for $C_7H_{12}O_2$, found 128.0870 and calcd 128.0838.

(3*Z*)-3-*tert*-Butoxyacrolein (10). ^1H NMR (acetone-d_6) δ 1.41 (s), 5.02 (dd, J = 8.8, 6.6 Hz), 7.52 (d, J = 6.6 Hz), 10.0 (d, J = 8.8 Hz). ^{13}C NMR (acetone-d_6) 27.9 (q, J = 127 Hz), 80.96 (s[a]), 109.14 (d, J = 176 Hz), 159.7 (d, J = 172 Hz), 188.0 (d, J = 171 Hz) [a indicates the assignment may be switched with carbon in **11**].

***tert*-Butylformate (11).** ^1H NMR (acetone-d_6) δ 1.45 (s), 8.05 (s). ^{13}C NMR δ 161.28 (d, J = 221 Hz), 81.14 (s[a]) (a indicates the assignment may be switched with carbon in **10**).

3,6-Di-*tert*-Butoxy-4,5-dioxene (7) gave a positive ammonium thiocyanate-ferrous ammonium sulfate test. Ammonia chemical ionization mass spectrum: M^+ found 230.1562 and calcd 230.1519; MH^+ found 231.1424 and calcd 231.1597; MNH_4^+ found 248.1700 and calcd 248.1863. Proton and carbon NMR data are found in Table II.

Photolysis Conditions. An acetone-d_6 solution that was 0.013 M in (1*E*,4*E*)-1,4-di-*tert*-butoxy-1,3-butadiene (**1**) and 1×10^{-5} M in rose bengal was placed in a 5 mm NMR tube. This solution was saturated with oxygen for 25 min at -78 °C while being protected from the room light. This reaction mixture was then irradiated for 25 min at -78 °C with a Sylvania 750 Q/Cl tungsten-halogen lamp through a 0.5% $K_2Cr_2O_7$ filter solution. The reaction was monitored at -80 °C by low-temperature NMR.

Kinetic Studies. The Young method[12] was utilized to measure $k_r + k_q$ for the reactions of singlet oxygen with dienes **1**, **2**, and **3**. The dye (mesoporphyrin IX dimethyl ester) was irradiated with a Sylvania 750 Q/Cl tungsten-halogen lamp operated at 36-45 V. This irradiation source was placed at right angles to the excitation (418 nm) and emission (460 nm) light paths and focused through a Corning 3-68 filter (cutoff 540 nm). Six stock solutions containing different concentrations of the three dienes were utilized for each determination. The S_0/S_x value was kept below 7.6 for **1**, 5.3 for **2**, and 2.9 for **3** in order to avoid quenching the fluorescent probe diphenylisobenzofuran. Each kinetic determination resulted in a S_0/S_x plot with a correlation coefficient (r) greater than 0.999. The k_d utilized for acetone was 2.17×10^4 s^{-1} [36] and that for methylene chloride 1.0×10^4 s^{-1}.[37]

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Registry No. 1, 86528-14-5; **2**, 86528-15-6; **3**, 79989-51-8; **4**, 97336-93-1; **5**, 97372-91-3; **6**, 97372-92-4; **7**, 97336-94-2; **8**, 97372-93-5; **9**, 97336-95-3; **10**, 97336-96-4; **11**, 762-75-4.

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Kinetics and Mechanism of the Acid Hydrolysis of Mitomycins

