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Aspects of the degradation kinetics of doxorubicin in aqueous solution

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Summary

The chemical stability of doxorubicin in aqueous solution has been investigated utilizing a stability-indicating HPLC assay with UV-VIS and fluorescence detection. The degradation kinetics were studied as function of pH (1–11), buffer composition (acetate, phosphate, carbonate), ionic strength ($\mu = 0.1$–0.4), temperature (30–70°C) and drug concentration (1–20 μg/ml). A pH–rate profile, obtained from first-order kinetic plots, corrected for buffer and ionic strength influences, was constructed. The pH profile shows maximum stability for doxorubicin at about pH 4. A theoretical description of the pH–rate profile is presented. In addition, some attention has been given to the degradation mechanism of doxorubicin in alkaline solution. The proposed mechanism is partly based on a comparison of the chemical stability of doxorubicin with daunorubicin and five related anthracycline agents.

Introduction

Over the past 15 years doxorubicin (Dx) (Fig. 1) unquestionably has proven to be of considerable medical value because of its outstanding activity on a number of human neoplasms (Arcamone, 1981). The importance of Dx is well documented in a number of publications (Arcamone, 1978, 1981; Brown and Imam, 1984; Myers, 1984). Considering the substantial interest in this oncochemotherapeutic drug, it is remarkable that hardly any systematic studies on the degradation of Dx have been reported. So far, systematic stability studies have only dealt with its degradation in acidic medium (pH < 3.5) (Wassermann and Bundgaard, 1983; Beijnen et al., 1985a). On the other hand, data of Dx stability in infusion fluids, of paramount importance for its clinical application, are available (Hoffman et al., 1979; Poochikian et al., 1981; Benvenuto et al., 1981; Beijnen et al., 1985b). However, these studies do not contribute to a better insight into the underlying mechanisms of the chemical degradation processes. An in-depth systematic approach of this matter is required in order to attain the necessary information.

The hesitation of investigators to study the degradation of Dx may be reduced to the fact that, from an analytical point of view, Dx is far from an ideal drug. It interacts with all kinds of ions (Porumb, 1978), chelates strongly with diva-

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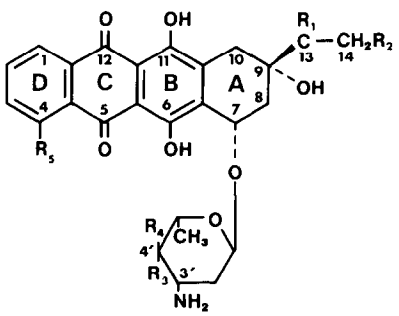

Fig. 1. Structures of anthracyclines.

	R1	R2	R3	R4	R5
doxorubicin	=O	OH	OH	H	OCH_3
4′-deoxydoxorubicin	=O	OH	H	H	OCH_3
4′O-methyldoxorubicin	=O	OH	OCH_3	H	OCH_3
4′-epidoxorubicin	=O	OH	H	OH	OCH_3
daunorubicin	=O	H	OH	H	OCH_3
4-demethoxydaunorubicin	=O	H	OH	H	H
doxorubicinol	-OH	OH	OH	H	OCH_3

lent and trivalent metal ions (Martin, 1985), adsorbs onto various materials amongst which glass surfaces (Benvenuto et al., 1981; Tomlinson and Malspeis, 1982), tends to self-associate in more concentrated solutions (Menozzi et al., 1984), is liable to photolytic decomposition (Daugherty et al., 1979; Tavoloni et al., 1980; Thoma et al., 1980) and to oxidation (Arcamone, 1978). In an earlier report on the degradation of daunorubicin (Dr) we evaluated these subjects and gave recommendations on how to cope with them in order to obtain reproducible and well-defined degradation patterns (Beijnen et al., 1986a).

This study focuses on the degradation kinetics of Dx in aqueous solution. The kinetics were investigated as a function of pH, buffers, temperature, ionic strength and drug concentration. As a result, proposals for the degradation mechanism could be developed.

Experimental

Chemicals

Doxorubicin (Dx), 4′-epidoxorubicin (4′-epiDx), 4′-O-methyldoxorubicin (4′-O-methylDx), 4′-deoxydoxorubicin (4′-deoxyDx), doxorubicinol (Dx-ol) and 4-demethoxydaunorubicin (4-demethoxyDr) were generous gifts from Dr. S. Penco, Farmitalia (Milan, Italy). Dr was donated by Rhône-Poulenc Nederland (Amstelveen, The Netherlands). The compounds were supplied as hydrochloric salts and were used as such.

