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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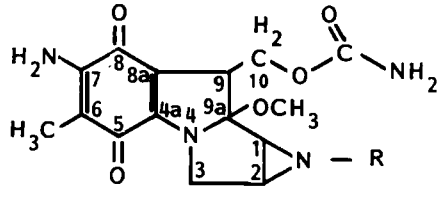
Received August 11, 1981 from the *Pharmaceutical Laboratory, Department of Analytical Pharmacy, State University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands.* Accepted for publication May 28, 1982.

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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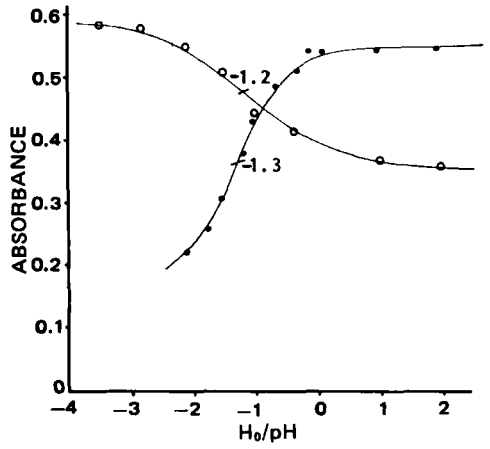


Figure 1—*Relation between absorbance and acidity; determination of pK_a (at room temperature) of the 7-amino group and N-4 nitrogen. Key: (●) mitomycin degradation product (IV), absorbance monitored at 310 nm, pK_{a2} −1.3; and (○) mitomycin analogue (III), absorbance monitored at 268 nm, pK_{a2} −1.2.*

mined by titration with acid to be 3.2 (2), but I in acid medium is very unstable. By a kinetic model, the pK_a of the protonated group in II was estimated to be ~1.5, and it was suggested that this pK_a could be assigned to the aziridine nitrogen in II (4). The present study was undertaken to determine the pK_a values of the other prototropic groups in I and II in an attempt to complete the prototropic characterization of the compounds.

EXPERIMENTAL

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

Methods—*Measurement of pK_a Values*—For the measurement of pK_a values of III and IV, the compounds were titrated with perchloric acid while the absorbances at 268 and 310 nm, respectively, were monitored as a function of acidity (pH or H_0). The pK_a values of the basic groups were calculated from these relationships. For the determination of the pK_a of the acidic function in I, a titration of the compound with sodium hydroxide was performed while monitoring the absorbance at 363 nm. Up to pH 13 the degradation was too slow to interfere with the

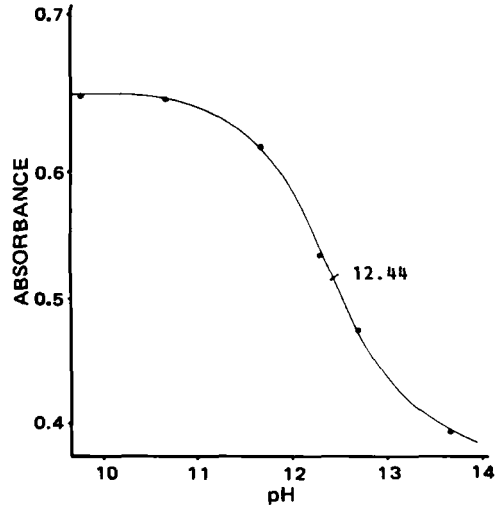


Figure 2—*Relation between absorbance at 363 nm and pH; determination of pK_a of the acidic function of mitomycin at room temperature, pK_{a4} 12.44.*

[1] Supplied by Bristol Myers B. V., Bussum, The Netherlands.
[2] Supplied by Cyanamid, Pearl River, N.Y.