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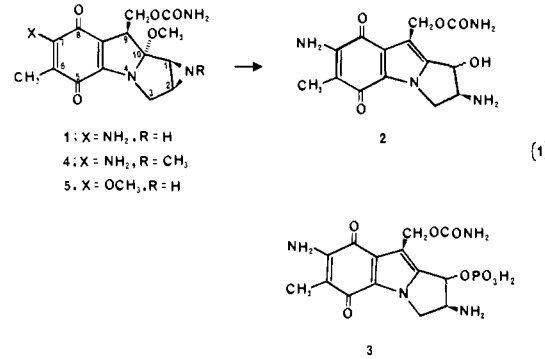
Abstract: First-order rate constants have been obtained as a function of pH and temperature for the hydrolysis of mitomycin C, mitomycin A, and porfiromycin to produce the corresponding 2-amino-1-hydroxymitosene. Rate constants are proportional to hydronium ion concentration at high pH (>4) and level at low pH when the aziridine ring of the mitomycin is protonated. Catalysis by added buffers is observed. A mechanism is proposed with the neutral mitomycin as the reactive species undergoing rate-limiting expulsion of methoxide in reactions catalyzed by hydronium ion and by added buffer acids. In a rapid step the cation so produced loses a proton to form an aziridinomitosene intermediate. This then undergoes rapid hydrolytic ring opening of the aziridine ring. This hydrolysis is triggered by the conjunction introduced in the methanol elimination.

Mitomycin C (**1**) is a potent antitumour antibiotic[1-3] which covalently binds[4] and cross-links[5,6] DNA. Since these effects are observed in the absence of cells only if a reducing agent is present,[7,8] the biologically active form of the drug is generally proposed to arise in the cell by reduction.[7,8] The term "bioreductive alkylating agent" has been introduced to designate agents of this type.[9] The possibility of using such drugs as agents specifically activated in hypoxic tumour cells has been discussed.[10]

Acidic conditions (pH 4) have been found to also activate the binding and cross-linking processes.[11] Mitomycin C under such conditions is converted into 2,7-diamino-1-hydroxymitosenes (**2**),[12-14] with the cis isomer predominating.[13,14] The alkylation of inorganic phosphate (structure **3**) and the phosphate group of various nucleotides can also be observed under these conditions.[15]



(1)

Interestingly these same substances have recently been observed after reoxidation of the products of reductive metabolism of

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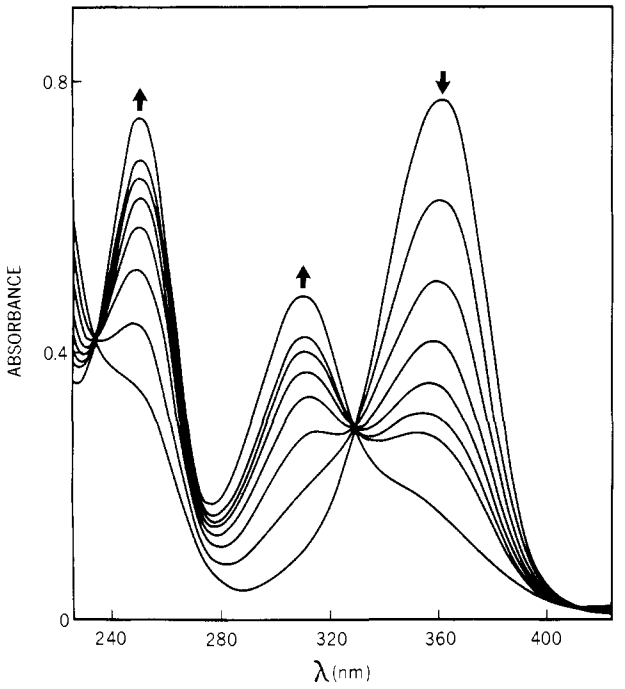

Figure 1. UV spectral change for the hydrolysis of mitomycin C in 0.001 M HCl at 25 °C. The first seven spectra were recorded at 5-min intervals. The last spectra was recorded after 1-h interval.

mitomycin C by rat liver microsomes.[16] A parallelism is thus implied between the acid and reductive activation pathways.

A mechanistic model for the activity of mitomycin C as a bifunctional alkylating agent was proposed in 1964 by Iyer and Szybalski[8] and is generally accepted with some modifications.[17] Little kinetic evidence is however available for this mechanism. As a first step to providing such evidence, we have studied the kinetics of the acid reactions of mitomycin C and two other mitomycins, porfiromycin (**4**) and mitomycin A (**5**), and we report here the results of that study.

