Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 1, pp. 59–69, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Quantitative aspects of the degradation of mitomycin C in alkaline solution

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Abstract: The alkaline hydrolysis of mitomycin C has been studied over a wide range of pH/H_(7–15). A stability-indicating high-performance liquid chromatographic (HPLC) method was used to separate the degradation products from the parent drug. The quantitative effects of temperature and buffers on the degradation of mitomycin C in alkali have been determined. A profile of log k_{obs} against pH/H_ was constructed after corrections had been made for buffer effects and after extrapolation to 25°C by application of the Arrhenius equation.

Keywords: *Mitomycin C stability in alkaline solution; degradation kinetics; buffer effects; temperature effects.*

Introduction

Mitomycin C (MMC) is an antineoplastic agent that is effective in the treatment of various solid tumours [1]. This antibiotic is produced by the growth of *Streptomyces caespitosus* [2]. Its structural formula, illustrated in Fig. 1, is characterized by the 1–2 fused aziridine ring system, a quinoid moiety, a carbamate group, a pyrrolidine nucleus and an indoline ring system. Doubts have been expressed recently about the absolute stereochemical configuration [3].

Like porfiromycin (PM), its 1a-*N*-methyl derivative, MMC degrades in acidic and alkaline media [4–6]. In acidic solution MMC degrades to 1–2 disubstituted mitosenes in which the aziridine ring has been opened and the 9a-methoxy group cleaved; this results in the formation of a double bond between C_{9-9a} [7]. One isomer is mainly formed; this isomer possesses the hydroxyl group at C_1 and the cis-amino group at C_2 [8]. Underberg

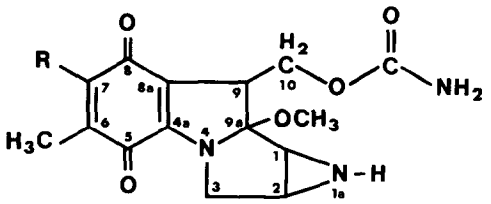

Figure 1
Structures of mitomycin C (R = NH_2) and X (R = OH).

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and Lingeman [6] have proposed a mechanism for this initial degradation step of MMC and PM on the basis of kinetic data.

Most stability studies have been performed on the decomposition of MMC or PM in acidic media and, except for fragmentary reports [4, 5, 9–11], the authors know of no systematic studies published on the instability of MMC in alkali. The present systematic study was undertaken to collect quantitative data and to obtain more detailed knowledge on the kinetics of the initial alkaline degradation step of MMC including the effects of ionic strength, temperature, pH and buffer components.

On the grounds of spectral shifts and the pK_a, Garrett [5] postulated that mild alkaline conditions should lead to substitution of a hydroxyl group for the 7-amino group to yield compound X (Fig. 1). The physicochemical and analytical properties of this compound are presented in the accompanying paper [12].

This project is a sequel to earlier studies in which the kinetics of the degradation of MMC in acidic media were examined and pK_a values of prototropic functions in MMC determined [6, 13].

Experimental

Materials

MMC was kindly provided by Prof. Dr Masaki Otagiri (Kumamoto University, Kumamoto, Japan). All other chemicals were of analytical grade; deionized water was used.

Buffer solutions

The following aqueous buffer solutions were used for the kinetic studies: pH 7–8.5, phosphate; pH 8.5–10.5, borate; pH 10.5–11.5, phosphate and pH > 11.5, sodium hydroxide. The pH was measured at the temperature of the experiment using a glass reference electrode and a pH meter (Metrohm Herisau E516 Tritriskop, Switzerland). pH values of 12.0 to 13.0 were calculated using the Debye–Hückel equation [14].

Further extension of the pH/H_- scale was accomplished by use of the Hammett function [14]. It was not necessary to keep the ionic strength constant because the degradation rate was not affected by ionic strength up to $\mu = 2.0$.

Kinetic measurements

A 10 µl sample of a solution of MMC in methanol was added to 5 ml of the pre-heated buffered solution so that the initial concentration was about 3×10^{-5}M. The solutions were kept in screw-capped test tubes in a water-bath at 25 ± 0.2°C for decomposition studies at $pH/H_- > 10.5$.

