Aspects of the Chemical Stability of Mitomycin and Porfiromycin in Acidic Solution

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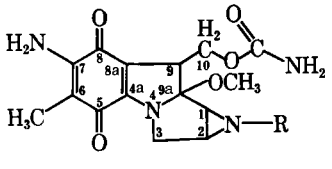
Abstract □ Aspects of the degradations of mitomycin and porfiromycin were studied. The initial degradation processes of the compounds in an acidic medium were investigated. Influences of pH, buffers, and other additives such as halogenides and dioctyl sodium sulfosuccinate [sodium 1,4-bis(2-ethylhexyl)sulfosuccinate] were studied. The hydrogen ion catalyzes the degradation of both the uncharged and the protonated species. Anions also promote the degradation of the compounds in an acidic medium. Rate constants for all of the catalytic reactions could be determined. From the pH profiles, after correction for buffer influences, accurate pK_a values for the aziridine nitrogens could be obtained. The protective influence of the dioctyl sulfosuccinate ion could be explained. From the data obtained a plausible mechanism for the initial acidic degradation reactions was developed.

Keyphrases □ Degradation—in acidic solution, mitomycin, porfiromycin, pK_a determination □ Mitomycin—degradation in acidic solution, rate constant determination □ Porfiromycin—degradation in acidic solution, rate constant determination □ pK_a determination—aziridine nitrogens, mitomycin, porfiromycin

Mitomycin (I), an important antitumor antibiotic originating from *Streptomyces caespitosus* (1), appears to be unstable in solution. Several investigators studied aspects of the degradation of I and of porfiromycin (II), which differs from I only in the methyl substituent on the aziridine nitrogen. The structure of I was determined by studying the products resulting from acid hydrolysis (2). Knowledge of the physicochemical properties of I was expanded by synthesizing derivatives and studying the acid hydrolysis (3). From these studies it became obvious that the degradations of I and II follow very complex patterns. Suggestions that the aziridine ring opens prior to the rapid solvolytic cleavage of the 9α-methoxyl group in acidic media have been reported previously (2). The degradation of II was studied using spectrophotometric techniques and the appearance of 12 different products during acidic and alkaline hydrolysis were reported (4, 5), in general agreement with other sources (2, 3). Kinetic data of the degradative transformation of II were also presented (4, 5). Most degradation processes followed pseudo first-order kinetics.



I: R = H
II: R = CH_3

The acid catalysis of the degradation of protonated II has a smaller specific rate constant than that of the nonprotonated species. This results in an estimated pK_a of the protonated group in II of ~1.5. Furthermore, a study of the influence of buffers on the degradation led to the conclusion that buffer components such as $H_2PO_4^-$ and CH_3COOH have catalytic effects on certain steps in the degradation process. Indications were found for both general acid and specific acid–base catalysis on the solvolysis. The primary degradation mechanism is the hydrolysis of the fused aziridine ring, while mild alkaline conditions lead to substitution of the amino group attached to the quinoid ring by a hydroxyl group (5). Although data on the degradation of I and II appear in the literature, no systematic study is available which includes the influences of external factors (ionic strength, buffer components, *etc.*). The present study was undertaken to elucidate the mechanism of the primary degradation of I and II in acidic solution and to establish the influence of a number of external factors on the process.

During previous work on the physicochemical properties of I and II, pK_a values of several groups in the molecules were determined (6). To complete the prototropic characterization of the compounds, more accurate pK_a values of the aziridine nitrogen in I and II will be presented as a result of a closer look at the kinetics of the initial acidic degradation.

EXPERIMENTAL

Materials—Mitomycin[1] and porfiromycin[2] were used as supplied. All other materials were reagent grade, and deionized water was used throughout.

Methods—*Kinetic Studies of I and II Degradation*—The appropriate compound was dissolved in methanol to a concentration of 3×10^{-3} *M*. This was used as the stock solution. For stability tests the stock solution was diluted with buffer solutions at the appropriate pH (after addition of any other additive) to a concentration of 3×10^{-5} *M*. The degradation of the compound was observed by monitoring the absorbance at 363 nm (4, 5) using an absorption spectrophotometer with an automatic cell positioner[3]. All measurements were performed at 40 ± 1°. The pH was measured with a glass-reference electrode using an appropriate pH meter[4]. Absorption spectra were taken with a recording spectrophotometer[5]. Calculation of the first-order rate constants were done using plots of log (percent undegraded compound) *versus* time.