Experimental Section

Mitomycin C was obtained from Aldrich Chemical Co. Porfiromycin and mitomycin A were kindly supplied by Bristol Laboratories, Syracuse, NY. Kinetic studies were conducted spectrophotometrically on a Unicam SP 1800 spectrophotometer with a thermostated cell compartment. To 2.5 mL of an aqueous solution previously thermostated in the UV cuvette, 10 μL of a solution of the mitomycin in methanol was added. The final mitomycin concentration was of the order of 10^{-4} M. The disappearance of the mitomycin was followed at its λ_{max} (see Figure 1), 360 nm for mitomycin C and profiromycin and 320 nm for mitomycin A. First-order rate constants were generally evaluated as the slopes of plots of ln $(A - A_\infty)$ vs. time. The Guggenheim treatment was applied in more concentrated acids where a subsequent reaction occurs (see Results) and at higher pH where the hydrolysis is very slow. Excellent linearity in the first-order plots was observed in all cases. Duplicate kinetic runs were performed with each solution and the results averaged.

Results

A typical UV spectral change for the hydrolysis of mitomycin C to the mitosenes **2** is shown in Figure 1. Corresponding to the disappearance of the mitomycin peak at 360 nm, the characteristic mitosene peaks at 310 and 250 nm appear. Excellent isosbestic points are observed at 330 and 240 nm. This latter observation indicates a clean reaction with no buildup of an intermediate. As reported previously,[18] a further UV spectral change does occur in more concentrated acids (pH <2). This presumably corresponds to the further reaction of the mitosene whereby the 7-amino group is replaced by hydroxy.[12,18] The hydrolysis of the carbamoyl group also occurs under strong acidic conditions.[12,18]

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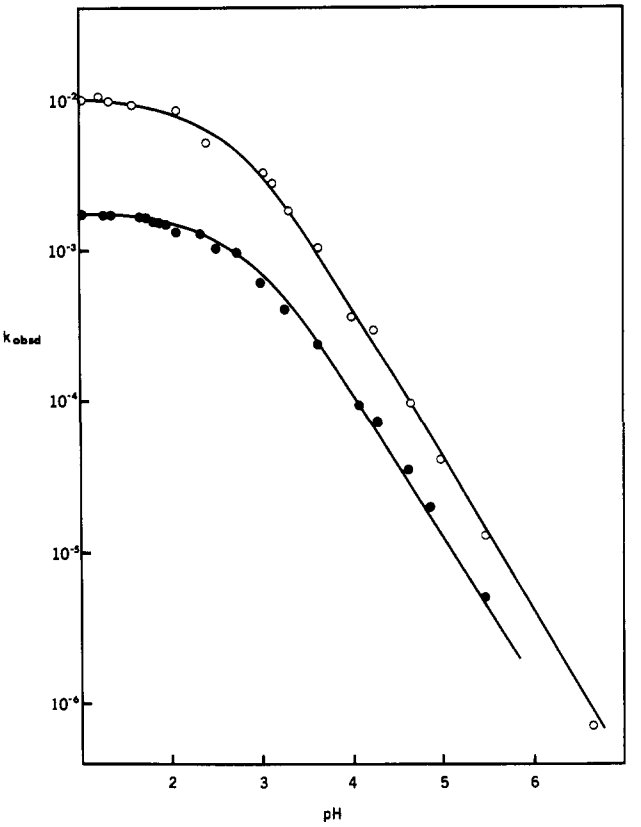


Figure 2. First-order rate constants for the hydrolysis of mitomycin C: (●) 25 °C, (○) 38 °C. All measurements at ionic strength 0.1. The points are experimental. The lines are drawn according to eq 2 with use of the parameters in Table I.

Table I. Rate Constants for the Hydrolysis of Mitomycins

substance	conditions	a, $M^{-1}\ s^{-1}$	$-\log b$ = pK_{BH^+}	$k_H(a/b)$, $M^{-1}\ s^{-1}$
mitomycin C	25°	0.0018	2.8	1.2
mitomycin C	38°	0.0098	2.7	4.5
mitomycin C	49.5°	0.042	2.50	13.3
mitomycin C	25°, D_2O	0.0019	3.3	3.6
porfiromycin	25°	0.0033	2.4	0.9
mitomycin A	25°	0.0038	2.7	2.1

[a] Ionic strength = 0.1.

