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Degradation kinetics of etoposide in aqueous solution

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Summary

The chemical stability of the anticancer drug etoposide in aqueous solution has been investigated utilizing a stability-indicating reversed-phase high-performance liquid chromatographic assay with ultraviolet detection. The degradation processes can adequately be described by pseudo-first order kinetics. The degradation kinetics have been studied as function of pH, buffer composition, ionic strength and temperature. A pH-rate profile, using rate constants extrapolated to zero buffer concentration, was constructed demonstrating that etoposide is most stable in the pH region 4–5. A comparison between the chemical stabilities of etoposide and podophyllotoxin in the pH region 9–11 has been made. The degradation mechanism of etoposide in aqueous solution is discussed.

Introduction

The semisynthetic epipodophyllotoxin derivative etoposide (VP 16-213; Vepesid) (Fig. 1) is considered to be one of the most effective agents against a diverse array of neoplastic diseases including small-cell anaplastic bronchial carcinoma, testicular teratomas, Hodgkin and non-Hodgkin lymphomas and neuroblastomas (Schmoll, 1982; Phillips and Lauper, 1983; Cavalli, 1982). Inherent to its clinical importance etoposide is currently experiencing increasing attention. Extensive studies have been conducted dealing with bioanalysis (Holthuis, 1985; Hersch and Ludden, 1986; Danigel et al., 1985 a and b; Duncan et al., 1986; Yamashita et al., 1987; Ho et al., 1985), pharmacokinetics (Issell et al., 1984; Holthuis, 1985), metabolism (Strife et al., 1980; Van Maanen et al.,

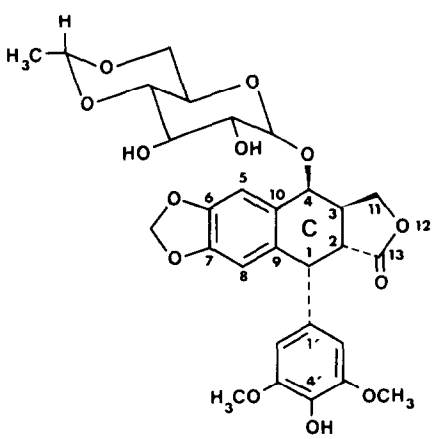

Fig. 1. Structure of etoposide.

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1982) and mode of action (Sinha and Myers, 1984). On the other hand it is surprising that data concerning the chemical stability of etoposide are scanty, especially quantitative information is lacking. Apart from some fragmentary reports (Strife et al., 1980; Floor et al., 1985) we are not aware of any systematic kinetic study about the degradation of etoposide in aqueous solution.

This study was initiated with the objective to collect quantitative data in order to obtain more detailed knowledge on the degradation kinetics of etoposide in aqueous solution, including the effects of several parameters (pH, buffers, temperature and ionic strength) on the degradation processes.

Materials and Methods

Chemicals

Etoposide and its aglycone (4′-demethylepipodophyllotoxin) were obtained from Bristol Myers BV, Weesp, The Netherlands. The *cis*-hydroxy acid derivative of etoposide, the *cis*-hydroxy acid derivative of the aglycone of etoposide and *cis*-etoposide were synthesized according to the procedures described by Strife et al. (1980). Podophyllotoxin was purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. All chemicals used were of analytical grade and deionized water was used throughout.

Buffer solutions

For the kinetic studies the following aqueous buffer solutions were used: H_0/pH − 1 to 2.5, perchloric acid; pH 2.5–6, acetate; pH 6–8.5, phosphate; pH 8.5–11, carbonate; pH 11–12, phosphate. The pH values between 1.5 and 12 were measured at the temperature of study by using an Ingold LOT-401 combined glass-reference electrode and a Radiometer PHM 64 research pH meter (Copenhagen, Denmark). Extension of the acidity scale below 1.5 was accomplished with the Hammett acidity function (Bates, 1973).

