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Degradation kinetics of 7-*N*-(*p*-hydroxyphenyl)mitomycin C (M-83) in aqueous solution in the presence of γ-cyclodextrin

O.A.G.J. van der Houwen [a], J. Teeuwsen [a], O. Bekers [b], J.H. Beijnen [c], A. Bult [a], W.J.M Underberg [a,*]

[a] *Department of Pharmaceutical Analysis, Faculty of Pharmacy, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands,* [b] *Department of Clinical Chemistry, Leyenburg Hospital, 's Gravenhage, The Netherlands;* [c] *Department of Hospital Pharmacy, Slotervaart Hospital, Amsterdam, The Netherlands*

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Abstract

The degradation kinetics of 7-*N*-(*p*-hydroxyphenyl)mitomycin C in the presence of cyclodextrin have been investigated over the pH region 0.3–11. Degradation mixtures were analysed by means of UV-Vis spectrometry and high-performance liquid chromatography with UV detection. The degradation kinetics have been modelled using a non-linear curve-fitting computer program.

Key words: Degradation kinetics; Mitomycin derivative; 7-*N*-(*p*-Hydroxyphenyl)mitomycin C; Cyclodextrin

1. Introduction

In a previous study (Beijnen et al., 1988) the degradation of the mitomycin C analogue, 7-*N*-(*p*-hydroxyphenyl)mitomycin C (M-83) (Fig. 1), an investigational antitumour agent, in aqueous solution was investigated. In both acidic and alkaline media M-83 showed fairly rapid degradation, while the pH of maximum stability was around 7.5. The solubility of M-83 in water is limited, which hampers the therapeutic use of high doses of the compound. Increase in solubility can be effected, for example, by the use of co-solvent, micelle formation or complexation. Each of these techniques may also influence the stability of the compound. In recent years much research has been focused on the use of cyclodextrins as complexing agents for cytostatic drugs, such as mitomycins and the influences of these agents on the stability of the guest molecules (Bekers et al., 1989, 1991a). For the mitomycins, γ-cyclodextrin (γ-CyD) proved to be the most effective complexing compound, as expressed in terms of increase in solubility and stability of the guest molecule. Recently, a general model for the interpretation of the degradation of compounds in the presence of ligands was developed (Van der Houwen et al., 1991). In the present study the degradation kinetics of M-83 in the presence of γ-CyD as complexing agent were investigated and interpreted, using this model.

* Corresponding author.

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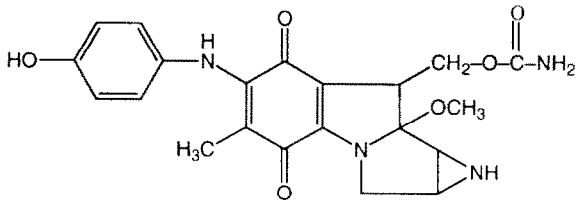

Fig. 1. Structure of 7-*N*-(*p*-hydroxyphenyl)-mitomycin C (M-83).

2. Experimental

2.1. Chemicals

M-83 was a gift from Dr K. Shirahata, Kyowa Hakko Kogyo Chemical Co. (Tokyo, Japan). γ-CyD came from Nihon Shokukin Kako Co. Ltd (Tokyo, Japan) and was used as received. All other chemicals were of analytical grade and deionized water was used throughout.

2.2. Buffer / CyD solutions

For the kinetic experiments the following aqueous buffer solutions were used: H_0/ pH 0.3–3, perchloric acid; pH 3–6, acetate (0.01 M); pH 6–8, phosphate (0.01 M); pH 8–11, carbonate (0.01 M). pH values between 1 and 11 were measured at the temperature of study with a suitable pH meter (Metrohm, E512, Titriskop, Herisau, Switzerland). pH values below 1 were calculated using the Hammett acidity function (Bates, 1973). A constant ionic strength ($\mu = 0.3$), adjusted with sodium chloride, was maintained for each degradation solution, except for the solutions where the hydrogen ion concentration exceeded 0.3 M. For the degradation studies where the influence of γ-CyD was investigated, various amounts of γ-CyD were added to the buffer solutions and the pH was adjusted to the desired value. The buffer/γ-CyD solutions were always prepared freshly before use. No significant changes in pH were observed throughout the degradation reactions.