Table II. Buffer Catalysis of the Hydrolysis of Mitomycin C (25 °C, Ionic Strength = 1.0)

buffer	pH	k_{cat}.[b]	k_{HA}[c]
H_3PO_4 (α = 0.5)[a]	2.1	1.35×10^{-3}	2.6×10^{-2}
$CH_2ClCOOH$ (α = 0.17)	3.2	4.6×10^{-4}	4.6×10^{-3}
HCOOH (α = 0.67)	3.3	1.9×10^{-4}	4.3×10^{-4}
HCOOH (α = 0.50)	3.6	1.56×10^{-4}	4.5×10^{-4}
HCOOH (α = 0.33)	3.9	1.15×10^{-4}	3.9×10^{-4}
CH_3COOH (α = 0.67)	4.3	3.3×10^{-5}	5.0×10^{-5}

[a] Fraction of acid component of buffer. [b] Slope of plot of k_{obsd} vs. total buffer concentration. [c] As calculated by eq 5.

Excellent first-order kinetics are observed for the disappearance of the mitomycins, and we have determined first-order rate constants under a variety of conditions. Actual values for these rate constants are given as Supplementary Material. Rate–pH profiles for mitomycin C at two temperatures are shown in Figure 2. These curves, as well as those for porfiromycin and mitomycin A, show an H^+-dependent reaction at high pH changing around pH 3 to a pH-independent reaction. The following empirical relationship describes such behavior.

$$k_{obsd} = \frac{a[H^+]}{b + [H^+]} \qquad (2)$$

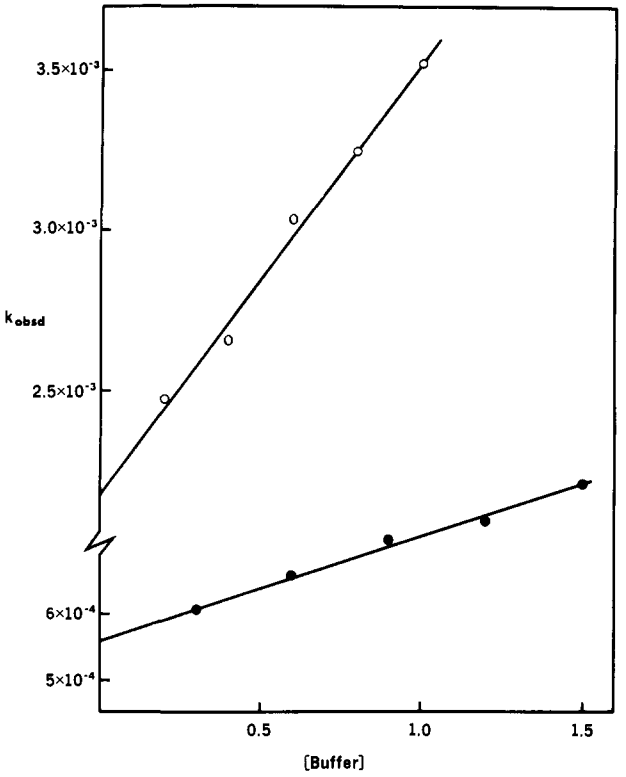


Figure 3. Dependence of the first-order rate constant on buffer concentration for the hydrolysis of mitomycin C at 25 °C and ionic strength 1.0. [Buffer] = [acid component] + [base component]. (○) H_3PO_4:$H_2PO_4^-$ = 1:1, pH 2.11; (●) HCOOH:HCOONa = 2:1, pH 3.11.

Values of *a* and *b* which provide the best fit to the experimental data are given in Table I.

Some rate acceleration by added buffer is observed. (The rate constants used for constructing the rate–pH profiles were obtained in very dilute buffers (<0.01 M) where the buffer contribution is negligible.) Rate constants as a function of total buffer concentration for mitomycin C in two buffers are shown in Figure 2. Table II lists the slopes of such plots for several buffers.