Assay of I and II—The percentage of remaining I and II during the degradation, in general, can be calculated from the relationship:

$$\% \text{ undegraded I or II} = \frac{A_t}{A_0} \times 100 \qquad \text{(Eq. 1)}$$

in which A_t is the absorbance at 363 nm at time t and A_0 is the absorbance at time zero. Equation 1 is valid when the resulting absorbance (A_∞) after completion of the degradation approaches zero.

In the case of the degradation of the mitosanes, however, the primary degradation product in acidic medium has an absorbance that is not negligible. Exact measurements of this absorbance revealed A_∞ to be 0.18

[1] Supplied by Bristol Myers B. V., Bussum, The Netherlands.
[2] Supplied by Cyanamid, Pearl River, N.Y.
[3] Shimadzu UV-140 Double Beam Spectrophotometer with ACP-140 Cell Positioner.
[4] Metrohm Herisau E516 Titriskop pH meter.
[5] Shimadzu UV-200 Double Beam Spectrophotometer with Kipp BD 40 Recorder.

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Table I—Determination of k_{obs} of the Degradation of I at Different Acidities and Total Phosphate Concentrations at 40° [a]

H_0/pH	Total Phosphate Concentration			
	10^{-2} M	5×10^{-2} M	10^{-1} M	5×10^{-1} M
−1.02	7.1×10^{-2}(−1.15)	7.2×10^{-2}(−1.14)	8.0×10^{-2}(−1.09)	7.4×10^{-2}(−1.13)
0.37	1.5×10^{-2}(−1.81)	1.5×10^{-2}(−1.81)	1.4×10^{-2}(−1.84)	1.4×10^{-2}(−1.84)
2.16	8.2×10^{-3}(−2.08)	8.3×10^{-3}(−2.08)	8.5×10^{-3}(−2.07)	9.6×10^{-3}(−2.02)
3.16	2.4×10^{-3}(−2.62)	3.1×10^{-3}(−2.50)	3.8×10^{-3}(−2.42)	5.5×10^{-3}(−2.26)
4.64	6.0×10^{-5}(−4.22)	1.1×10^{-4}(−3.95)	1.4×10^{-4}(−3.85)	3.4×10^{-4}(−3.47)

[a] Table values expressed as k_{obs} in sec^{-1} (log k_{obs}).

A_0. A first approximation of the exact percentage of the undegraded compound was made by modifying Eq. 1 to:

$$\% \text{ undegraded I or II} = \frac{A_t - \left(\frac{A_0 - A_t}{A_0}\right) A_\infty}{A_0} \times 100 \qquad \text{(Eq. 2)}$$

Four repetitions of this approximation led to a refinement of Eq. 2 to:

$$\% \text{ undegraded I or II} = \frac{A_0^3(A_t - A_\infty)(A_0 + A_\infty) + A_0(A_0 + A_\infty)(A_t - A_\infty)A_\infty^2 + A_t(A_\infty^4)}{A_0^5} \times 100 \qquad \text{(Eq. 3)}$$

which has been used to calculate the percent undegraded compound as a function of time.

RESULTS

Degradation Kinetics of I and II in Acidic Solution—Studies on the stability of II in both acidic and alkaline solutions have been published (4, 5). The structures of a number of degradation products were determined, the pK_a of the aziridine nitrogen was estimated, rate constants for the degradation in acidic as well as in alkaline solution were determined, and an overall mechanism for the degradation of II was described. The influence of several buffer components on the degradation was also studied. Some data on the kinetics of the degradation of I have been published (7). So far no data are available on the mechanism of the initial degradation steps of I and II nor the nature of the buffer influences on the degradation. Determination of the exact pH profiles for the degradation of both compounds should lead to more accurate pK_a values for the aziridine nitrogen.

Standard Deviation in k_{obs}—The standard deviation in the overall rate constant, k_{obs}, for degradation of I in an acid solution was determined at pH 3.0 and buffer concentration 10^{-2} M total phosphate. At 40 ± 1° all experiments showed linearity between log [I] and time, indicating the process as pseudo first-order, which is in agreement with the literature data (2, 4, 5, 7). The value of k_{obs} (and log k_{obs}) and the standard deviation, calculated from 16 observations, is $2.3 \pm 0.1 \times 10^{-3}$ sec^{-1} (and −2.64 ± 0.03).

pH Profiles for the I and II Degradations—In unbuffered solutions pH values of partially degraded solutions of I will differ from the starting value due to the disappearance of a compound with pK_a ~1.5 and the appearance of a compound with pK_a ~7. Starting at pH 5.0, for instance, a solution with [I] = 3.0×10^{-5} M, after being kept at 40° for 4 hr, has a pH of 5.6. This pH shift results in a change in k_{obs}.