Table I—First-Order Rate Constants for Deprotonation (k_{dep}) and half-lives ($t_{1/2}$) of I at Various pH Values at 40°

pH	k_{dep}, sec^{-1}	$t_{1/2}$, sec
12.0	$8.2 \pm 0.5 \times 10^{-2}$	8.5 ± 0.6
12.7	$5.5 \pm 0.6 \times 10^{-2}$	13 ± 1
13.0	$3.4 \pm 0.4 \times 10^{-2}$	20 ± 1
13.4	$2.8 \pm 0.2 \times 10^{-2}$	25 ± 1

change in absorbance due to deprotonation. At pH values >13 a continuous decrease in absorbance at 363 nm was observed, which was due to degradation of the compound in a strong alkaline solution (5). Consequently, the absorbance–pH relationship could only be obtained incompletely and A_{I^-}, the absorbance of the deprotonated species, was not measurable. In terms of absorbance, the acidity constant K_a can be expressed as

$$K_a = \frac{(A_I - A)[H^+]}{(A - A_{I^-})} \qquad \text{(Eq. 1)}$$

where A_I is the absorbance of uncharged I, A_{I^-} is the absorbance of deprotonated I, and A is the absorbance of the solution at a given pH. Equation 1 can be rearranged to:

$$(A_I - A)[H^+] = K_a(A - K_a)(A_{I^-}) \qquad \text{(Eq. 2)}$$

A plot of A *versus* $(A_I - A)[H^+]$ yields a straight line with slope K_a and intercept $(-K_a)(A_{I^-})$, thus leading to the values of K_a, pK_a, and A_{I^-}.

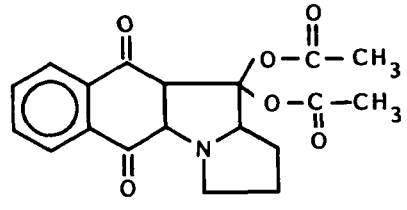
Kinetic Study of I Deprotonation—For the study of the kinetics of I deprotonation, several solutions in water with concentrations varying from 1.5×10^{-6} *M* to 1.8×10^{-4} *M* were prepared. After mixing equal volumes of sodium hydroxide solutions at various pH with the aforementioned solutions of I, the decrease in absorbance at 363 nm was monitored using stopped-flow equipment[3]. Measurements of pH were done with a glass-reference electrode using an appropriate pH meter[4]. Rate constants and half-lives were calculated using plots of log (percent uncharged I) *versus* time.

RESULTS

The basic groups of I and II, apart from the aziridine function, are the N-4 nitrogen and the 7-amino group. The pK_a of the N-4 nitrogen can be established by titration of an analogue of I (III) with perchloric acid. The absorption spectrum of uncharged III has a maximum at 280 nm. During protonation this maximum shifts to 268 nm, with an increase in absorptivity. Thus it is possible to follow the degree of protonation by monitoring the absorption at 268 nm, provided that no degradation occurs during the process. A solution of III in 1 *M* perchloric acid (H_0 −0.3) does not show changes in absorptivity at 268 nm when kept at 40° for 1 hr. The relation between the absorbance at 268 nm and the acidity, expressed in terms of the Hammett acidity scale, is shown in Fig. 1. The pK_a of the nitrogen in III calculated from this relationship is −1.2 and almost certainly this value can also be assigned to the N-4 nitrogen in I and II.

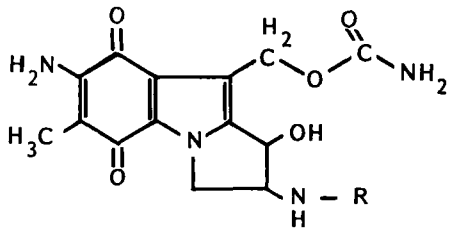
The spectra of I and II quickly change in acidic solution due to cleavage of the 9a-methoxyl group and opening of the aziridine ring, thus producing degradation products IV and V, respectively. Compound IV in acidic solution also undergoes degradative processes but at a much slower rate (4, 5). The pK_a of the 2-amino group in the compound is ~7 (5) so that degradations of I and II at pH 5 result in the formation of protonated IV and V, respectively. These compounds are sufficiently stable to be titrated with perchloric acid to establish the pK_a of the other basic functions. The absorption maximum of IV in the neutral form is 310 nm; on acidification this maximum disappears.