Degradation studies at pH values <10.5 were performed at several elevated temperatures. At appropriate times samples were analysed for undegraded MMC by a stability-indicating assay by high-performance liquid chromatography (HPLC). The pH of samples obtained from partly degraded solutions at pH/H_- values higher than 10.5 was reduced before chromatographic analysis.

Degradation at pH/H_- values >13 was followed spectrophotometrically by continuously monitoring the absorbance at 365 nm. In these stability tests, 5 µl of a solution of MMC in methanol (1.5×10^{-2}M) was added to 2.5 ml of the pre-heated buffered solution in a stoppered cell at 25 ± 0.2°C in the spectrophotometer. MMC degradation was monitored at H_- values >13 by HPLC, but the assays were performed only at lower

temperatures in order to decrease the degradation rate. The observed rate constants were extrapolated to room temperature (25°C) by application of the Arrhenius equation.

Apparatus and experimental conditions

The HPLC analysis was performed by the procedure of den Hartigh *et al.* [15] with modifications.

A model M-45 liquid chromatograph was used equipped with a UV 440 dual wavelength detector (Waters Associates, Milford, MA, USA) operating at 365 and 313 nm with a sensitivity of 0.05 and 0.02 a.u.f.s., respectively, for most samples. The 300 × 3.9 mm i.d. column was packed with Lichrosorb 10 RP-18 (Merck, Darmstadt, FRG). The eluent comprised methanol–water (30:70, m/m). To this mixture 0.5% v/m of a 0.5 M sodium phosphate buffer solution (pH 7.0) was added.

The flow rate was 1.0 ml min^{-1} and the column was used at ambient temperature. The column pressure was approximately 1900 psi. Samples of 20 μl were injected into the chromatograph. Peak area measurement was used to quantitate undegraded MMC. Standard curves exhibited linear response ($r > 0.999$) in the concentration range of interest, 3×10^{-5} to 9×10^{-7}M. Relative standard deviations of 0.7–9.0% were obtained for replicate injections of 3×10^{-5} to 9×10^{-7}M solutions, respectively.

Ultraviolet and visible spectra were recorded on a Shimadzu UV-200 double-beam spectrophotometer equipped with a Kipp BD 40 Recorder. Kinetic studies were performed with a Shimadzu UV-140 double-beam absorption spectrophotometer operating at 365 nm; 1-cm quartz cells were used.

Choice of analytical methodology

Figure 2 represents the spectra of undegraded MMC (A) and of MMC at the stage of about 50% decomposition (B) in 0.1 M phosphate buffer solution pH 11.0. The spectral changes due to MMC degradation were too slight for the adequate determination of rate constants. Therefore an HPLC method was used so that decomposition products could be resolved from the parent MMC ($k' = 1.9$). Compound X is eluted with the solvent front. Retention of X for quantification purposes can be achieved by adding a quaternary

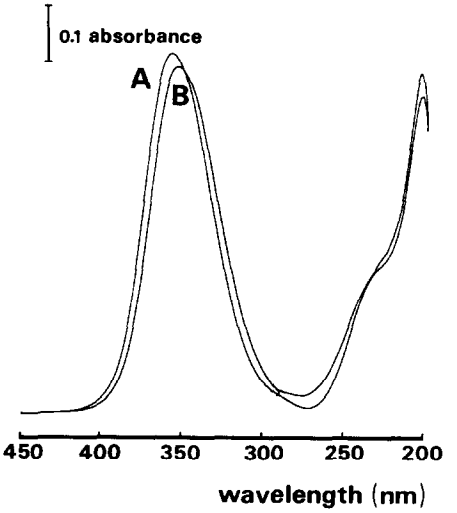

Figure 2
Spectra of 3×10^{-5}M mitomycin C in 0.1 M phosphate buffer solution (pH 11.0) at $t = 0$ (A) and $t = t_{1/2}$ (B).

ammonium pairing ion to the mobile phase [12]. However, the present HPLC method has been found to be adequate for study of the degradation of the parent compound.