To keep the pH constant during the process, buffer solutions of various compositions and concentrations were used. Since the influences of buffer components were already recognized (4), accurate pH profiles for the degradation processes have to be corrected for these influences. For these corrections k_{obs} was determined at several H_0/pH values. In the H_0/pH region <3.0, the pH was adjusted with perchloric acid and various concentrations of sodium biphosphate were added. At pH values >3.0, the pH was adjusted using various concentrations of sodium biphosphate and dropwise addition of either dilute perchloric acid or sodium hydroxide. At each pH value and each phosphate concentration, k_{obs} was determined (Table I). From Table I it becomes clear that at pH <2 the influence of phosphate or phosphoric acid on k_{obs} becomes negligible. At pH >3 this influence is very important.

Considering the reported value for the pK_a of I of ~1.5 (4) at pH >3.5, the compound should be exclusively in the unprotonated form. In that case k_{obs} can be expressed as:

$$k_{obs} = k_0^I + k_H^I[H^+] + k_{buffer}^I[\text{buffer}] \qquad \text{(Eq. 4)}$$

Table II—Rate Constants for Catalyzed Degradation Reactions of I and II at 40°

Rate Constant	I	II
k_0^I	~1×10^{-6} sec^{-1}	~6×10^{-6} sec^{-1}
$k_{H^+}^{IH^+}$	1.0×10^{-2} $mole^{-1}$ sec^{-1}	5.7×10^{-3} $mole^{-1}$ sec^{-1}
$k_{H_3PO_4}^{IH^+}$	0	0
$k_{H_2PO_4^-}^{IH^+}$	5.7×10^{-1} $mole^{-1}$ sec^{-1}	9.6×10^{-1} $mole^{-1}$ sec^{-1}
k_H^I	4.07 $mole^{-1}$ sec^{-1}	2.89 $mole^{-1}$ sec^{-1}
$k_{acetic\ acid}^I$	0	0
$k_{acetate^-}^I$	8.7×10^{-4} $mole^{-1}$ sec^{-1}	
$k_{H_3PO_4}^I$	0	0
$k_{H_2PO_4^-}^I$	5.4×10^{-4} $mole^{-1}$ sec^{-1}	4.7×10^{-4} $mole^{-1}$ sec^{-1}
$k_{citric\ acid}^I$	0	0
$k_{H_2\text{-}citrate^-}^I$	~8×10^{-3} $mole^{-1}$ sec^{-1}	~9×10^{-3} $mole^{-1}$ sec^{-1}
$k_{Cl^-}^I$	3.8×10^{-4} $mole^{-1}$ sec^{-1}	5.9×10^{-3} $mole^{-1}$ sec^{-1}
$k_{Br^-}^I$	5.1×10^{-4} $mole^{-1}$ sec^{-1}	
$k_{I^-}^I$	6.4×10^{-4} $mole^{-1}$ sec^{-1}	
$k_{SO_4^{2-}}^I$	3.2×10^{-4} $mole^{-1}$ sec^{-1}	