2.3. Kinetic experiments

All kinetic degradation experiments were performed in the dark at 25°C. At pH < 3.8 degradations were followed by monitoring the decrease in absorbance at 385 nm. Degradations at pH > 3.8 were followed by the described stability-indicating high-performance liquid chromatography (HPLC) method (Beijnen et al. 1988). The degradation reactions were initiated by adding 10 μl of a methanolic stock solution of M-83 to 2.5 ml preheated buffer, resulting in an initial concentration of 6×10^{-5} M. In experiments where the γ-CyD influences on the M-83 degradation were studied the γ-CyD concentrations varied from 0 to 8×10^{-2} M. It appears that in alkaline medium the presence of oxygen resulted in irreproducible degradation processes. This difficulty could be eliminated by using boiling water and purging the buffer/γ-CyD solutions with nitrogen. After addition of M-83 the test tubes were closed under a nitrogen atmosphere. At appropriate intervals samples were taken and directly analysed for the content of undegraded M-83.

2.4. Ultraviolet-visible (UV-Vis) spectrometry

UV-Vis absorbance spectra were recorded on a Perkin Elmer Lambda 5 UV-Vis spectrophotometer (Perkin Elmer, Oak Brook, IL, U.S.A.). Absorbance measurements were performed with a Shimadzu UV-140 Double Beam Spectrophotometer equipped with an APC-140 Thermostated Cell-positioner (Shimadzu Corp., Kyoto, Japan).

2.5. HPLC

The conditions and equipment of the HPLC assay were identical to those described in an earlier report by Beijnen et al. (1988).

3. Results

3.1. Analytical procedures and degradation kinetics

The degradation of M-83 in the presence of γ-CyD follows the same mechanism as that of the free compound in aqueous solution, as indicated by the occurrence of a pseudo-first order pattern in acidic and alkaline solution and the appear-

ance of identical degradation products. Both the spectrophotometric and the HPLC assays are not influenced by the presence of the ligand or the occurrence of unexpected interfering degradation products. The stability-indicating properties of both assays, consequently, are equal for the included and the free M-83. In Table 1 a summary is given of the degradation rate constants, k_{obs}, for M-83 at various pH values and [γ-CyD]. The data from Table 1 indicate a tendency to a decrease in k_{obs} on inclusion of M-83 in γ-CyD, especially at pH > 2. In the region pH < 2 this influence diminishes. These results are in agreement with those obtained for mitomycin C and demonstrate that on protonation of the aziridine function in M-83 the stabilizing effect of γ-CyD disappears. Using the data of Table 1 attempts have been made to calculate the values for the specific reaction constants, acid dissociation constants and ligand binding constants, using the theoretical model described earlier (Van der Houwen et al., 1991)

3.2. Calculation of rate constants and equilibrium constants

M-83 has been shown to be subject to three succesive protolytic equilibria (Beijnen et al. 1988). Therefore, the relation between k_{obs} and the macro reaction constants, acid dissociation constants and ligand binding constants is given by Eq. 1 (Van der Houwen et al., 1991).

$$k_{obs} = \frac{\sum_{i=0}^{5} \left\{ \left(M_i^F + [L] \cdot M_i^L \right) / [H^+]^{i-1} \right\}}{\sum_{i=0}^{3} \left\{ \prod_{j=0}^{i} K_j + [L] \cdot \prod_{j=0}^{i} K_j \cdot K_i^L \right\} / [H^+]^i} \quad (1)$$

The constants for the degradation reactions of the free M-83 are combined in the macro reaction constants M_i^F according to Eq. 2–7, those for the degradation reactions of the complexed M-83 in the macro reaction constants M_i^L according to Eq. 8–13.