The kinetics of decomposition of MMC at H_- values >13 can be followed spectrophotometrically, since the spectral changes are sufficiently great. The graph of change in absorptivity at 365 nm on degradation consists of two parts. The first part can be attributed to achievement of the keto-enol tautomeric equilibrium and deprotonation of the 7-amino quinoid moiety [13] (Fig. 3). The second part of the curve represents the decrease in absorptivity due to decomposition of deprotonated MMC (MMC^-). Both processes occur simultaneously but the differences between the rates of tautomerism and degradation allow the second part of the curve to be used for the determination of rate constants after correction for the resulting absorbance at complete degradation.

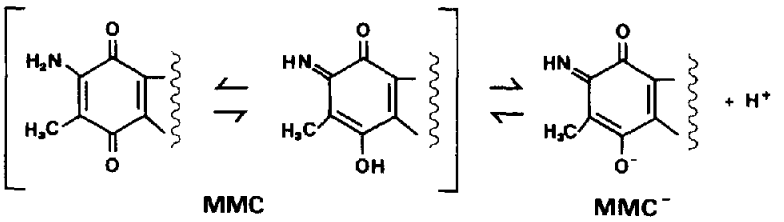

Figure 3
Tautomerization and deprotonation of mitomycin C [13].

Results

Order of reactions

The disappearance of MMC or its deprotonated form in buffered solution follows strict pseudo first-order kinetics over several half-lives. This is indicated by the linearity ($r > 0.99$) of plots of log [MMC] and log [MMC^-] against time.

From the slopes of these straight lines values for k_{obs}, the pseudo first-order rate constant, can be obtained (Fig. 4).

The rate equation may be written as:

$$-\frac{d\,[MMC]}{dt} = k_{obs}\,[MMC]. \qquad (1)$$

Standard deviation of k_{obs}

The standard deviation (SD) of the overall rate constant, k_{obs}, was determined for the HPLC and spectrophotometric assays. These statistical assessments were performed at 25°C. The value of k_{obs} (±SD) for the spectrophotometric assay was determined at H_- 14.9; the value of k_{obs} was $8.8 \pm 0.4 \times 10^{-3}\ s^{-1}$ ($n = 8$). For the HPLC assay, values of k_{obs} were determined at pH 11.1 and at several phosphate concentrations. The results of these experiments are listed in Table 1. Corrections for the effects of the buffer ions were made by plotting the mean values of k_{obs} against total phosphate concentration; this yielded a straight line ($r = 0.9992$) with an intercept (k'_{obs} at zero buffer concentration) of $2.0 \times 10^{-5}\ s^{-1}$. SD values were of the same order of magnitude as those for the spectrophotometric method. Other rate constants are mean values of duplicate determinations.

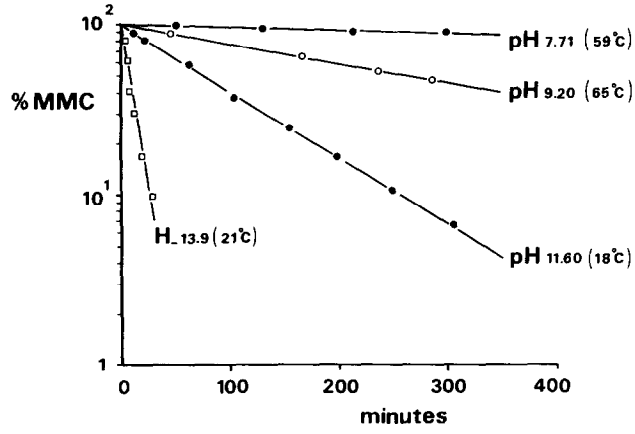

Figure 4
Semilogarithm apparent first-order plots for the degradation of MMC in 0.1 M phosphate (●), 0.05 M borate (○), and 1.0 M sodium hydroxide (□) buffer solutions.

Table 1
k_{obs} values for MMC degradation at pH 11.1 and various phosphate concentrations at 25°C

[Total phosphate] (M)	k_{obs}* (s^{-1})	SD† (s^{-1})
0.25	9.8×10^{-5}	0.3×10^{-5}
0.10	5.3×10^{-5}	0.2×10^{-5}
0.05	3.5×10^{-5}	0.7×10^{-6}

* Mean values of six observations.
† Standard deviation.