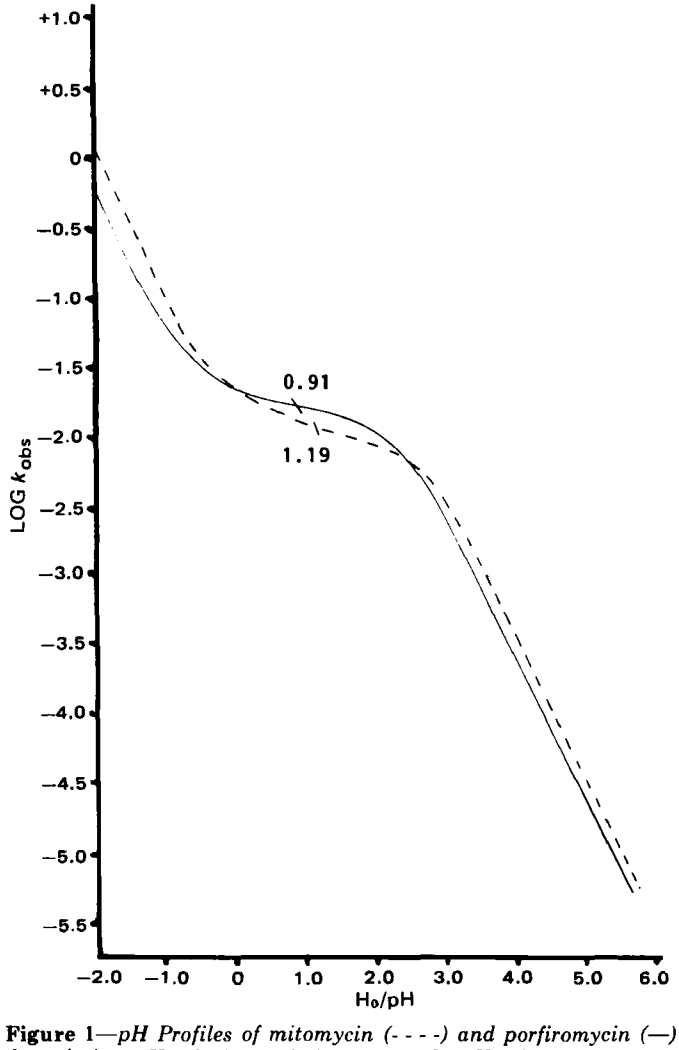


Figure 1—*pH Profiles of mitomycin (- - - -) and porfiromycin (—) degradation; pK_a of mitomycin is 1.19 and the pK_a of porfiromycin is 0.91.*

Table III—Influence of Dioctyl Sodium Sulfosuccinate on k_{obs} for Degradation of I and II in an Acidic Solution at 40° [a]

pH	k_{obs}(log k_{obs}), sec^{-1} [b]		k_{obs}(log k_{obs}), sec^{-1} [c]		Δlog k_{obs}
	I	II	I	II	
1.97	$8.3 \times 10^{-3}(-2.08)$		$6.3 \times 10^{-3}(-2.20)$		−0.12
2.10		$1.04 \times 10^{-2}(-1.98)$		$7.9 \times 10^{-3}(-2.10)$	−0.12
2.49	$5.5 \times 10^{-3}(-2.26)$		$3.2 \times 10^{-3}(-2.49)$		−0.23
2.91	$3.0 \times 10^{-3}(-2.55)$		$1.7 \times 10^{-3}(-2.77)$		−0.22
3.08		$2.3 \times 10^{-3}(-2.63)$		$8.8 \times 10^{-4}(-3.05)$	−0.42
3.47	$7.8 \times 10^{-4}(-3.11)$		$5.0 \times 10^{-4}(-3.30)$		−0.19
3.84		$4.0 \times 10^{-4}(-3.40)$		$1.5 \times 10^{-4}(-3.81)$	−0.41
4.23	$2.5 \times 10^{-4}(-3.89)$		$1.2 \times 10^{-4}(-3.93)$		−0.04

[a] The [I] and [II] was 3×10^{-5} *M*; the dioctyl sodium sulfosuccinate concentration was 1.25×10^{-3} *M*. [b] In the presence of dioctyl sodium sulfosuccinate. [c] In the absence of dioctyl sodium sulfosuccinate.

where k_0^I is the first-order rate constant for degradation in water only, k_H^I is the second-order rate constant for proton-catalyzed degradation of the unprotonated compound, and $[H^+]$ is the hydrogen ion concentration. The third term of Eq. 4 is the sum of several terms representing the products of the second-order rate constants for the degradation catalyzed by each of the buffer components and the concentrations of these buffer components. For each pH this sum can be written as the product of an overall second-order rate constant (k'_{buffer}) and the total buffer concentration ([buffer]), expressed in terms of total phosphate concentration. For each pH the pseudo first-order rate constant for [buffer] = 0, $k' = k_0^I + k_H^I[H^+]$, can be calculated as the intercept from a plot of k_{obs} *versus* [buffer]. The corrected pH profiles for the degradation of I and II are obtained by plotting log k' *versus* pH. These pH profiles are given in Fig. 1. The curves show log k' *versus* pH to have a slope of negative unity both in the regions $H_0 < -0.8$ and pH >3.2 indicating that there is specific proton catalysis on the degradation of the protonated as well as the unprotonated forms of I and II, while, in agreement with other findings (4), the rate constants for the proton-catalyzed degradation of the protonated species are smaller than those for the uncharged forms. This phenomenon permits the calculation of the pK_a for the aziridine nitrogen in I and II from the curves of Fig. 1. For I this pK_a turns out to be 1.19, while for II the pK_a is 0.91. The latter pK_a value is substantially different from a previously determined value (4), probably due to the corrections made for the buffer influence.