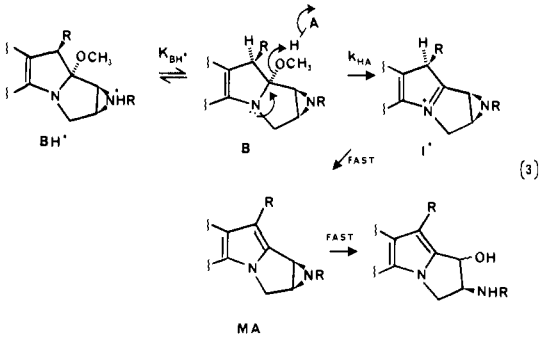
Although the products of the hydrolysis are well established,[12-14] the following NMR experiments were carried out to provide further mechanistic information. In one experiment the hydrolysis of mitomycin C was carried out in D_2O at pD 5.0 for a time corresponding to one half-life, at which point a 360-MHz NMR spectrum was recorded. Spectra were also recorded for the same solution after complete hydrolysis and for the mitomycin itself at pD 7.5. Although the spectrum of the half-hydrolyzed solution is complex, it contains only signals observed in the mitomycin solution and the fully hydrolyzed solution. This establishes that within the accuracy of the NMR method no intermediate accumulates during the hydrolysis. In a second experiment hydrolysis was carried out to completion for an H_2O solution at pH 5. After removal of the water by lyophilization, the spectrum was recorded in D_2O. Comparison of this spectrum with that obtained for hydrolysis directly in D_2O revealed that the two were identical. This establishes that no C–H exchange occurs during the hydrolysis in D_2O.

Discussion

The mitomycin hydrolysis actually combines two quite different reactions—the elimination of a methanol molecule and the regiospecific but nonstereospecific hydrolytic ring opening of the aziridine ring. The observation of excellent isosbestic points in the UV spectra of hydrolyzing solutions (Figure 1) implies that no intermediate accumulates. This conclusion is obviously supported by the experiment in which an NMR spectrum was recorded for a half-hydrolyzed solution. This suggests that whichever of these two reactions occurs first, that process triggers the other.

The rate–pH profiles (Figure 2) show a reaction which is first order in hydronium ion concentration in solutions with pH greater than 3.5. Extrapolation to pH 7 reveals that the mitomycins are very stable toward this hydrolysis under physiological conditions, mitomycin C for example having a half-life of 18 days at pH 7 and 38 °C. The rate constants do level in more acidic solutions, a behavior which can be attributed to the protonation of the aziridine ring nitrogen. A dissociation constant (pK_{BH^+}) of 3.2 has been determined titrimetrically for mitomycin C.[12] This value is considerably lower (4–5 pK units) than those normally found for protonated aziridines.[19] The mitomycins contain a number of electron-withdrawing groups; moreover a molecular model shows that the aziridine nitrogen is placed under the middle ring of the molecule, where it can be influenced by the electron poor quinone system. As will be seen presently, regardless of the kinetic model the constant *b* of the empirical rate equation, eq 2, is the dissociation constant K_{BH^+}. The values determined from the fit to the kinetic data are slightly less than 3, in reasonable agreement with the previously obtained value.

Proposed Mechanism. We present first the mechanistic model which best satisfies the data. This mechanism has the methanol elimination as the initial reaction, this elmination occurring by way of a cation I^+. The overall reaction is directly analogous



to an acid-catalyzed alcohol dehydration. The rate-limiting step is the formation of the cation, in a general acid catalyzed expulsion of the methoxy group. This step has an analogy in the formation of oxocarbocations in the general acid catalyzed hydrolyses of acetals and ortho esters.[20,21] A number of investigations[22] have now shown that the general acid version of this hydrolysis involves proton transfer to the departing group concerted with C–O bond breaking. A similar concerted mechanism is therefore proposed for the mitomycins. The departure in the mitomycin case is assisted by the adjacent nitrogen (see later for a discussion of this effect) and for this reason the cation I^+ is written in eq 3 as an imminium ion.