The absorption band at ~250 nm changes in shape while the absorp-



III

[3] Durrum 110 with Durrum 13000 Spectrophotometer.
[4] Metrohm Herisau E516 Titriskop pH meter.



IV: R = H
V: R = CH_3

tivity increases. The pK_a can be established from the relation between the absorbance at 310 nm and the acidity (Fig. 1). The more acidic part of the curve is unreliable because degradation of IV in that acidity region cannot be neglected. The pH of the protonated group, determined using Eq. 2 after rearrangement, appears to be −1.3. The N-4 nitrogen in IV is much less basic than in I due to the introduction of the 9=9a double bond, as can be seen from indole (pK_a −2.5) (7). Therefore, the pK_a of IV can be assigned to the 7-amino group. From the similarity in the structures of I, II, and IV, it becomes clear that this pK_a value also can be assigned to the 7-amino groups in I and II.

The absorption spectrum of I does not change between pH 6 and 10. At pH < 6 a gradual shift in spectrum with time takes place due to degradation, while at pH > 10.5 a sudden change in the absorption spectrum occurs. The maximum at 363 nm (pH 10.5) shifts to 295 nm (pH 13.0) with a sharp decrease in absorptivity. This shift could be due to a configurative change in the molecule, stabilized by deprotonation of I at the higher pH. The pK_a of the acidic function can be determined from the relation between the absorbance at 363 nm and the pH, provided no degradation occurs. Although alkaline degradation of II takes place at rates comparable to those in acidic medium (4), the stability of I in the region pH < 13 is sufficiently high to permit the determination of the pK_a using Eq. 2. The curve cannot be completed because the degradation of I cannot be neglected at pH > 13. The relation between the absorbance at 363 nm and pH, represented in Fig. 2, leads to the calculation of a pK_a of 12.44.

Although the change in absorptivity with change in pH at 363 nm in the region of pH > 10.4 is very rapid, it is not instrumentally instantaneous. The kinetic process involved can be monitored using stopped-flow equipment. These experiments indicate that the reaction follows (pseudo) first-order kinetics. The relation between the apparent rate constant for deprotonation, k_{dep}, and the pH is presented in Table I. The influence of the initial [I] at a pH > pK_a and at a pH < pK_a is listed in Table II. The data of Table I show that k_{dep} decreases with increasing pH.

At pH 13.0, k_{dep} is independent of [I] in the chosen concentration range but at pH 12.0 an increase of [I] causes a decrease in k_{dep} as presented in Table II. The data obtained at pH > pK_a indicate that the process follows first-order kinetics, but the data at pH < pK_a contradict such a conclusion. A plausible explanation for the results is that aggregation occurs between the molecules at higher concentrations of uncharged I. At pH < pK_a the majority of the molecules appears in the protonated neutral form so that in this pH region the possibility of aggregation will be relatively high. Other properties, such as the absorptivity, may change also with an increase in the concentration. In Fig. 3 the relation between the absorbance at 290 nm and [I] at pH 10 is presented. The curve shows linearity between absorbance and concentration up to 1.5×10^{-5} *M* I. At concentrations over 2.0×10^{-5} *M* the absorbance is no longer linear with [I], which means that the chromophore is influenced by interaction with other molecules.

Table II—Rate Constants (k_{dep}) and Half-Lives ($t_{1/2}$) of I at Various Concentrations at Two Different pH Values at 40°

pH	[I] × 10^5 *M*	k_{dep}, sec^{-1}	$t_{1/2}$, sec
12.0	1.5	8.9×10^{-2}	7.7
	3.0	8.7×10^{-2}	8.0
	6.0	6.8×10^{-2}	10.2
	9.0	4.7×10^{-2}	15.0
13.0	3.0	3.5×10^{-2}	20.0
	6.0	3.3×10^{-2}	21.0
	9.0	3.4×10^{-2}	20.5

DISCUSSION

The data presented above lead to the following conclusions on the prototropic nature of I and II. Both compounds have three basic functions:

1. The 7-amino group, with pK_{a1} − 1.3 at room temperature.
2. The N-4 nitrogen, with pK_{a2} −1.2 at room temperature. It is possible that pK_{a2} is a little higher, due to mesomeric effects of the 7-amino group.
3. The aziridine nitrogen, with pK_{a3} ~1.5 (4).