The difference between the pK_a values for I and II are in agreement with the usual differences in pK_a between secondary and tertiary amines (8). It is also possible to calculate the second-order rate constants $k_H^{IH^+}$ and k_H^I of the proton-catalyzed degradation of the protonated and unprotonated species. $k_H^{IH^+}$ can be determined from:

$$k_{obs} = k_0^{IH^+} + k_H^{IH^+}[H^+] \quad \text{(Eq. 5)}$$

which is valid at $H_0 < -0.8$. At these high acidities $k_0^{IH^+} \ll k_H^{IH^+}[H^+]$ so that Eq. 5 simplifies to:

$$k_{obs} = k_H^{IH^+}[H^+] \quad \text{(Eq. 6)}$$

from which $k_H^{IH^+}$ can be calculated.

The rate constants k_H^I for the proton-catalyzed degradation of uncharged I, and k_0^I for the degradation in water only, can be determined from the expression:

$$k' = k_0^I + k_H^I[H^+] \quad \text{(Eq. 7)}$$

obtained from Eq. 4 after correction for buffer influences. Equation 7 is valid at pH > 3.2, where $[IH^+]$ becomes extremely small. A plot of k' *versus* $[H^+]$ yields a straight line with slope k_H^I and intercept k_0^I. The values of $k_H^{IH^+}$, $k_H^{IIH^+}$, k_H^I, k_H^{II}, k_0^I and k_0^{II} are listed in Table II.

Influence of Buffer Components—From the previous experiments it is obvious that biphosphate ions have a positive influence on the degradation of I and II. Other sources (4, 5) also indicate positive catalytic effects of buffer components on the degradation rate of II in an acid solution. A catalytic effect of undissociated acetic acid has been suggested (5), although the experimental conditions also permit the conclusion that acetate ions produced the catalytic effect. Even though the aziridine ring is not attacked by the strong nucleophilic hydroxyl ion (4), it is known that other nucleophiles, such as chloride, promote the opening of the aziridine ring in an acidic medium (9–11) with the formation of chlorinated degradation products. Therefore, it appears that the acetate ion, rather than acetic acid, promotes the cleavage of the aziridine group. The influence of uncharged acetic acid on the degradation of I is studied at pH 2.77, where acetic acid exists almost completely in the uncharged form while $[IH^+] \ll [I]$. All other conditions kept constant, variation of the [acetic acid] is the only factor influencing k_{obs}, according to Eq. 4. However, no significant changes in k_{obs} occur on changing the [acetic acid] from 10^{-2} *M* to 5×10^{-1} *M*. The term k'_{buffer}[buffer] in that case must be zero and, consequently, $k^I_{acetic\ acid}$ must be zero.

Influences of the phosphate buffer components of pH 1–5 can be determined as follows. Phosphoric acid has pK_{a1} 2.16 and pK_{a2} 7.21. In the region of 4.2 < pH < 5.2, phosphate buffers almost exclusively consist of $H_2PO_4^-$ ions, so that this ion can be considered the only potential catalytic species. In this case, Eq. 4 can be rewritten as:

$$k_{obs} = k_0^I + k_H^I[H^+] + k^I_{H_2PO_4^-}[H_2PO_4^-] \quad \text{(Eq. 8)}$$

and a plot of k_{obs} *versus* $[H_2PO_4^-]$ yields a straight line with slope $k^I_{H_2PO_4^-}$.

The influence of molecular H_3PO_4 on the degradation of both the protonated and the unprotonated species as well as the influence of $H_2PO_4^-$ on the degradation of the protonated species also can be established. To achieve this, solutions of pH 3.2–3.6 were prepared using different concentrations of total phosphate. At a given pH $[H_3PO_4] = (f)$ [total phosphate], and $[H_2PO_4^-] = (1 - f)$ [total phosphate], where f is the fraction remaining. For each pH, f can be calculated using the Henderson–Hasselbach equation.