Influence of pH

At pH 7.0–10.5 accelerated stability tests were performed because of the slow reaction rates in this pH range. By application of the Arrhenius equation the observed rate constants were extrapolated to room temperature (25°C). At higher pH/H_ values degradation took place at 25°C or lower temperatures. The rate constants determined at lower temperatures were also extrapolated to 25°C similarly.

It is necessary to keep the pH constant by use of a buffer because decomposition leads to the formation of compound X which has acidic properties ($pK_a \sim 4.3$) [5]. However, the possible catalytic effects of buffer components [4, 9] have to be taken into account. Hence, the k_{obs} value can be expressed as

$$k_{obs} = k_o^{MMC} + k_{OH}^{MMC}[OH^-] + k_H^{MMC}[H^+] + k_{buffer}^{MMC}[buffer] \quad (2)$$

where k_o^{MMC} is the first-order rate constant for degradation in water only, k_{OH}^{MMC} and k_H^{MMC} second-order rate constants for hydroxyl- and proton-catalysed degradation, respectively. The term k_{buffer}^{MMC} [buffer] represents the sum of the second-order rate constants for degradation catalysed by each of the buffer components, multiplied by its concentration. At pH > 7.5 the contribution to k_{obs} of the term $k_H^{MMC}[H^+]$ (equation 2)

can be neglected because of the low hydrogen ion concentration. Equation (2) is only valid when virtually all MMC is present in the undissociated form, that is at pH values <10.44, since the acid pK_a has been reported to be 12.44 [13].

For each pH value, a plot of k_{obs} against buffer concentration yields a graph with an intercept that corresponds to the observed rate constant (k_{obs}^{I-III}) at zero buffer concentration. These rate constants were used in the construction of the pH/H_ profile (Fig. 5). Theoretically, the values of k_{obs}^{I-III} in the different parts of this graph can be described by three equations, based on the pK_a for MMC of 12.44 [13].

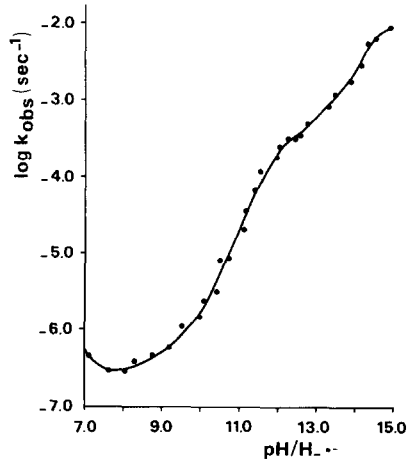

Figure 5
pH/H_ rate constant profile for mitomycin C degradation at 25°C.

Where 7.5 < pH < 10.44

$$k_{obs}^{I} = k_o^{MMC} + k_{OH}^{MMC}[OH^-] \tag{3}$$

Where 10.44 < pH/H_ < 14.44

$$k_{obs}^{II} = [k_o^{MMC} + k_{OH}^{MMC}[OH^-]] \cdot \frac{[MMC]}{[MMC]_t} + [k_o^{MMC^-} + k_{OH}^{MMC^-}[OH^-]] \cdot \frac{[MMC^-]}{[MMC]_t} \tag{4}$$

Where H_ > 14.44

$$k_{obs}^{III} = k_o^{MMC^-} + k_{OH}^{MMC^-}[OH^-]. \tag{5}$$

The total MMC concentration is given by: $[MMC]_t = [MMC] + [MMC^-]$. The mole fraction of each species at a specific pH can be calculated using the Henderson–Hasselbalch equation [14].

Plots of k_{obs}^{I} and k_{obs}^{III}, respectively, against $[OH^-]$ yield straight lines with slopes equal to k_{OH}^{MMC} and $k_{OH}^{MMC^-}$ respectively and intercepts equal to k_o^{MMC} and $k_o^{MMC^-}$ respectively.