The compounds also have an acidic function, with pK_{a4} 12.44 at room temperature. This pK_a must be assigned to the 7-amino–quinoid moiety in the compounds, as is shown in Scheme I. The keto–enol tautomerism in this structure is responsible for the spectral changes, while the absorbance value marks the position of the equilibrium. The tautomeric reaction has a finite rate.

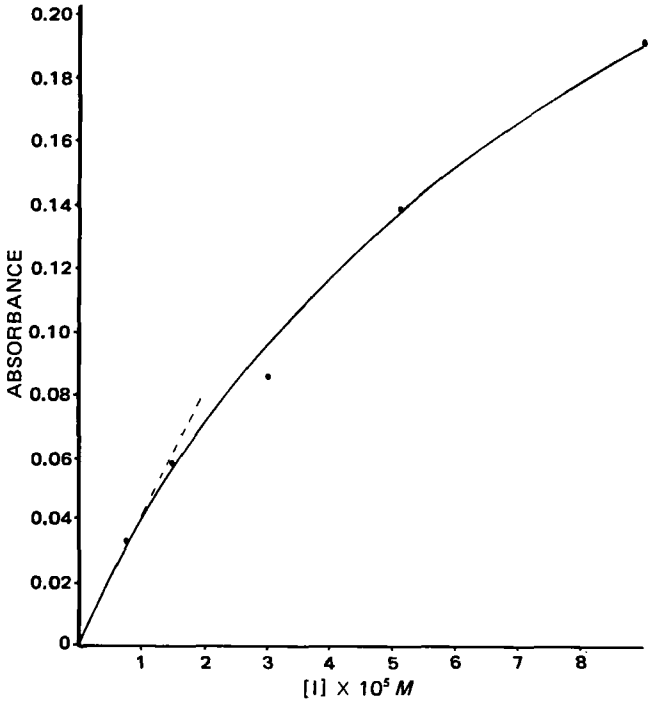
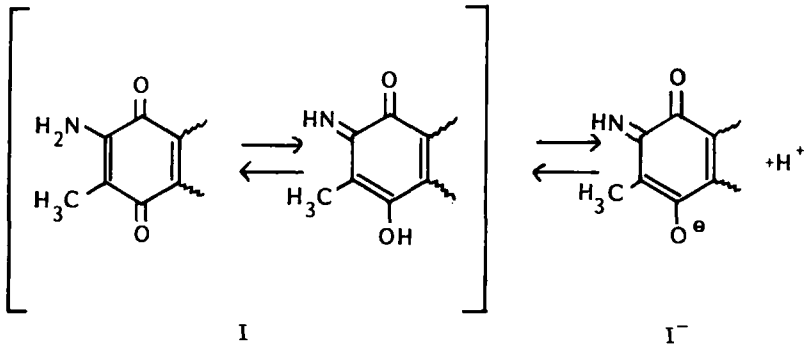


Figure 3—*Relation between absorbance at 290 nm and mitomycin concentration at pH 10 at room temperature.*



I I⁻

Scheme I—Deprotonation of mitomycin, Key: I, uncharged mitomycin; I⁻, deprotonated mitomycin.

The degree of protonation (α) of the reaction can be written as:

$$\alpha = \frac{[I^-]}{[I + I^-]} = \frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 3)}$$

The overall rate constant k_{dep} consists of:

$$k_{dep} = k_t^{I}[I] + k_t^{I^-}[I^-] = k_t^{I}(1 - \alpha) + k_t^{I^-}(\alpha) \quad \text{(Eq. 4)}$$

where k_t^{I} is the rate constant for tautomerization of uncharged I and $k_t^{I^-}$ the same constant for I^- (deprotonated I). Combination of Eqs. 3 and 4 yields:

$$k_{dep} = (k_t^{I})\frac{[H^+]}{K_a + [H^+]} + (k_t^{I^-})\frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 5)}$$