Buffer solutions / kinetic measurements

The composition of the buffer solutions used and the procedures followed in the kinetic measurements were described extensively in a previous report on the degradation kinetics of Dr, a structure analog of Dx (Fig. 1) (Beijnen et al., 1986a).

Apparatus and analytical procedures

The conditions and equipments of the HPLC assay were described earlier (Beijnen et al., 1986a), except for the mobile phase. The mobile phase used in this study consisted of 0.01 M sodium chloride–acetonitrile (45 : 55, v/v). The pH of the 0.01 M sodium chloride solution was adjusted to 2.25 with perchloric acid (70%, w/v) before mixing with the organic modifier. Peak height measurements were used for the quantitation of undegraded Dx. Standard solutions of Dx in 0.005 M perchloric acid were co-chromatographed daily and calibration curves were constructed. These calibration curves were linear ($r > 0.999$) within the concentration range of interest, 3.5×10^{-5} to 3.5×10^{-6} M. Relative standard deviations of 0.9–5.1% were obtained for replicate injections ($n = 8$) of solutions of the upper and lower limits of the standard curve, respectively.

TLC of chloroform extracts of degradation mixtures was performed on 60F254 silica gel plates (Merck, Darmstadt, F.R.G.) with chloroform–methanol–6 N ammonia (90 : 10 : 1, v/v/v) as the solvent.

Results and Discussion

Degradation products

In acidic solution (pH < 4) the initial degradation step of Dx is its conversion into the 7-hydroxyaglycone, doxorubicinone (Fig. 2, compound I) and the amino sugar, daunosamine (Arcamone,

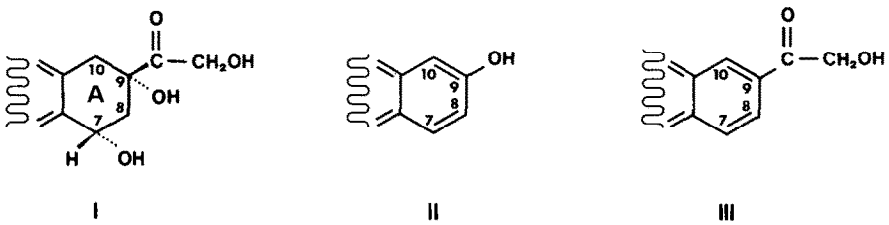

Fig. 2. Structures of doxorubicin degradation products.

1978). Similar conversions occur upon acidic hydrolysis of the related anthraquinone glycosides, shown in Fig. 1 (Beijnen et al., 1985a). Compound I was effectively separated from the parent anthracycline Dx, using the HPLC assay.

At pH > 4 the degradation pattern of Dx is not elucidated completely. Decomposition mixtures were extracted with chloroform and the extracts were analyzed with TLC showing one pink coloured major degradation product ($R_f = 0.5$), compound II (Fig. 2), and minor quantities of fluorescing compounds. The structure elucidation and characteristics of II are discussed elsewhere (Beijnen et al., 1986b). II also appeared as the main degradation product in degradation mixtures (pH 8.0) of 4'-epiDx, 4'-O-methylDx and 4'-deoxyDx. In Dr decomposition mixtures II was also found as well as another aglycone with a fully aromatized A ring possessing an acetyl function at C9. The analogous bisanhydro derivative of Dx (III, Fig. 2) was not found as a Dx degradation product at pH > 4. III could only be obtained if Dx was subjected to severe acidic conditions (1 N HCl, 100°C, 24 h). The structure of III was confirmed with field desorption mass spectrometry. The capacity factors of Dx, I and II in the HPLC system are 1.6, 0.7 and 5.0, respectively. During the course of the degradation the ratio between the UV (280 nm) and VIS (546 nm) HPLC detector signals remained constant, demonstrating the stability indicating capability of the HPLC assay.

Degradation kinetics

Order of reactions

Semilogarithmic plots of the concentration of residual Dx versus time were linear and indicated the degradation of the drug to follow pseudo-first-order kinetics at constant pH, temperature and ionic strength under the various pH conditions. The observed rate constants (k_{obs}) are extracted from the slopes of the first-order plots.