In the mechanism of eq 3 the protonated aziridine BH^+ is unreactive, since the loss of methanol from this species would produce a dication. The equilibrium assumption produces the following equation for k_{obsd}, with the first term representing ca-

$$k_{obsd} = \frac{k_{H^+}K_{BH^+}[H^+]}{K_{BH^+} + [H^+]} + \frac{k_{HA}K_{BH^+}[HA]}{K_{BH^+} + [H+]} \quad (4)$$

talysis by the hydronium ion and the second term catalysis by added buffer acids. According to this expression the constant "*a*" of the empirical equation is equal to $k_{H^+}K_{BH^+}$ with *b* being equal to K_{BH^+}, so that k_{H^+} can be calculated as *a*/*b*. For the buffer catalysis the following relationship is obtained,

$$k_{HA} = \frac{k_{cat.}}{\alpha}\left(\frac{K_{BH^+} + [H^+]}{[H+]}\right) \quad (5)$$

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Table III. Hydronium Ion Catalytic Coefficients and Brønsted α Values for Acetal Hydrolysis (25 °C)

acetal	k_{H^+}, M^{-1} s^{-1}	α	k_{D^+}/k_{H^+}
acetaldehyde dimethyl acetal[a]	0.29	specific acid	2.7
mitomycin C[b]	1.2	1.0	3.0
benzaldehyde diethyl acetal[c]	1.8×10^2	0.87	3.2
tropone diethyl ketal[d]	1.5×10^5	0.75	1.5

[a] Reference 23. [b] This work. [c] Reference 24. [d] Reference 25.

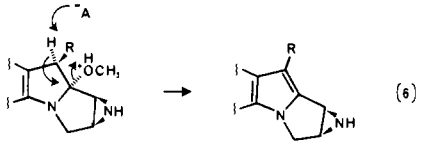
where α is the fraction of the buffer present in the acid form. Values of k_{HA} calculated from this expression with use of the K_{BH^+} values determined in the kinetic analysis are listed in Table II. The formic acid buffer data obtained at three different buffer ratios produce within experimental error the same value of k_{HA}, so that the analysis is consistent.

A Brønsted plot (not shown) for the acid catalysts exhibits excellent linearity for the four buffer acids employed, with the point for the proton showing a negative deviation. The α value for the four buffers is 1.0, indicative of a transition state in which the proton is almost completely transferred. This is the typical situation for acetals where the intermediate cation is of moderate stability. An example would be benzaldehyde dimethyl acetal whose hydrolysis is general acid catalyzed but which has a very high α value.[24] Acetals where the cation is considerably more stable, for example, tropone diethyl ketal, show pronounced general acid catalysis with a lower α value. In cases where the cation is less stable only specific acid catalysis is observed; the proton must be completely transferred before C–O bond cleavage commences. The overall relationship between cation stability and the nature of the acid catalysis is illustrated in Table III, where the k_{H^+} value is used as a measure of cation stability. Mitomycin C can be seen to be on the borderline between the specific acid and general acid catalyzed reactions.

An inverse solvnet isotope effect of 3.0 (k_{D^+}/k_{H^+}) is observed. This is also consistent with the proposed mechanism. This value is typical of a specific acid catalyzed acetal hydrolysis (Table III), or as illustrated by the benzaldehyde acetal example a general acid catalyzed reaction with a large amount of proton transfer in the transition state.

Finally, the entropy of activation for the hydrolysis can be determined by using the data obtained at three temperatures for mitomycin C. A value of +2.3 cal $°C^{-1}$ mol^{-1} is obtained for k_{H^+}. This is again consistent with a dissociative rate-limiting step. Acid-catalyzed acetal hydrolyses have $\Delta S^{\ddagger}$ values ranging from −3.8 to +16.7.[26]

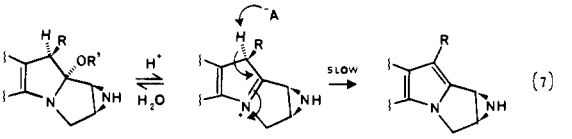
Alternative Mechanisms. A concerted syn elimination of the methoxy-protonated mitomycin does satisfy the observed general



(6)

acid kinetics, since this would represent the kinetically equivalent specific acid–general base catalysis. For this mechanism, the Brønsted α value of 1 would correspond to a β value of 0 for the slow step, or in other words, a situation with very little proton transfer in the transition state. This seems unlikely for an E2 reaction.