These rate constants are listed in Table 2. Figure 5 shows the pH/H_ profile for the hydrolysis of MMC at 25°C. It transpires that the k_o^{MMC} term is the principal contributor to the observed rate constants in the pH range 7.5–9.0. The first-order rate constant for hydrolysis in this pH range is 5×10^{-7} s^{-1}, corresponding to a half-life of 385 h at room temperature. The slope of the straight line segment between pH 10 and 11.5 is +1.06;

Table 2
Rate constants* for catalysed degradation reactions of MMC and MMC^- at 25°C

MMC		MMC^-
k_o^{MMC}	$5 \times 10^{-7}\ s^{-1}$	$k_o^{MMC^-} \sim 4 \times 10^{-3}\ s^{-1}$
k_{OH}^{MMC}	$1.2 \times 10^{-2}\ mol^{-1}\ s^{-1}$	$k_{OH}^{MMC^-} \sim 1 \times 10^{-3}\ mol^{-1}\ s^{-1}$
$k_{H_2PO_4^-}^{MMC}$	$2 \times 10^{-6}\ mol^{-1}\ s^{-1}$	$k_{HPO_4^{2-}}^{MMC^-}$ 0
$k_{HPO_4^{2-}}^{MMC}$	$5.4 \times 10^{-6}\ mol^{-1}\ s^{-1}$	$k_{PO_4^{3-}}^{MMC^-}$ 0
$k_{PO_4^{3-}}^{MMC}$	$\sim 1 \times 10^{-4}\ mol^{-1}\ s^{-1}$	
$k_{H_3BO_3}^{MMC}$	0	
$k_{BO_2^-}^{MMC}$	$1.3 \times 10^{-5}\ mol^{-1}\ s^{-1}$	
$k_{Ac^-}^{MMC}$	0	
$k_{NO_3^-}^{MMC}$	0	
$k_{Cl^-}^{MMC}$	0	

* Second-order rate constants, except for the first-order rate constants reported for k_o^{MMC} and $k_o^{MMC^-}$

this is close to unity and indicates the occurrence of specific base catalysis. However, the slope of the linear portion of the curve above approximately pH/H_ 12.5–13 is not equal to unity. It appears that hydrolysis is not simply due to specific base catalysis at these high pH/H_ values.

Influence of buffer components

Other investigators [4–6] have pointed out the catalytic effects of buffer components on the degradation of MMC in acidic media. In alkaline media the degradation of MMC is subject to general base catalysis. This catalytic effect was determined by measurement of the decomposition rate at constant pH and temperature, but at different buffer concentrations. This implies that only the fourth term in equation (2) is varied at a specific pH.

A linear relationship was found to exist between k_{obs} and the total borate concentration up to 0.06 M. However, the relationship between the rate constant of MMC and the total phosphate concentration is linear within a wider concentration range (up to 0.5 M). The second-order rate constants for buffer-catalysed degradation can be determined as follows. The pK_a values of phosphoric acid are: $pK_{a_1} = 2.15$; $pK_{a_2} = 7.10$; and $pK_{a_3} = 12.32$ [16]. In the range $9.1 < pH < 10.3$, phosphate buffers consist almost exclusively of HPO_4^{2-} ions, so that this ion can be considered to be the only potential catalytic species. Since in this pH region the $k_H^{MMC}[H^+]$ term is negligible, equation (2) can be written as:

$$k_{obs} = k_o^{MMC} + k_{OH}^{MMC}[OH^-] + k_{HPO_4^{2-}}^{MMC}[HPO_4^{2-}] \tag{6}$$

and a plot of k_{obs} against $[HPO_4^{2-}]$ yields a straight line with a slope equal to $k_{HPO_4^{2-}}^{MMC}$. The second-order rate constant $k_{PO_4^{3-}}^{MMC^-}$ was determined by measurement of the observed rate constants as a function of total phosphate concentration at H_ 14.5. At this high value of H_, MMC is present almost exclusively in its deprotonated form and phosphate buffers are present largely as PO_4^{3-} ions. Consequently, k_{obs} can be expressed as

$$k_{obs} = k_o^{MMC^-} + k_{OH}^{MMC^-}[OH^-] + k_{PO_4^{3-}}^{MMC^-}[PO_4^{3-}]. \tag{7}$$

A plot of k_{obs} against $[PO_4^{3-}]$ yields a horizontal line, so that $k_{PO_4^{3-}}^{MMC^-} = 0$. In the region of $5.1 < pH < 9.1$, phosphate buffers contain two anionic species, $H_2PO_4^-$ and HPO_4^{2-}. Therefore equation (2) must be modified to:

$$k_{obs} = k_o^{MMC} + k_{OH}^{MMC}[OH^-] + k_H^{MMC}[H^+] + k_{H_2PO_4^-}^{MMC}[H_2PO_4^-] + k_{HPO_4^{2-}}^{MMC}[HPO_4^{2-}]. \quad (8)$$