Standard deviation in k_{obs}

The standard deviation in k_{obs} was determined at pH 8.0 and 0.01 M phosphate buffer containing 5×10^{-4} M EDTA ($\mu = 0.3$). The value of k_{obs} and the standard deviation (n = 6) is $3.0 \pm 0.1 \times 10^{-5}$ s^{-1}. Other experiments were duplicated.

Influence of buffers

In the region up to pH 9.5 catalysis by acetate, phosphate and carbonate buffer components could be demonstrated. At pH values ≥ 10 no buffer catalysis was observed. In Table 1 some examples are presented. The relation between k_{obs} and the buffer concentration, at constant pH and ionic

TABLE 1

k_{obs} VALUES (in s^{-1}) FOR THE DEGRADATION OF DOXORUBICIN IN VARIOUS BUFFER SOLUTIONS [a] AT 50°C

pH	[buffer]	k_{obs}	pH	[buffer]	k_{obs}
4.0	0.1	5.1×10^{-7}	8.0	0.1	4.9×10^{-5}
	0.075	4.5×10^{-7}		0.075	5.0×10^{-5}
	0.05	4.2×10^{-7}		0.04	4.6×10^{-5}
	0.025	3.3×10^{-7}		0.02	3.7×10^{-5}
	0.01	2.7×10^{-7}		0.01	3.0×10^{-5}
6.0	0.075	2.1×10^{-6}	10.0	0.0625	1.9×10^{-3}
	0.04	1.6×10^{-6}		0.0375	2.0×10^{-3}
	0.025	1.3×10^{-6}		0.02	1.9×10^{-3}
	0.01	1.0×10^{-6}		0.01	1.9×10^{-3}
	0.005	9.3×10^{-7}		0.005	1.9×10^{-3}

[a] pH 4.0, acetate; pH 6.0 and 8.0: phosphate; pH 10.0, carbonate. The buffer solutions contained 5×10^{-4} M EDTA ($\mu = 0.3$).

strength, deviates from linearity at higher buffer concentrations and then levels. Using the linear part of the curve it was possible to extrapolate to zero buffer concentration, yielding k′ which is only influenced by $[H^+]$, $[H_2O]$ and $[OH^-]$.

Influence of ionic strength

The influence of ionic strength on the degradation rate of Dx was ascertained over the entire pH region studied. At each pH, experiments were performed where the buffer concentration and temperature were kept constant while the ionic strength, adjusted with sodium chloride, was varied. At pH < 4 the logarithm of k_{obs} was found to be linearly related to $\sqrt{\mu}$ (Beijnen et al., 1985a). At higher pH values the effect was negligible in the μ range 0.1–0.4.

Influence of temperature

The effect of temperature on the degradation rate of Dx was studied in 0.01 M buffer solutions ($\mu = 0.3$) in the pH range 4.0–10.0 and temperature range 30–70°C. The Arrhenius relationship is obeyed. The results, in terms of activation energies (E_a) and frequency factors (A), are summarized in Table 2. The variation of E_a with pH indicates that the nature of the rate-limiting decomposition step varies with pH. As no change in the degradation pattern in the pH region 4.0–10.0 was found, the pH dependence of E_a may be related with the change in the degree of protonation of the Dx molecule in this pH region.

Influence of drug concentration

In the literature there is no common opinion whether the decomposition of Dx is dependent on its initial concentration. Both Tavoloni et al. (1980) and Poochikian et al. (1981) found no evidence for the occurrence of a concentration-dependent Dx degradation. However, Janssen et al. (1985) ascertained that Dx degrades faster in solutions containing 500 μg/ml than 50 μg/ml at pH 7.4. We found no indications for the existence of a concentration-dependent degradation rate of Dx in 0.01 M phosphate buffers pH 8 (50°C; 5×10^{-4} M EDTA; $\mu = 0.3$) in the range 1–20 μg/ml. In solutions of higher Dx concentrations (50, 100, 500 μg/ml) precipitates were formed during the course of the degradation process. Analysis of the precipitate revealed the presence of considerable amounts of undegraded Dx, apart from degradation products. When Dx precipitates arise, no proper degradation kinetics can be established. The formation of Dx precipitates at higher concentrations is probably related to the high ionic strengths and the presence of buffer ions with specific catalytic properties in the reaction solutions, promoting sedimentation as time elapses (Porumb, 1978).

