ methanolic solution (5 mL) and deaerated (Ar, 15 min). Bulk electrolysis of the solution at −1.0 V (300 s) led to the precipitation of 7-aminoaziridinomitosene as a purple solid. The reaction was quenched by the admission of air into the reaction system. The "pH" of the methanolic solution changed from 7.6 to 10.8 during the electrolysis. The solution was filtered through a sintered glass funnel and rapidly dried *in vacuo*. HPLC analysis of a DMSO solution of the precipitate using program 2 gave two peaks identified by coinjection as **8** and **9**. HPLC analysis using program 1 revealed the presence of two peaks eluting at 18.6 and 20.2 min (44.6%), respectively, in a ratio of 1.6:1 in addition to **8** and **9** (55.4%). Coinjection (program 1) with the pH 8.5 reaction solution with limiting amounts of $Na_2S_2O_4$ revealed the coelution of the two unknown peaks with the 18.6- and 20.2-min peaks.

Procedure for Reduction of Dilute Aqueous Solutions of Mitomycin C with Limiting Amounts of $Na_2S_2O_4$ Followed by Addition of Aniline. To a deaerated (Ar), aqueous buffered (0.2 M Tris·HCl, pH 8.5) solution (10 mL) of **1a** (1 mg, 2.99 μmol, 0.3 mM) was added a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.2 equiv). In protocol A, the solution was stirred at room temperature (6 min) prior to the removal of an aliquot (10 μL) for HPLC analysis. A deaerated, aqueous solution (30 mM, 100 μL) of aniline (10 equiv) was then added to the remaining reaction solution. In protocol B, the solution was stirred at room temperature (6 min) and quenched by administration of O_2 (30 s). An aliquot (10 μL) was removed for HPLC analysis prior to the addition of a solution (30 mM, 100 μL) of aniline (10 equiv) into the reaction vessel. In protocol C, the solution was stirred at room temperature (20 s) and quenched by administration of O_2 (30 s), and then an aqueous solution (30 mM, 100 μL) of aniline (10 equiv) was added to the reaction solution. Each reaction solution was stirred at room temperature (40 min), filtered through a 0.45-μm filter (Millipore), and analyzed by HPLC (UV detection). The pH of the solution at the conclusion of the reaction did not change by more than 0.1 pH unit. The integrated areas of the product peaks and **1a** in the HPLC chromatogram at 313 nm were adjusted to account for the differences in the absorption coefficients of **1a,b** and the mitosene products[4b,33] and normalized to 100%. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, and the data were averaged. The observed experimental results are listed in Table V.

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(30) (a) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275–283. (b) Kohn, H.; Li, V.-S.; Tang, M.-s. *Ibid.* **1992**, *114*, 5501–5509. (c) Kohn, H.; Li, V.-S.; Schiltz, P. S.; Tang, M.-s. *Ibid.* **1992**, *114*, 9218–9220.

(31) Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399–1407.

(32) Keyes, S. R.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1985**, *45*, 3642–3645.

(33) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* **1979**, *101*, 6061–6067.

Preparation of *trans*-10-(Decarbamoyloxy)-1-anilino-2,7-diaminomitosene-10-sulfonate (26). To a deaerated (Ar), aqueous solution (20 mL) of **22** (20 mg, 51 μmol) and K_2SO_3 (161 mg, 1.02 mmol) was added a freshly prepared, deaerated (Ar) aqueous solution (100 μL) of $Na_2S_2O_4$ (4.5 mg, 26.0 μmol). The reaction was stirred at room temperature (10 min), quenched by filtration, lyophilized, and purified by Sephadex chromatography to give **26**. Compound **26** was obtained as the only detectable product of the reaction.

***trans*-10-(Decarbamoyloxy)-1-anilino-2,7-diaminomitosene-10-sulfonate (26)**: HPLC t_R program 1 21.9 min, program 2 20.9 min; ^1H NMR (CD_3OD) δ 1.78 (s, C(6)CH_3), 3.88–3.95 (m, C(2)H), 4.04 (dd, J = 3.1, 13.0 Hz, C(3)H_β), 4.19 ($^1/_2AB_q$, J = 13.4 Hz, C(10)*H*H′), 4.29 ($^1/_2AB_q$, J = 13.4 Hz, C(10)H*H′*), 4.47 (dd, J = 6.0, 13.0 Hz, C(3)H_α), 4.61 (d, J = 2.5 Hz, C(1)H), 6.63 (t, J = 7.3 Hz, C(4)PhH), 6.79 (d, J = 8.1 Hz, C(2)PhH), 7.13 (dd, J = 7.3, 8.1 Hz, C(3)PhH); ^{13}C NMR (D_2O + dioxane) 8.48 (C(6)*C*H_3), 47.60 (C(10)), 54.14 (C(3)), 60.57 (C(1) or C(2)), 61.57 (C(2) or C(1)), 107.12 (C(6)), 116.03 (C(2)Ph), 120.46 (C(4)Ph), 122.1 (C(8a)), 123.47 (C(9)), 129.95 (C(9a)), 130.80 (C(3)Ph), 142.22 (C(7)), 148.07 (C(5a)), 149.40 (C(1)Ph), 179.26 (C(8)), 179.42 (C(5)) ppm; MS (–FAB) m/z 418 $[M + 2H]^-$, 417 $[M + H]^-$, 416 $[M]^-$; UV–visible (H_2O) λ_{max} 253, 313, 353 nm.

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