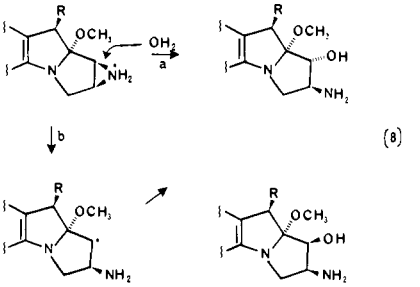
This argument also applies for the mechanism of eq 7, which is identical with the proposed mechanism of eq 3, but with a different rate-limiting step. Again the observed α value would correspond to a β value of 0 for the proton removal and this seems unlikely. It can also be noted that with this mechanism the



(7)

methoxy group would undergo exchange with water. This exchange would probably not be detected in the UV spectra, since there would be little difference in the mitomycin spectrum whether an OMe group or an OH group were attached at C_{10}. However, this exchange would be observed in the NMR spectra of partially hydrolyzed solutions as methanol was released more rapidly than hydrolysis was occurring. This is not observed. Interestingly, the mechanism of eq 7 is that normally observed for alcohol dehydration, that is, a mechanism with rate-limiting C–H deprotonation.[27] That this step is not rate limiting in the elimination in the mitomycin C case may be associated with some geometrical effect, proton removal being easier than water addition to C-10. Proton removal also results in a conjugated system.

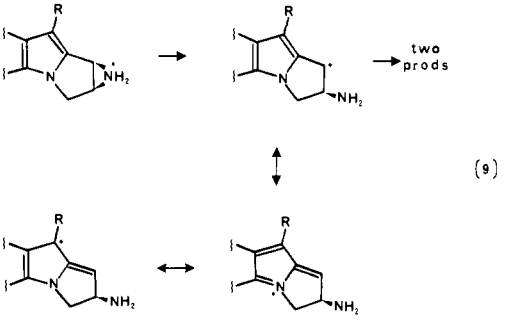
Mechanisms where the aziridine hydrolysis occurs initially can also be considered. The protonated aziridine is now the reactive form,[28] and two reactions are possible,[29] a direct nucleophilic substitution (eq 8a) or a two-step reaction with a carbocation intermediate (eq 8b). The direct displacement mechanism is immediately ruled out since this must give stereospecifically a trans product. The unimolecular reaction would have as its slow step



(8)

the ionization to form the carbocation. The principal piece of evidence against this is the finding of buffer catalysis, since there can be no involvement of buffer in the ionization process.

Mechanism of Aziridine Ring Opening. The proposed mechanism of eq 3 requires that upon elimination the aziridine ring is activated toward hydrolysis. The sterochemistry of the hydrolysis products indicates a unimolecular mechanism, with the regiospecificity indicating that it is the cation **4** which is formed. As



(9)

indicated in eq 9 this cation is resonance stabilized, with a second carbocationic structure (on tertiary carbon) and even with a structure where the charge is placed on nitrogen. the "triggering" effect of the elimination can therefore be seen in terms of the introduction of the double bond so that this conjugation is possible.

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(28) The rate law now becomes $k_{obsd} = k[H^+]/(K_{BH^+} + [H^+])$ where k refers to the rate-limiting step. This also satisfies the experimental equation (eq 2) with $a = k$.
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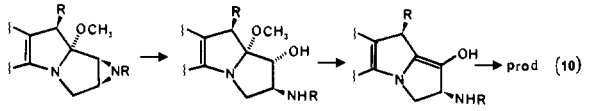
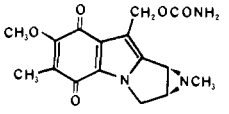
Stevens and co-workers[12] have proposed the mechanism of eq 10. Here a stereospecific trans aziridine ring opening occurs as



(10)