TABLE 2

ACTIVATION ENERGIES (E_a) AND FREQUENCY FACTORS (A) FOR THE DEGRADATION OF DOXORUBICIN IN 0.01 M BUFFERS ($\mu = 0.3$; 5×10^{-4} M EDTA)

pH	E_a [a]	A [b]
4.0	89.7	8.2×10^7
5.0	86.6	3.3×10^7
6.0	90.4	4.0×10^8
7.0	104.1	2.6×10^{11}
7.4	105.5	1.2×10^{12}
8.0	95.7	8.5×10^{10}
9.0	69.1	2.3×10^7
10.0	67.3	5.0×10^7

[a] Activation energy in $kJ \cdot mol^{-1}$.
[b] Frequency factor in s^{-1}.

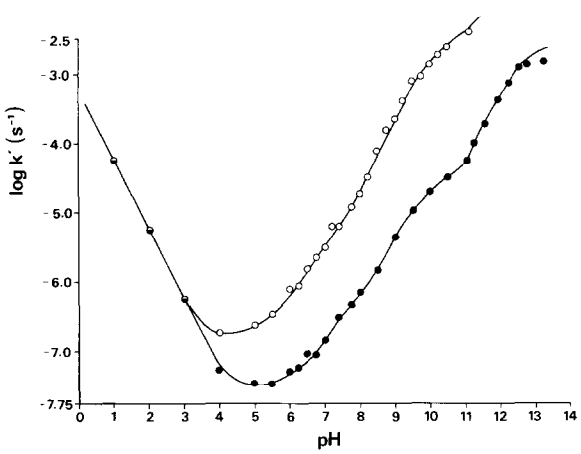

Fig. 3. Log k′–pH profiles for doxorubicin (○) and daunorubicin (●) degradation at 50°C, corrected for buffer and ionic strength influences.

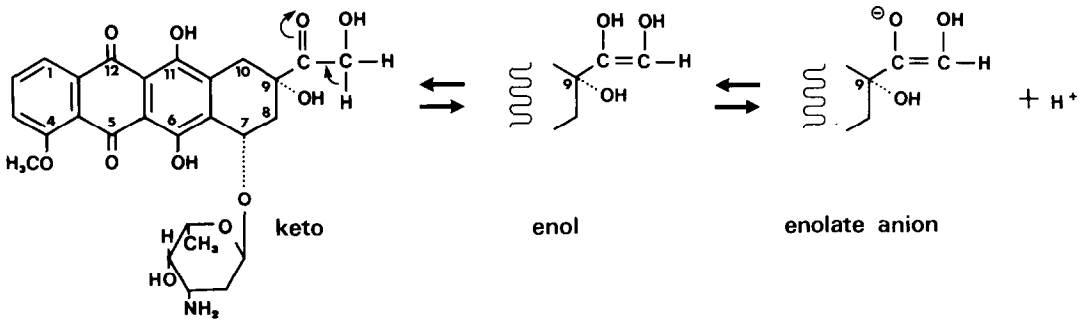

Fig. 4. Keto–enol tautomerization and deprotonation of doxorubicin.

Influence of pH

The degradation rate of Dx is strongly influenced by the pH of the medium. The effects of pH were explored over the region 1–11. At each pH the influences of buffer components and ionic strength were checked and k_{obs} values were corrected for these influences, yielding k′ values incorporating only H^+, H_2O and OH^- as catalysts. The log k′–pH profile for the degradation of Dx is shown in Fig. 3.

Anthracyclines contain three prototropic functions: the C3′ ammonium group and the two phenolic hydroxyl groups (C6, C11). In an earlier study the overlapping macro-ionization constants of Dr at 50°C were determined (Beijnen et al., 1986a), using the spectrophotometric procedures advocated by Sturgeon and Schulman (1977). The results, also applicable to Dx, were: $pK_1 = 7.4$ and $pK_2 = 9.5$. The second phenolic group does not undergo deprotonation until pH 12.5. This deprotonation step is therefore left out of consideration in this study, because of the pH region investigated. An exact assignment of the first phenolic deprotonation step (C6 or C11) cannot be made due to the lack in distinction of the acidic properties of both phenolic groups (Martin, 1985).