In the chosen pH region, $[IH^+]$ approaches zero and $[I] \approx [I_{total}]$. In this case Eq. 4 is valid with one adjustment. The value of $k^{IH^+}_{H_2PO_4^-}$ can be expected to be large due to the electrostatic attraction between ions of opposite charge. The term $k^{IH^+}_{H_2PO_4^-}[H_2PO_4^-][IH^+]$ cannot be neglected *a priori*, but has to be included in Eq. 4, which then must be written as:

$$k_{obs} = k_0^I + k_H^I[H^+] + k^I_{H_3PO_4}[H_3PO_4] + k^I_{H_2PO_4^-}[H_2PO_4^-] + k^{IH^+}_{H_2PO_4^-}[H_2PO_4^-]\frac{[IH^+]}{[I_{total}]} \quad \text{(Eq. 9)}$$

or, after rearrangement:

$$k_{obs} = k_0^I + k_H^I[H^+] + [\text{total phosphate}]\left(f\,k^I_{H_3PO_4} + (1-f)k^I_{H_2PO_4^-} + \frac{[IH^+]}{[I_{total}]}(1-f)k^{IH^+}_{H_2PO_4^-}\right) \quad \text{(Eq. 10)}$$

Graphical treatment of Eq. 10 by plotting k_{obs} *versus* [total phosphate] yields a straight line with slope:

$$\left(f\,k^I_{H_3PO_4} + (1-f)k^I_{H_2PO_4^-} + \frac{[IH^+]}{[I_{total}]}(1-f)k^{IH^+}_{H_2PO_4^-}\right)$$

Knowing the value of $k^I_{H_2PO_4^-}$, combination of the slopes of plots at two different pH values in the region 3.2–3.6 yields both the values of $k^I_{H_3PO_4}$ and $k^{IH^+}_{H_2PO_4^-}$. These values, and the corresponding rate constants for II, are listed in Table II.

Influence of Other Additives—The influence of halide ions has been established by adding various concentrations of the appropriate sodium salts to solutions of I at pH 4.2, while $[H_2PO_4^-]$ is kept constant at 10^{-2}

Table IV—Enthalpy of Activation (ΔH) for the Initial Degradation of I at Various pH Values in the Acidic Region

H_0/pH	ΔH, kJ/mole
−1.02	78
1.34	77
2.06	77
2.80	76
3.66	73
4.74	76
5.02	68
5.20	68

M. In this case one extra term involving the influence of the halide ion has to be included in Eq. 8, yielding (in the case of chloride):

$$k_{obs} = k_0^I + k_H^I[H^+] + k_{H_2PO_4^-}^I[H_2PO_4^-] + k_{Cl^-}^I[Cl^-] \quad \text{(Eq. 11)}$$

From Eq. 11 $k_{Cl^-}^I$ can be obtained either by plotting k_{obs} *versus* $[Cl^-]$, yielding a straight line with slope $k_{Cl^-}^I$, or by calculating the rate constant using various concentrations of Cl^- and the data in Table II. The specific rate constants for the halide ions and sulfate are also listed in this table.

The influence of ion-pairing compounds like dioctyl sodium sulfosuccinate [sodium 1,4-bis(2-ethylhexyl)sulfosuccinate] on the degradation was also studied. At various pH, using phosphate buffers with [total phosphate] = 10^{-2} M, dioctyl sodium sulfosuccinate was added to a concentration of 1.25×10^{-3} M while [I] and [II] both were adjusted to 3×10^{-5} M. In Table III the pseudo first-order rate constants, k_{obs}, for degradation in the presence as well as in the absence of dioctyl sulfosuccinate anion at various pH are listed. The data illustrate clearly the decrease in k_{obs} on addition of dioctyl sodium sulfosuccinate in the pH range of 1.5 to 4.0. The decrease for II is even more dramatic than for I, probably due to the more lipophilic properties of II and, hence, the increased lipophilic character of the ion pair. At pH > 5 the inhibition of the degradation disappears, probably because the compounds are virtually not in the protonated form at these acidities. At pH < 1.5 the inhibition also vanishes due to protonation of the sulfonic acid group and subsequent loss of the ion-pairing properties of the additive.

Influence of Temperature—The temperature influence on the degradation rate is determined using an Arrhenius plot from:

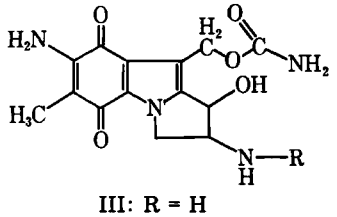
$$\ln k_{obs} = \ln A + \frac{\Delta H}{RT} \quad \text{(Eq. 12)}$$

in which A represents the frequency factor, ΔH the enthalpy of activation, R the gas constant, and T the temperature in °Kelvin. The ΔH values for the initial degradation of I at various pH values in the acidic region, using buffers with [total phosphate] = 10^{-2} M, are listed in Table IV. ΔH appears to be constant in the region pH < 5 and the values are in good agreement with those previously obtained (7). In the region pH > 5 ΔH tends to lower somewhat. This may be an indication that other degradation processes occur in this pH region.