$$M_0^F = k_0^H \quad (2)$$

$$M_1^F = k_1^H \cdot K_1 + k_0^S \quad (3)$$

$$M_2^F = k_2^H \cdot K_1 \cdot K_2 + k_1^S \cdot K_1 + k_0^{OH} \cdot K_w \quad (4)$$

$$M_3^F = k_3^H \cdot K_1 \cdot K_2 \cdot K_3 + k_2^S \cdot K_1 \cdot K_2 + k_1^{OH} \cdot K_w \cdot K_1 \quad (5)$$

$$M_4^F = k_3^S \cdot K_1 \cdot K_2 \cdot K_3 + k_2^{OH} \cdot K_w \cdot K_1 \cdot K_2 \quad (6)$$

$$M_5^F = k_3^{OH} \cdot K_w \cdot K_1 \cdot K_2 \cdot K_3 \quad (7)$$

$$M_0^L = K_0^L \cdot k_{l0}^H \quad (8)$$

$$M_1^L = K_1^L \cdot k_{l1}^H \cdot K_1 + K_0^L \cdot k_{l0}^S \quad (9)$$

Table 1
Degradation rate constants, k_{obs} (s^{-1}), for M-83 at various pH and [γ-CyD] at 25°C

H_0/ pH	[γ-CyD] (M)					
	0	5×10^{-3}	1×10^{-2}	2×10^{-2}	4×10^{-2}	8×10^{-2}
0.3	2.9×10^{-3}	2.5×10^{-3}	2.4×10^{-3}	2.2×10^{-3}	2.1×10^{-3}	2.0×10^{-3}
0.8	2.1×10^{-3}	1.9×10^{-3}	1.9×10^{-3}	1.8×10^{-3}	1.7×10^{-3}	1.7×10^{-3}
1.3	1.7×10^{-3}	1.6×10^{-3}	1.6×10^{-3}	1.6×10^{-3}	1.5×10^{-3}	1.4×10^{-3}
1.8	1.8×10^{-3}	1.6×10^{-3}	1.4×10^{-3}	1.3×10^{-3}	1.2×10^{-3}	1.1×10^{-3}
2.8	8.0×10^{-4}	5.1×10^{-4}	4.2×10^{-4}	3.4×10^{-4}	2.8×10^{-4}	2.4×10^{-4}
3.8	1.3×10^{-4}	6.9×10^{-5}	5.2×10^{-5}	3.9×10^{-5}	3.1×10^{-5}	2.7×10^{-5}
4.8	1.3×10^{-5}	6.7×10^{-6}	4.4×10^{-6}	3.4×10^{-6}	2.8×10^{-6}	2.6×10^{-6}
6.1	5.4×10^{-7}	3.6×10^{-7}	2.8×10^{-7}	2.7×10^{-7}	2.4×10^{-7}	2.0×10^{-7}
6.8	1.2×10^{-7}	9.8×10^{-8}	1.7×10^{-7}	1.6×10^{-7}	1.3×10^{-7}	1.0×10^{-7}
8.0	2.6×10^{-7}	1.0×10^{-7}	9.2×10^{-8}	8.8×10^{-8}	7.6×10^{-8}	9.8×10^{-8}
9.0	1.1×10^{-5}	5.4×10^{-6}	3.3×10^{-6}	2.8×10^{-6}	2.4×10^{-6}	1.1×10^{-6}
10.0	5.2×10^{-4}	2.6×10^{-4}	6.4×10^{-5}	5.0×10^{-5}	1.7×10^{-5}	5.2×10^{-6}
11.0	2.2×10^{-3}	6.2×10^{-4}	3.4×10^{-4}	2.0×10^{-4}	2.1×10^{-5}	4.7×10^{-6}

$$M_2^L = K_2^L \cdot k_{l2}^H \cdot K_1 \cdot K_2 + K_1^L \cdot k_{l1}^S \cdot K_1 + K_0^L \cdot k_{l0}^{OH} \cdot K_w \quad (10)$$