The total phosphate concentration is equal to the sum of $[H_2PO_4^-]$ and $[HPO_4^{2-}]$; furthermore: f[total phosphate] = $[H_2PO_4^-]$ and consequently (1-f) [total phosphate] = $[HPO_4^{2-}]$, where the symbol f represents the mole fraction of a buffer species to the total buffer concentration, calculated according to the Henderson–Hasselbalch equation [14]. Therefore, equation (8) can be transformed into:

$$k_{obs} = k_o^{MMC} + k_{OH}^{MMC}[OH^-] + k_H^{MMC}[H^+] + [(k_{H_2PO_4^-}^{MMC} - k_{HPO_4^{2-}}^{MMC}) f + k_{HPO_4^{2-}}^{MMC}][\text{total phosphate}]. \quad (9)$$

A plot of k_{obs} against [total phosphate] yields a straight line with a slope $(k_{H_2PO_4^-}^{MMC} - k_{HPO_4^{2-}}^{MMC}) f + k_{HPO_4^{2-}}^{MMC}$. Plots were drawn for several pH values in the region 7.5–9.1. The values of the calculated slopes were plotted against f, calculated by the Henderson–Hasselbalch equation at the pH of the experiment. This graphical treatment results in a straight line with an intercept $k_{HPO_4^{2-}}^{MMC}$ and a slope $(k_{H_2PO_4^-}^{MMC} - k_{HPO_4^{2-}}^{MMC})$. The values $k_{H_3BO_3}^{MMC}$ and $k_{BO_2^-}^{MMC}$ were determined in a similar way.

In the pH region near the dissociation constant of MMC ($pK_a = 12.44$) this compound exists in a neutral and anionic form and the phosphate ions as HPO_4^{2-} and PO_4^{3-} ions. Therefore, equation (4) is extended to:

$$k_{obs} = [k_o^{MMC} + k_{OH}^{MMC}[OH^-] + k_{HPO_4^{2-}}^{MMC}[HPO_4^{2-}] + k_{PO_4^{3-}}^{MMC}[PO_4^{3-}]] \cdot \frac{[MMC]}{[MMC]t}$$

$$+ [k_o^{MMC^-} + k_{OH}^{MMC^-}[OH^-] + k_{HPO_4^{2-}}^{MMC^-}[HPO_4^{2-}] + k_{PO_4^{3-}}^{MMC^-}[PO_4^{3-}]] \cdot \frac{[MMC^-]}{[MMC]t}. \quad (10)$$

Rearrangement of equation (10) and substituting $k_{PO_4^{3-}}^{MMC^-} = 0$ leads to:

$$k_{obs} = (k_o^{MMC} + k_{OH}^{MMC}[OH^-]) \frac{[MMC]}{[MMC]_t} + (k_o^{MMC^-} + k_{OH}^{MMC^-}[OH^-] \frac{[MMC^-]}{[MMC]_t}$$

$$+ \{[(k_{HPO_4^{2-}}^{MMC} - k_{PO_4^{3-}}^{MMC}) f + k_{PO_4^{3-}}^{MMC}] \cdot \frac{[MMC]}{[MMC]_t}$$

$$+ k_{HPO_4^{2-}}^{MMC^-} \cdot f \cdot \frac{[MMC^-]}{[MMC]_t}\} \cdot [\text{total phosphate}]. \quad (11)$$

At a fixed pH in the region pK_a (MMC) ±2, a plot of k_{obs} against [total phosphate] yields a straight line with slope:

$$([(k^{MMC}_{HPO_4^{2-}} - k^{MMC}_{PO_4^{3-}})\,f + k^{MMC}_{PO_4^{3-}}] \cdot \frac{[MMC]}{[MMC]_t} + k^{MMC^-}_{HPO_4^{2-}} \cdot f \cdot \frac{[MMC^-]}{[MMC]_t}).$$

With knowledge of the value of $k^{MMC}_{HPO_4^{2-}}$ and after calculation of the terms [MMC]/[MMC]$_t$, [MMC$^-$]/[MMC]$_t$ and f at the pH of the experiment, an equation remains with the two unknown terms $k^{MMC}_{PO_4^{3-}}$ and $k^{MMC^-}_{H_2PO_4^-}$. When this procedure is repeated with data obtained at another pH in the region near the pK$_a$ of MMC, $k^{MMC}_{PO_4^{3-}}$ and $k^{MMC^-}_{H_2PO_4^-}$ can thus be calculated from the combined equations.