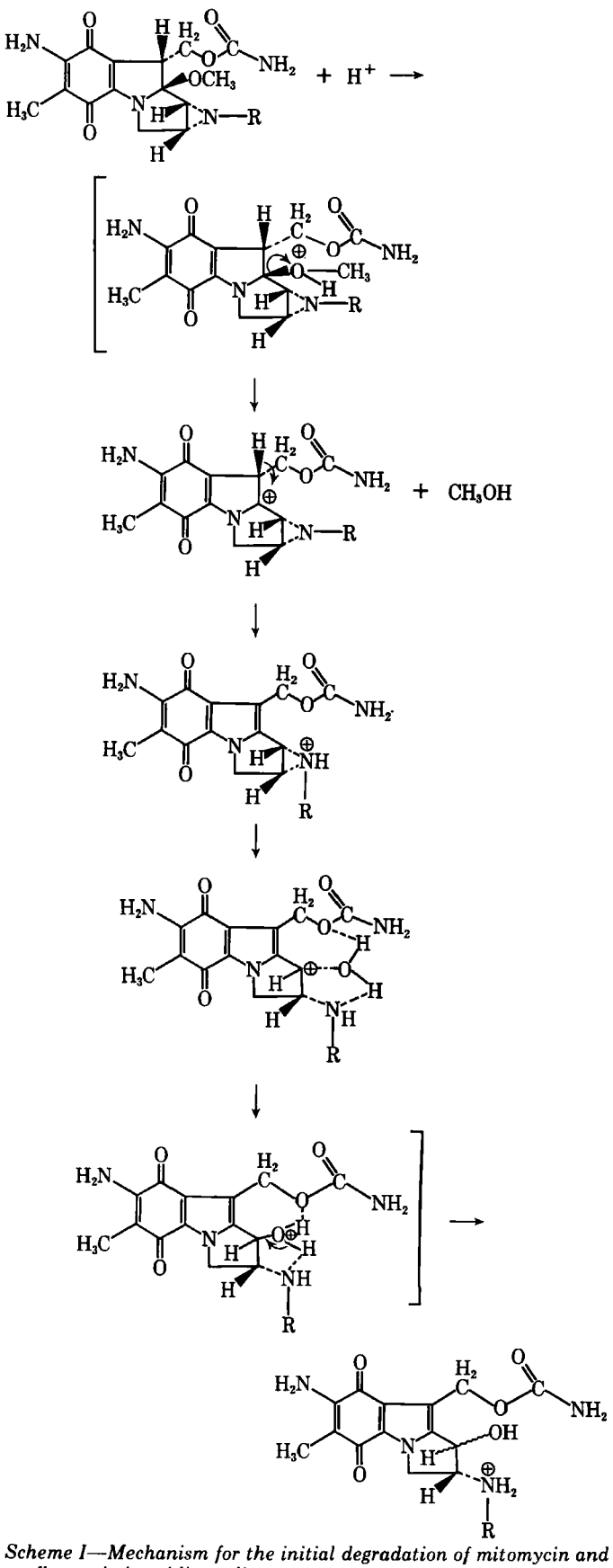
DISCUSSION

Although an overall scheme for the degradation of II (4) and mechanisms for the aziridine ring to open in deuteroacetic acid (2) and in water (12) have been published, no explanation for the very fast opening of the aziridine ring in I and II in comparison with other aziridines (13) has been given. The first degradation products of I and II isolated after degradation in an acidic solution appear to be compounds III and IV, respectively, where the 9-methoxyl group is cleaved, the 9=9a double bond is formed, and the aziridine ring is opened to form a 2-amino-1-hydroxy compound. The C-9 carbon carries two hetero atoms, N-4 and the methoxy oxygen, and this configuration can be considered as an amino–acetal. It is most likely that, as in acetal hydrolysis, cleavage of the methoxyl group starts with protonation of the oxygen, followed by the formation of methanol and a 9a-carbenium ion (14). Protonation of the aziridine nitrogen destabilizes this carbenium ion, which explains the shape of the pH profiles of the degradation. Subsequent deprotonation of C-9 results in the formation of the 9=9a double bond. It has been indicated that compound IV exists in one isomer, always with the hydroxyl group at C-1 and the amino group at C-2 (15). The positions of the groups at C-1 and C-2 are mainly *cis*-orientated. Thus the cleavage of the aziridine ring starts with protonation of the nitrogen followed by opening of the ring and formation of a carbenium ion at C-1.