$$M_3^L = K_3^L \cdot k_{l3}^H \cdot K_1 \cdot K_2 \cdot K_3 + K_2^L \cdot k_{l2}^S \cdot K_1 \cdot K_2 + K_1^L \cdot k_{l1}^{OH} \cdot K_w \cdot K_1 \quad (11)$$

$$M_4^L = K_3^L \cdot k_{l3}^S \cdot K_1 \cdot K_2 \cdot K_3 + K_2^L \cdot k_{l2}^{OH} \cdot K_w \cdot K_1 \cdot K_2 \quad (12)$$

$$M_5^L = K_3^L \cdot k_{l3}^{OH} \cdot K_w \cdot K_1 \cdot K_2 \cdot K_3 \quad (13)$$

The subscripts 0–3 of the reaction constants refer to the successive deprotonation steps, the subscript 0 indicating the fully protonated species. The superscripts H, S and OH refer to the proton, solvent and hydroxyl catalyzed reactions, respectively. The subscript l is used to indicate the reaction constants of the complexed species. The successive protolytic dissociation constants are referred to as K_i, the ligand binding constants of the successive species as K_i^L.

Eq. 1 was used as a model equation for the calculation of the macro reaction constants, the acid dissociation constants and the ligand binding constants in two successive steps. The constants M_i^F and the macro dissociation constants were calculated by non-linear regression with Eq. 14 as model equation from the observed rate constants in the absence of cyclodextrin. The values are listed in Table 2.

$$k_{obs} = \frac{\sum_{i=0}^{5} \left\{ \left(M_i^F \right) / [H^+]^{i-1} \right\}}{\sum_{i=0}^{3} \left\{ \left(\prod_{j=0}^{i} K_j \right) / [H^+]^i \right\}} \quad (14)$$

The values obtained correspond well with those reported in a previous study (Beijnen et al., 1988). The values were subsequently substituted into Eq. 1 as the model equation for the second non-linear regression calculation. This approach is less laborious than the alternative of calculating the constants A and B with model Eq. 15 from degradation rate measurements at fixed pH and varying cyclodextrin concentrations and fitting the calculated constants A and B with the model Eq. 16 and 17.

Table 2
Macro reaction constants, acid dissociation constants and ligand binding constants at 25°C

M_0^F	6.3×10^{-3} M^{-1} s^{-1}	M_0^L	nsc
M_1^F	6.3×10^{-2} s^{-1}	M_1^L	8.7×10^{-3} s^{-1}
M_2^F	nsc	M_2^L	1.3×10^{-8} M^{-1} s^{-1}
M_3^F	2.5×10^{-18} M^{-2} s^{-1}	M_3^L	nsc
M_4^F	3.2×10^{-26} M^{-3} s^{-1}	M_4^L	nsc
M_5^F	2.5×10^{-38} M^{-4} s^{-1}	M_5^L	–
pK_{a1}	2.7		
pK_{a2}	9.5		
pK_{a3}	11.0		
K_0^L	3×10^{-1} M^{-1}		
K_1^L	1.3×10^{2} M^{-1}		
K_2^L	nsc		
K_3^L	–		

nsc, no significant contribution; –, no calculation possible.

$$k_{obs} = \frac{k_{obs}^0 + A \cdot [L]}{1 + B \cdot [L]} \quad (15)$$

$$A = \sum_{i=0}^{n+2} \left(M_i^L / [H^+]^{i-1} \right) \times \Big/ \sum_{i=0}^{n} \left\{ \left(\prod_{j=0}^{i} K_j \right) / [H^+]^i \right\} \quad (16)$$

$$B = \sum_{i=0}^{n} \left\{ \left(\prod_{j=0}^{i} K_j \right) \cdot K_i^L / [H^+]^i \right\} \times \Big/ \sum_{i=0}^{n} \left\{ \left(\prod_{j=0}^{i} K_j \right) / [H^+]^i \right\} \quad (17)$$

4. Discussion

The binding of a degrading substance to ligands influences the log k_{obs}-pH profile in two different ways. Firstly, it causes a shift of any inflection point to lower or higher pH values depending on the values of the binding constants of subsequent species of the solute and the ligand concentration. Secondly, it results in a shift to lower or higher values of the observed rate constant. The latter effect is quantitatively described by Eq. 15–17.