In analogy with corticosteroids possessing a C17 α-ketol side chain, i.e. hydrocortisone (Hansen and Bundgaard, 1979; Johnson, 1982) Dx should also contain an extra deprotonation site, located in its C9 α-ketol substituent. Reversible enolization and ionization of the α-ketol side chain of Dx is illustrated in Fig. 4. For hydrocortisone an apparent pK_a of 11.05 (25°C) is reported (Hansen and Bundgaard, 1979). The determination of the corresponding pK_a of Dx is hampered by overlap with the deprotonation of the phenolic group.

The deprotonation possibilities of Dx suggest the presence of four types of species in the pH region under investigation, based on the overall charge of the compound. The species are: Dx monocation (Dx^+); Dx neutral molecule (Dx^0); Dx monoanion (Dx^-) and Dx dianion (Dx^{2-}). Inherent to the assumption of the existence of keto–enol tautomerization Dx^+, Dx^0 and Dx^- molecules may occur in the keto as well as the enolic form. The total Dx concentration ($[Dx]_{tot}$) is defined by:

$$[Dx]_{tot} = [Dx^+] + [Dx^0] + [Dx^-] + [Dx^{2-}] \quad (1)$$

The groups in the Dx molecule involved in protonation/deprotonation equilibria are designated as A, P, K and E, where A^0 and A^+ refer to the C3′-amino function and its protonated form while P^0 and P^- refer to a C6 or C11 phenol group and its deprotonated form, respectively. K, E^0 and E^- refer to the status of the C9 side chain: keto form (K), enol form (E) or enolate form (E^-). In this case:

$$[Dx^+] = [A^+P^0P^0K] + [A^+P^0P^0E^0] \quad (2)$$

$$[Dx^0] = [A^0P^0P^0K] + [A^+P^-P^0K] + [A^+P^0P^-K] + [A^0P^0P^0E^0] + [A^+P^-P^0E^0] + [A^+P^0P^-E^0] + [A^+P^0P^0E^-] \quad (3)$$

$$[Dx^-] = [A^0P^-P^0K] + [A^0P^0P^-K] + [A^0P^-P^0E^0] + [A^0P^0P^-E^0] + [A^0P^0P^0E^-] + [A^+P^-P^0E^-] + [A^+P^0P^-E^-] \quad (4)$$

$$[Dx^{2-}] = [A^0P^-P^0E^-] + [A^0P^0P^-E^-] \quad (5)$$

An overview of the possible Dx species and deprotonation/protonation equilibria in the pH region studied is depicted in Fig. 5. The pH independent keto/enol equilibria as well as equilibria coherent with the overlapping ionization constants, e.g. the equilibrium between the neutral molecule $A^0P^0P^0K$ and the neutral zwitter ionic species $A^+P^-P^0K$ are not visualized in Fig. 5. In principle, each suggested species can be subjected to a solvent-, hydroxyl- or proton-catalyzed degradation yielding 54 possible reactions. However, the reactivity of species with equal charges cannot be distinguished. Therefore, the degradation of Dx can be represented as reactions of Dx^+, Dx^0, Dx^- and Dx^{2-} with H^+, H_2O and OH^-. As none of these reactions can be precluded a priori, all possible reactions were included in the evaluation of the pH profile of Dx. The summation of the contributions of all reactions of each Dx species yields k′ which is defined by Eqn. 6,

$$k' = \sum (k_0 + k_{OH}[OH] + k_H[H^+]) \cdot f_1 \quad (6)$$

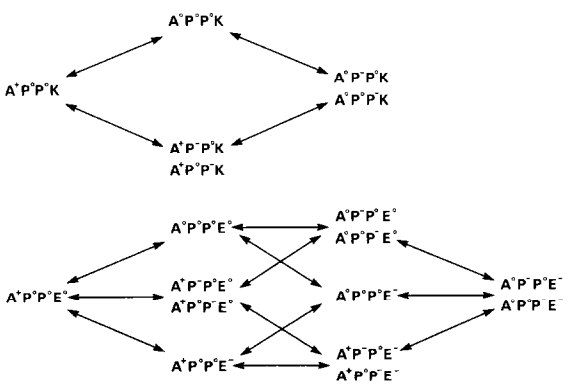

Fig. 5. Protolytic equilibria of doxorubicin in aqueous solution (pH 1–12).

where k_0 is the first-order rate constant for the solvent-catalyzed degradation and k_{OH} and k_H represent the specific second-order rate constants for hydroxyl- and proton-catalyzed degradation, respectively, and f_1 is the fraction of the species involved. Most of the suggested reactions are kinetically indistinguishable except for the reactions between H^+ and Dx^+ and between OH^- and Dx^-. The reactions involving the same total charge in the activated state can be considered as kinetically equivalent, e.g. the reactions $Dx^+ + OH^-$; $Dx^0 + H_2O$; $Dx^- + H^+$ are kinetically indistinguishable, yielding an overall rate constant consisting of a combination of the constants for the three reactions involved.