For each pH, the mole fraction f can be calculated using the Henderson–Hasselbalch equation, where: f[total phosphate] = [HPO_4^{2-}], and consequently: (1 − f) [total phosphate] = [PO_4^{3-}]. The calculated rate constants are listed in Table 2.

Influence of additives

The effects of acetate, chloride and nitrate ions were determined by adding various concentrations of the appropriate sodium salts to solutions of MMC at pH 8.4 while the total phosphate concentration was kept constant at 5×10^{-2}M. No significant changes in k_{obs} occurred when the additive concentration was increased to 2.0 M and consequently $k^{MMC}_{Cl^-}$, $k^{MMC}_{Ac^-}$ and $k^{MMC}_{NO_3^-}$ are zero.

Influence of temperature

The temperature dependence of the degradation of MMC was investigated under conditions where MMC existed exclusively in the neutral form (pH < 10.44), as the deprotonated species (H_- > 14.44) or in both forms (10.44 < pH/H_- < 14.44).

The temperature dependences of the observed rate constants can be represented in the form of Arrhenius plots. Such plots are described by equation (12):

$$\ln k_{obs} = \ln A - \frac{\Delta H^{\ddagger}}{RT} \tag{12}$$

where A represents the frequency factor, $\Delta H^{\ddagger}$ the enthalpy of activation, R the gas constant and T the temperature (Kelvin).

The $\Delta H^{\ddagger}$ values of the most important reaction rate constants were determined as follows. In the region where the MMC degradation is independent of pH, reaction rates were determined at a fixed pH (pH 7.71); only temperature and buffer concentration were varied. At each temperature, plots of k_{obs} against [total phosphate] were drawn, yielding intercepts corresponding to ($k^{MMC}_o + k^{MMC}_{OH}$ [OH^-]). At pH 7.71 [OH^-] is low; and therefore, if the value of k^{MMC}_{OH} (Table 2) is taken into account, the term k^{MMC}_{OH} [OH^-] << $k^{MMC}_o \cdot k^{MMC}_{OH}$ [OH^-] vanishes and the intercepts are approximately equal to the value of k^{MMC}_o. A plot of the natural log function of k^{MMC}_o against the reciprocal of the absolute temperature yields a straight line with slope $-\Delta H^{\ddagger}/R$ and intercept ln$A$. The enthalpy of activation of the water-catalysed degradation, together with its frequency factor, were calculated from these results (Table 3).

The temperature dependence of k^{MMC}_{OH} was determined at pH 10.20. At this pH the k^I_{obs} value at [buffer] = 0 can be described as $k^I_{obs} = k^{MMC}_o + k^{MMC}_{OH}$ [OH^-]. k^I_{obs} was determined at several temperatures; for each temperature k^{MMC}_o was calculated from the Arrhenius plot obtained from the experiment at pH 7.71.

This procedure is correct if it is assumed that $\Delta H^{\ddagger}$ for k^{MMC}_o is constant over the temperature range of the experiment. The [OH^-] is calculated from the difference between [H^+] (=10^{-pH}) and the pK$_s$ of water.

Table 3
Enthalpies of activation ($\Delta H^{\ddagger}$) and frequency factors (A) of the most important rate constants and observed apparent first-order rate constants (k_{obs}) of MMC degradation in alkaline solution

	$\Delta H^{\ddagger}$ (kJ mol^{-1})	A
k_o^{MMC}	52	5.4×10^2 s^{-1}
k_{OH}^{MMC}	66	4.7×10^9 mol^{-1} s^{-1}
$k_{HPO_4^{2-}}^{MMC}$	101	2.5×10^{12} mol^{-1} s^{-1}
$k_{obs}^{pH\ 7.7*}$	84	6×10^7 s^{-1}
$k_{obs}^{pH\ 8.4*}$	72	1.8×10^6 s^{-1}
$k_{obs}^{pH\ 9.2*}$	69	1.6×10^6 s^{-1}
$k_{obs}^{pH\ 10.4*}$	67	9×10^6 s^{-1}
$k_{obs}^{pH\ 12.44}$	59	8×10^6 s^{-1}
$k_{obs}^{H_-\ 14.3}$	51	3×10^6 s^{-1}

* Temperature dependence of k_{obs} was determined in 0.05 M phosphate buffer solution.