Assuming that, with respect to tautomerization, I is much more active than I^- or that $k_t^{I} \gg k_t^{I^-}$, it is obvious that on increasing the pH, the term $k_t^{I}([H^+]/K_a + [H^+])$ will decrease more rapidly than the term $k_t^{I^-}([H^+]/K_a + [H^+])$ increases which will result in a lower value of k_{dep}. Under the assumption that $k_t^{I^-}$ is so small that the term $k_t^{I^-}(K_a/K_a + [H^+])$ can be neglected, Eq. 5 permits the estimation of k_t^{I}, using pK_a 12.44 of the acidic group and the values for k_{dep} listed in Table I. The calculation results in a k_t^{I} of $1.4 \pm 0.4 \times 10^{-1}$ sec^{-1} and a half-life ($t_{1/2}$) of the process of 5.0 sec. The explanation of the concentration dependence of k_{dep} at $pH < pK_a$ lies in the aforementioned possibility of interaction between the 7-amino–quinoid moieties on increasing [I]. The occurrence of these interactions is shown by the changes in absorptivity at higher [I] at pH 10, where the compound is present almost completely in the uncharged form. The decrease in the molar absorptivity must be due to interaction of the 7-amino–quinoid chromophores. Since the tautomerization occurs in the same functional group in the molecule, this interaction most likely also causes a decrease in the overall rate constant k_{dep} at higher [I] at pH 12.0, as the concentration of the unaffected 7-amino–quinoidal function decreases due to this interaction. Equation 4 shows that on decreasing [I] and $[I^-]$, k_{dep} will decrease. At pH 13.0, where $[I] = 0.2\ [I + I^-]$, the concentration of uncharged I in the range studied is too low for aggregation to occur.

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NOTES

Tissue Distribution of [^{14}C]Bretylium Tosylate in Rats

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Received March 2, 1982, from the *Department of Pharmaceutical Development, Research and Development Department, American Critical Care, McGaw Park, IL 60085.* Accepted for publication April 21, 1982. *Present address: Xavier University, New Orleans, LA 70125.

Abstract □ The distribution of [^{14}C]bretylium tosylate in the body and the relationship between tissue and plasma concentrations was determined following intravenous administration of the drug to Charles River rats. The renal excretion of bretylium was rapid in rats and follows an active process. On the average, 50% of the administered dose was excreted in the urine within 1 hr. In the postequilibrium phase, the plasma concentration declined with a half-life of 5 hr. Bretylium concentrations in all tissues, except the heart, declined rapidly according to a triexponential equation. The liver and kidney bretylium concentrations declined in parallel to the plasma concentration with mean tissue–plasma concentration ratios of 6.04 and 12.3, respectively, in the β phase. However, the concentration of bretylium in the heart increased gradually and peaked at 2 hr, with a tissue–plasma concentration ratio of 121, which, in turn, declined to a value of >60 after 8 hr. The data indicated that (*a*) bretylium is rapidly distributed into the liver and kidney immediately after reaching the systemic circulation; (*b*) the distribution into the heart occurs at a slower rate compared with the other organs, and the drug has a high affinity to the myocardium; and (*c*) since the heart is the site of action and there is no direct correlation between the concentrations in myocardium and plasma, the antiarrhythmic effect of bretylium may not be related to the plasma concentration.

Keyphrases □ Bretylium tosylate—^{14}C-labeled, tissue distribution, rats, plasma, renal excretion □ Distribution—tissue, [^{14}C]bretylium tosylate in the rat, plasma, renal excretion □ Excretion, renal—tissue distribution of [^{14}C]bretylium tosylate in rats, plasma

Bretylium tosylate is a quaternary ammonium salt given in the treatment of cardiac arrhythmia. It suppresses *ventricular* fibrillation within minutes of intravenous infusion (1). The suppression of ventricular tachycardia, however, developed more slowly, usually 20–120 min after intramuscular administration of 4 mg/kg of bretylium tosylate in humans (2). The effect of bretylium in the intact heart was shown to be biphasic, with an initial transient increase in blood pressure and heart rate followed by an opposite and more prolonged depression of these same

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