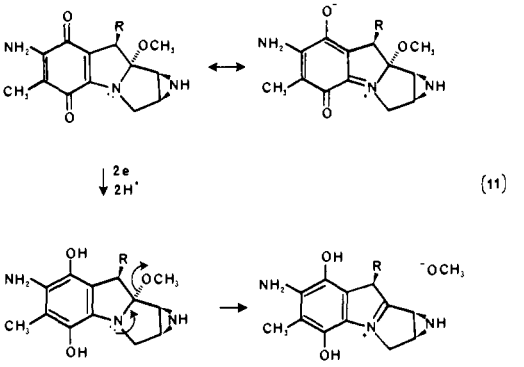
the first step and the elimination follows initially involving the hydrogen at C1. This mechanism was invoked specifically to explain deuterium incorporation at C1 during mitomycin C solvolysis in deuterated acetic acid, but it obviously also accounts for the lack of stereospecificity in the aziridine hydrolysis. We feel that this mechanism is not accommodated by the present results for the hydrolysis reaction, and our NMR experiments in fact establish that during D_2O hydrolysis no exchange occurs. Cheng and Remers[30] have prepared the mitosene MA with an intact aziridine ring from *N*-methylmitomycin A and find it hydrolyzes also to give a 3:1 cis:trans ratio very similar to that found with the mitomycin itself. As noted by these authors, the Stevens



MA

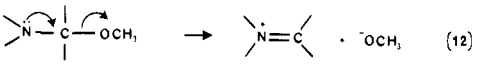
mechanism is not possible starting from MA. A unimolecular reaction of this mitosene, however, does explain the results.

Reductive Activation. A parallelism does exist between the acid activation and the reductive activation.[11] The aziridinomitosene MA was prepared by the hydrogenation of the mitomycin in ethyl acetate followed after a short reaction time by reoxidation. This is strong evidence that elimination precedes the aziridine ring opening under reductive conditions as well, with the important differences that under the conditions employed in the synthesis the aziridine solvolysis of the eliminated product is slower than the initial elimination. In terms of the mechanism of the acid hydrolysis the "activation" afforded by reduction can be quite easily understood. In the oxidized mitomycin the nitrogen lone pair is strongly conjugated with the quinone ring. The system can be



(11)

regarded as a homoconjugated amide. This nitrogen lone pair is required to stabilize the carbocation center at C10 which forms during hydrolysis. The conjugation with the quinone ring works against this so that the departing alcohol must be converted to an excellent leaving group by protonation for the hydrolysis to occur. In the reduced hydroquinone form, however, the conjugation with the aromatic ring is greatly reduced, and the nitrogen electron pair becomes much more available to initiate the elimination. A comparison here is with α-amino ethers and amide acetals, species which are very reactive toward C–O cleavage even in the absence of acid, readily expelling the alcoxide as an anion.[31]



(12)

We propose therefore that mitomycin reduction triggers the loss of alcoxide which initiates the elimination and the entire reaction sequence. The double bond produced by the elimination is now conjugated with a very electron rich aromatic ring, so that as proposed by Iyers and Szybalski[5] the subsequent aziridine ring opening should also be significantly accelerated in the reduced form.

Supplementary Material Available: Tables of first-order rate constants (4 pages). Ordering information is given on any current masthead page.

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Ultrasound-Promoted Selective Perfluoroalkylation on the Desired Position of Organic Molecules

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Abstract: Perfluoroalkylzinc iodides or bromides which were prepared from perfluoroalkyl iodides or bromides and zinc powder in *N,N*-dimethylformamide or tetrahydrofuran with ultrasonic irradiation were found to behave as potential perfluoroalkylating reagents for the preparation of a wide variety of perfluoroalkylated compounds. Especially, the ultrasound-promoted asymmetric induction with perfluoroalkyl group on the asymmetrical carbon was achieved by the reaction of perfluoroalkyl halides with optically active enamines in the presence of zinc powder and a catalytic amount of dichlorobis(π-cyclopentadienyl)titanium.

The utility of organometallic reagents has been generally recognized to be useful in organic synthesis.[1] However, very little synthetic application of perfluoroalkylmetallic reagents has been studied,[2-8] probably due to their low stability or low reactivity. For example, perfluoroalkylmagnesium or lithium compounds readily decompose into perfluoroolefins and magnesium or lithium halides,[9] and they are not used practically to introduce the per-

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