The complex binding constants can be calculated from both effects. By using linear regression with Eq. 1 as a model equation both effects are taken into account at the same time. This is a more straightforward approach than that used for the computation of the complex stability constant of mitomycin C and γ-cyclodextrin (Van der Houwen et al., 1993). The calculated value of the complex binding constant of M-83 is not very precise. This is due to the fact that the change in reaction rate on increasing the ligand concentration is relatively small (and therefore subject to a relatively large experimental error) and to the fact that the inflection points are shifted from pH values of 2.7 and 9.5 to 1.7 and 10.5, respectively. Determination of the inflection points requires a number of measurements of degradation rates at a range of pH values under 1.7 and a range of pH values above 10.5, respectively. At these pH extremes the degradation is very rapid and difficult to reproduce accurately. This is particularly difficult at pH values above 10 where traces of oxygen can enhance degradation. Moreover, the shift of the inflection point of pH 9.5 to 10.5 is obscured by the occurrence of an inflection point due to the second deprotonation step. No reliable values for the ligand binding constants of the deprotonated species of M-83 can therefore be obtained from the shape of the curve. The equilibrium complexation constant of the uncharged species of M-83 to γ-cyclodextrin was found to be 1.3×10^{-2}. The binding constant determined for the fully protonated species was found to be low and hardly significant. This is in accordance with the hydrophobic character of the inclusion of drugs in the cavity of cyclodextrins (Bekers et al., 1991b). The calculated macro reaction constants indicate that the log k_{obs}-pH profile for the degradation of M-83 in the absence of cyclodextrin is mainly determined by the macro reaction constants M_1^F and M_4^F (Fig. 2). In the presence of cyclodextrin there is also some contribution of M_1^L and M_2^L (Fig. 3). The calculated values are given in Table 2. The effect of ligand binding on the overall degradation rate profile is shown in Fig. 4. The contribution of the degradation of the ligand bound species to the overall degradation rate, as indicated by the contribution of the macro reac-

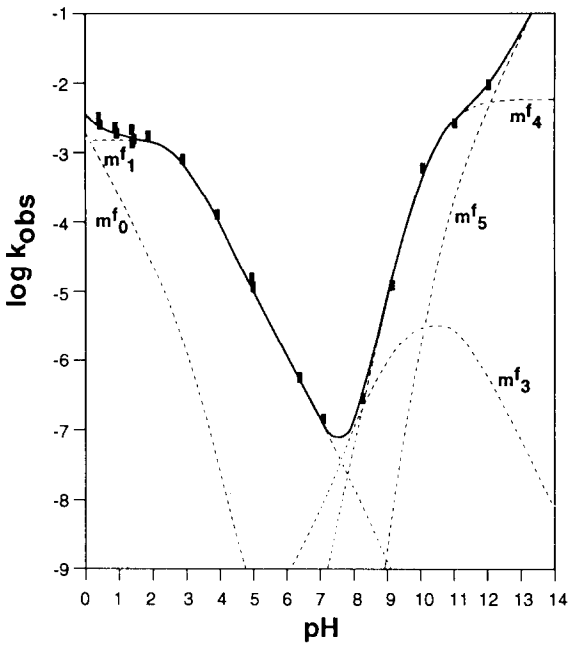

Fig. 2. Contribution of the macro reaction constants to the overall rate profile of M-83 at zero ligand concentration.