The macro-ionization constants are defined by:

$$K_1 = \frac{[Dx^0][H^+]}{[Dx^+]} \quad (7)$$

$$K_2 = \frac{[Dx^-][H^+]}{[Dx^0]} \quad (8)$$

$$K_3 = \frac{[Dx^{2-}][H^+]}{[Dx^-]} \quad (9)$$

The fractions of Dx^+, Dx^0, Dx^- and Dx^{2-} are derived from Eqns. 1 and 7–9 and are given by:

$$f^+ = [H^+]^3/([H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3) \quad (10)$$

$$f^0 = [H^+]^2K_1/([H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3) \quad (11)$$

$$f^- = [H^+]K_1K_2/([H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3) \quad (12)$$

$$f^{2-} = K_1K_2K_3/([H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3) \quad (13)$$

Combining Eqns. 6, 10–13 and inserting $[OH^-] = K_w/[H^+]$ yields

$$k' = \left(c_1[H^+]^4 + c_2[H^+]^3 + c_3[H^+]^2 + c_4[H^+] + c_5 + c_6[H^+]^{-1}\right)\left([H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3\right)^{-1} \quad (14)$$

where:

$$c_1 = k_H^+$$

$$c_2 = k_0^+ + k_H^0 K_1$$

$$c_3 = k_{OH}^+ K_w + k_0^0 K_1 + k_H^- K_1 K_2$$

$$c_4 = \left(k_{OH}^0 K_w + k_0^- K_2 + k_H^{2-} K_2 K_3\right) K_1$$

$$c_5 = \left(k_{OH}^- K_w + k_0^{2-} K_3\right) K_1 K_2$$

$$c_6 = k_{OH}^{2-} K_w K_1 K_2 K_3$$

c_2–c_5 incorporate the reaction constants of kinetically indistinguishable reactions. The superscripts of the specific reaction constants refer to the appropriate Dx species.

The experimental kinetic data were fitted in Eqn. 14. The macro-ionization constants K_1 and K_2 were fixed at the values obtained from spectrophotometric experiments with the structural analog Dr (Beijnen et al., 1986a). An optimal fit was achieved with the values for c_1–c_6 and the macro-ionization constants listed in Table 3. In Fig. 3 the graph for Dx is a simulation, using Eqn. 14 and the constants listed in Table 3, showing Eqn. 14 to be a proper description of the pH profile. From the fitting experiments it became apparent that c_1 contributes only in the pH region 1–4, c_2 in the pH region 3–8, c_3 in the pH region 4–10, c_4 in the pH region 7–10, c_5 in the pH region 8–11 and c_6 in the pH region 10–11. The macro-ionization constant K_3 contributes significantly to an appropriate fit of the data, implying the necessity of the involvement of this ionization step in the description of the pH profile.

TABLE 3

RATE CONSTANTS FOR THE DEGRADATION OF DOXORUBICIN AND IONIZATION CONSTANTS AT 50°C

c_1	5.6×10^{-4} $M^{-1}\cdot s^{-1}$
c_2	1.5×10^{-7} s^{-1}
c_3	4.0×10^{-13} $M\cdot s^{-1}$
c_4	4.5×10^{-21} $M^2\cdot s^{-1}$
c_5	3.5×10^{-30} $M^3\cdot s^{-1}$
c_6	3.5×10^{-41} $M^4\cdot s^{-1}$
K_1	3.2×10^{-8}
K_2	4.0×10^{-10}
K_3	1.0×10^{-10}

TABLE 4

k_{obs} VALUES (in s^{-1}) OF ANTHRAQUINONE GLYCOSIDES AT pH 8.0 [a] (50°C)

Compound	k_{obs}
doxorubicin	3.0×10^{-5}
4′-deoxydoxorubicin	2.5×10^{-5}
4′-O-methyldoxorubicin	2.6×10^{-5}
4′-epidoxorubicin	2.7×10^{-5}
daunorubicin	9.5×10^{-7}
4-demethoxydaunorubicin	1.8×10^{-6}
doxorubicinol	1.1×10^{-6}

[a] 0.01 M phosphate buffer (μ = 0.3; 5×10^{-4} M EDTA).