The stability of this carbenium ion is enhanced by the presence of the 9=9a double bond. This serves as an explanation for the stereospecificity of the reaction with respect to the positions of the hydroxyl and amino groups. Attack of a water molecule on the carbenium ion is the final step in the reaction. The major compound formed is *cis*-2,7-diamino-1-hydroxymitosene (15) which indicates that this attack must have some stereospecificity. An explanation for the predominant existence of the *cis*-isomer might be the possibility of the existence of weak hydrogen bonds between the attacking water molecule and the ester oxygen of the



III: R = H
IV: R = CH_3



Scheme I—Mechanism for the initial degradation of mitomycin and profiromycin in acidic medium.

9-methylcarbamoyl and 2-amino groups. In the entire process the formation of the 9a-carbenium ion is the rate-determining step (16). The mechanism for acid degradation is presented in Scheme I.

The decrease in k_{obs} on addition of dioctyl sodium sulfosuccinate in the pH region 1.5–4 can be explained by the formation of an ion pair between the dioctyl sulfosuccinate anion and the protonated species. The octyl groups of the anion sterically hinder the protonation and subsequent cleavage of the 9a-methoxyl group. At pH > 5 the degree of protonation of the aziridine group, with pK_a 1.19 and 0.91 for I and II, respectively, becomes so small that the ion pair is virtually nonexistent and, hence, the protective influence of the additive disappears.

All buffer components and other additives with a negative charge had positive influences on the rate constants. Uncharged buffer species, such as phosphoric acid, acetic acid, and citric acid did not have a catalytic influence on the degradation processes, which differs from previous findings (4). The influences of the anions on the degradation can be associated with influences on the aziridine ring opening. In general, nucleophiles have negative effects on the stability of the aziridine moiety (9–11, 14). Degradation products containing chlorine, for instance, were isolated (9, 10). Analogues of these products in the degradation of mitosanes like I and II, however, could not be isolated. All rate constants for catalysis by singly charged anions are of the same order of magnitude (Table II) with the exception of dihydrogen citrate (H_2-citrate$^-$). This suggests a general ionic strength effect rather than more specific anionic catalysis on the degradation. In the case of H_2-citrate$^-$ the rate constant is determined at pH 3.06, being the pK_{a1} of citric acid. Although the pK_{a2} of this acid is 4.74, the [H-citrate^{2-}] was considered to be negligible for the calculation of $k_{H_2\text{-citrate}^-}$ of I and II. This is of course very approximate and makes the reported values of $k_{H_2\text{-citrate}^-}$ only a rough estimate which most likely includes an influence of H-citrate^{2-}. The rate constants for the $H_2PO_4^-$-catalyzed degradation of the protonated species are several orders of magnitude bigger than the other constants. This must be due to the fact that as a result of the opposite charges of the reacting species, electrostatic attraction causes a much greater possibility of encounter and, therefore, reaction.

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Determination of pK_a Values of Some Prototropic Functions in Mitomycin and Porfiromycin

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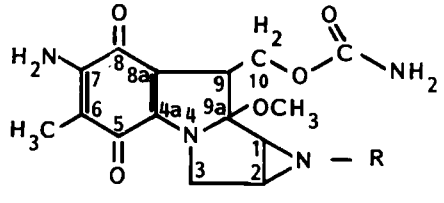
Abstract □ The prototropic properties of mitomycin and porfiromycin were studied. pK_a values for two potentially basic groups and one acidic function could be established by titration. The kinetics of the tautomerization preceding the prototropic reaction in an alkaline medium were investigated.

Keyphrases □ Prototropic properties—mitomycin, porfiromycin, alkaline medium, kinetics of tautomerization □ Mitomycin—prototropic properties, pK_a determinations, tautomerization kinetics □ Porfiromycin—prototropic properties, pK_a determinations, tautomerization kinetics □ Kinetics—tautomerization, mitomycin, porfiromycin

Mitomycin (I), originating from *Streptomyces caespitosus* (1), is an important antitumor antibiotic. The compound is unstable in solution and aspects of the degradation of I and porfiromycin (II), which differs from I only in the methyl substituent on the aziridine nitrogen, have been studied by several investigators (2–6). For a systematic study of the chemistry of these compounds, including the stability, it is necessary to characterize the prototropic properties of I and II.

From the structures it can be seen that the compounds have several basic groups, including the aziridine nitrogen, the N-4 nitrogen, and the 7-amino function. In the literature data are available only on the protonation of one basic function; these data are either unreliable or are approximations. The pK_a of a basic group in I has been deter-



I: R = H
II: R = CH_3

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