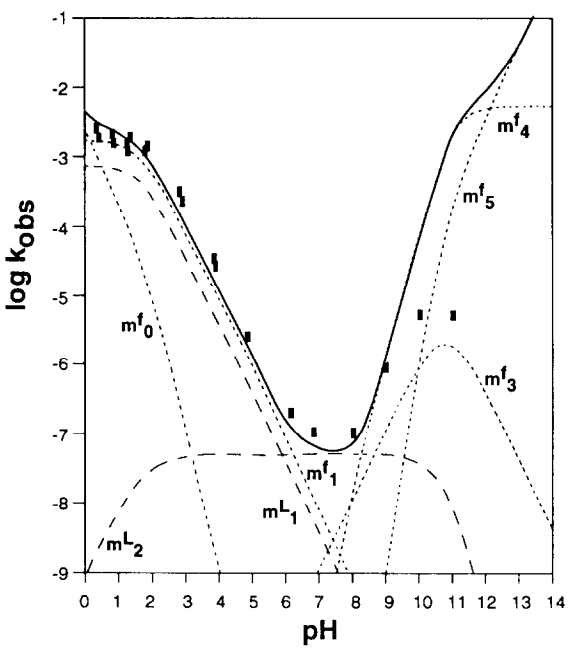

Fig. 3. Contribution of the macro reaction constants to the overall rate profile of M-83 at ligand concentration 8×10^{-2} M.

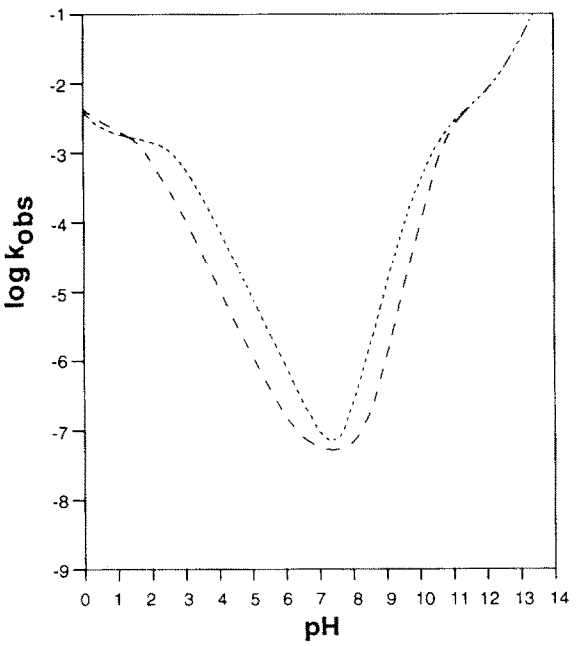

Fig. 4. Degradation rate profile of M-83 for ligand concentrations of 0 and 8×10^{-2} M γ-cyclodextrin.

tion constant M_1^L, suggests that the reaction center for this degradation reaction might be located outside the conical hole of the cyclodextrin molecule. This would indicate that the M-83 molecule is bound to cyclodextrin with the hydroxyphenyl group and the chinoid part in the conical hole and the aziridine group outside it, permitting the formation of mitosene degradation products at low pH. At higher pH the reaction product is mitomycin. The center for this reaction is the chinoid structure. The fact that no significant contribution of the macroreaction constant M_4^L could be found (which can be expected to be correlated which the formation of mitomycin at higher pH values) is in accordance with the assumption for the orientation of the included M-83.

5. Conclusions

The addition of M83 increases the stability of M-83 over a large pH range (Fig. 4), analogous to the result found for MMC (Bekers et al., 1991a). The macro reaction constants associated with the complexed M-83 suggest that M-83 is bound with the hydroxyphenyl group located in the conical hole of the cyclodextrin molecule. The observed pH degradation profile is in accordance with the general equation developed for such profiles. However, the precision of the calculated values for the constants in the equation is limited by the large number of constants involved. When all constants are calculated from observed degradation rates the error in each estimated constant adds to the error in the estimation of the other constants. The precision of the calculation therefore decreases as the number of constants increases. For more precise measurement of the macro reaction constants, known precise values of protolytic dissociation constants and complex binding constants obtained by independent methods are a prerequisite. The lack of stability of substances such as M-83 makes it very difficult to obtain such information by the traditional methods which justifies the approach used in this study.

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