The slope of the straight line segment ($0 < pH < 3$) in the pH profile is -1, indicative of specific proton catalysis. At $pH > 4$ the slopes of the graph do not reach unity. The shape of this part of the pH profile suggests that the overall degradation rate is strongly influenced by dissociations of Dx.

Comparison of the stability of 7 anthracycline cytostatics at pH 8.0

The chemical stability of various anthraquinone glycosides was determined at pH 8. The k_{obs} values are listed in Table 4. It appears that Dx and its 4′-congeners all degrade with almost the same velocity, while Dr, 4-demethoxyDr and Dx-ol are much more stable. These findings are different from observations on the acid hydrolysis ($pH < 3.5$). Under acidic conditions the rate of the cleavage of the glycosidic bond is strongly dependent on structure modifications in the sugar moiety while the rate is unaffected by structure modifications located in the aglycone part (Beijnen et al., 1985a). At pH 8.0, however, structure differences in the sugar moiety have no effect on the degrada-

tion rate, contrarily to the nature of the C9 substituent.

Degradation mechanism

In Fig. 3 the pH profile of Dr at 50°C, obtained in another study (Beijnen et al., 1986a), is incorporated. In the region $0 < pH < 3$ the straight line segments in the profiles for Dx and Dr coincide while at $pH > 3$ Dx is less stable than Dr. As the only difference between the drugs is a C14 proton in Dr versus a C14 hydroxyl group in Dx, this structural feature must hold the key for the explanation of the difference in stability between both drugs. This conclusion also appears from the experiments where the degradation of related anthracyclines was studied (Table 4). The α-ketol side chain in Dx and its 4′-analogs favours enolization while in Dr, 4-demethoxy-Dr and Dx-ol this tautomerization is not likely to occur. In analogy with the degradation of corticosteroids possessing a dihydroxyacetone side chain at C17 (Hansen and Bundgaard, 1979; Dekker, 1980) the enolization step is assumed to be the first step of the degradation reaction of Dx. A mechanism for the conversion of Dx into II is proposed (Fig. 6). The Dx enolate anion (X, Fig. 6), arising from keto/enol tautomerization and deprotonation (Fig. 4), may be involved in a tautomeric equilibrium with its 13-ol-14-aldehyde derivative (Y, Fig. 6). The enolic structure in this mesomeric form has vanished leading to an increased basicity of the deprotonated 13-ol-14-aldehyde derivative (Y, Fig. 6). Abstraction of a C10 benzylic proton, protonation of the C13 O^- function in Y (Fig. 6), cleavage of the C9 side chain and the aminosugar part yields II. The high stability of the resulting degradation product is the driving force of the full aromatization of the A ring after cleavage of the C9 side chain.

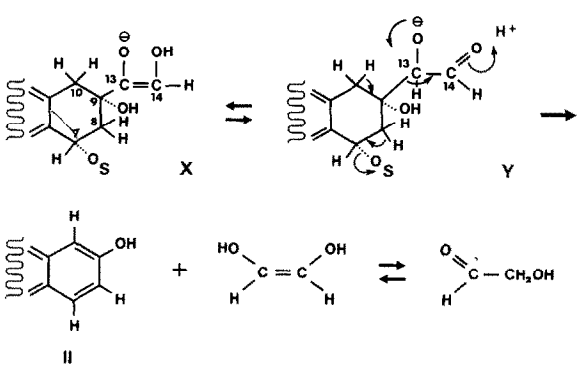

Fig. 6. Proposed degradation scheme for the conversion of doxorubicin into II. S refers to the daunosamine sugar moiety.

The fact that III (Fig. 2) is not traced in alkaline Dx decomposition mixtures, while, in the case of Dr, 7,8,9,10-bisanhydrodaunorubicinone is the principal degradation product (Beijnen et al., 1986a), further supports the hypothesis that enolization of the C9 side chain in Dx destabilizes the compound in comparison with the C9 acetyl function in Dr.

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