This procedure makes it possible to calculate k_{OH}^{MMC} at the several temperatures used in the experiment. The relationship between k_{OH}^{MMC} and $1/T$ followed the Arrhenius equation.

In phosphate buffer at pH 10.20, HPO_4^{2-} ions are the only catalytic buffer species. Thus the k_{obs} values can be described according to equation (6). The values of $k_{HPO_4^{2-}}^{MMC}$ were determined from the slopes of plots of k_{obs} against $[HPO_4^{2-}]$ at several temperatures. A plot of $\ln k_{HPO_4^{2-}}^{MMC}$ against $1/T$ yielded the values for $\Delta H^{\ddagger}$ and the frequency factor A. The temperature dependence of the decomposition of MMC was also studied where the pH was within the region pK_a (MMC) ± 2. Under these conditions MMC exists in the neutral and anionic forms.

At each pH the ratio of these species can be calculated using the Henderson–Hasselbalch equation. Arrhenius plots were constructed within the temperature range 5–30°C. A plot of the calculated $\Delta H^{\ddagger}$ values against the percentage MMC^- yielded a straight line ($r = 0.99$) and can be described as:

$$\Delta H^{\ddagger} = 67.0 - 0.16 \cdot (\text{percentage } MMC^-). \quad (13)$$

Equation (13) appears only to be valid in the pH/H_- region 10.4–14.3. Since rate constants calculated from experiments at decreased temperatures are in agreement with those obtained at 25°C, equation (13) appears to be valid. This suggests that changes in the pK_a value of MMC due to temperature variation are negligible under the chosen experimental conditions.

Discussion

At pH 8 to 13 only one degradation product (X) exists. In the scheme for the degradation of porfiromycin, postulated by Garrett [5], a scheme that also appears valid for MMC, the substitution of a hydroxyl group for the 7-amino group was proposed. The physicochemical and analytical properties of X are evaluated in the accompanying paper [12].

The pH profile of the degradation of MMC shows an inflection around pH 12.4 (Fig. 5). This inflection point is consistent with the pK_a of 12.44 as found by Underberg and Lingeman [13] which is due to keto-enol tautomerism in the MMC molecule (Fig. 3). This tautomerization influences both the nature of the degradation and the rate constants. At H_- values >13, X could not be detected either chromatographically or spectrophotometrically. Instead, other products absorbing at 313 nm, probably anionic compounds also, were eluted with the solvent front. Unlike X and MMC these products did not show significant UV absorbance near 365 nm (Fig. 2). This indicates a dramatic change in the chromophore during the degradative process at these high H_- values. It appears that the deprotonated form of MMC follows a different degradation pattern, which is induced by the enolic structure of the compound. Certain buffer anions, such as borate and phosphate ions, can greatly influence the observed rate constants. This is indicated by the linear relationships between k_{obs} and [buffer] in a certain concentration range, for borate up to 0.06 M and for phosphate up to 0.5 M. Within a series of closely related buffer anions such as the different phosphate ions a trend exists for increased activity with increased anionic charge (Table 2); other anionic compounds, such as acetate, nitrate or chloride ions, do not influence the degradation rate. General base catalysis, shown by borates and phosphates, is attributed to their strength, as expressed by their pK_a values. Borates and phosphates can act as intermediates in transferring protons during degradation reactions in alkaline solution since their pK_a values permit the existence of protonated and deprotonated species of the buffer in this pH region. Weaker bases such as acetates, nitrates and chlorides are unable to act as intermediates in this pH region; thus these bases do not exert any catalytic effect on degradation.

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[*First received for review 24 July 1984; revised manuscript received 4 October 1984*]