Kinetic measurements

The degradation reactions were initiated by adding a 60-μl sample of a solution of etoposide in methanol to 4 ml of the preheated buffered solution to obtain an initial etoposide concentration of 50 μg/ml (8.5×10^{-5}M). The stock solution of etoposide in methanol (5.7×10^{-3}M) kept at 4° C was found to be stable for 4 weeks at least. The reaction solutions were kept in screw-capped polypropylene test tubes in a thermostatically controlled waterbath in the dark. At appropriate time intervals 20-μl samples were withdrawn and directly analyzed for undegraded etoposide and the degradation products by a stability-indicating high-performance liquid chromatographic assay (HPLC). All kinetic experiments have been performed in duplicate.

Apparatus and analytical procedures

The chromatographic system consisted of a model M-45 solvent delivery system, a U6K septumless injection device and a model 440 dual wavelength UV detector (all from Waters Assoc., Milford, MA, U.S.A.) with fixed wavelength filters for detection at 254 and 280 nm. UV spectra of etoposide and the degradation products were recorded on line using a PU 4021 photo diode array detector (Pye Unicam Ltd., Cambridge, U.K.). The stainless-steel analytical column (30 cm × 3.9 mm i.d.) was filled with μ-Bondapak phenyl material (particle size 10 μm). For the degradation studies of etoposide a mobile phase (I) was used, consisting of methanol/water (50/50, w/w) to which 0.5% (v/w) 0.5 M sodium phosphate buffer solution (pH 6.5) and 0.5% (v/w) of a 20% (w/v) tetrabutylammonium bromide solution were added. For the analysis of the degradation mixtures of podophyllotoxin a mobile phase (mobile phase II) was used with the same composition as eluent I except for the methanol/water ratio (60/40, w/w). The mobile phases were used at a flow rate of 1.0 ml/min with the column at 25° C, thermostatically controlled. Quantitation of etoposide and the degradation products was based on peak area measurements using a SP4000 integrator (Spectra Physics, Santa Clara, CA, U.S.A.). Calibration curves of standard etoposide solutions in methanol showed excellent linearity ($r > 0.999$) in the concentration range of interest (5–60 μg/ml). The precision of the method was determined by repetitive injections ($n = 6$) of a methanolic etoposide

solution (25 μ/ml) and the relative standard deviation was found to be 1%.

The pK_a of the C4′ phenolic function of etoposide was determined spectrophotometrically by absorbance measurements at 264 nm with a Shimadzu UV-140 double-beam spectrophotometer (Shimadzu Corp., Kyoto, Japan). 20-μl samples of a stock solution of etoposide in methanol (5 mg/ml) were added to 2.00 ml of 0.01 M buffer solutions ($\mu = 0.1$) in the pH range 6–12 and the absorbance was measured. The pK_a of etoposide was determined graphically as the inflection point of the sigmoid titration curve obtained by plotting the absorbance at 264 nm against pH.

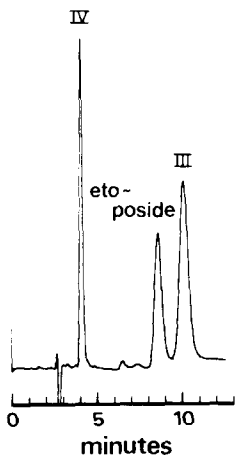

Fig. 3. HPLC chromatogram of a decomposition mixture of etoposide in alkaline medium.

Results and Discussion

Analytical procedures

Degradation of etoposide yields products in which the chromophore is unchanged. Consequently, UV spectrophotometry is an inadequate technique for the quantitation of the degradation reactions and a HPLC method was used in order to resolve decomposition products from the parent etoposide (Figs. 2 and 3). Compounds **II** and **IV** are eluted with the solvent front when tetrabutylammonium-bromide is omitted in the mobile phase. Retention of these degradation products, for quantitation purposes, can be achieved by adding a quaternary ammonium pairing ion to the eluent. Podophyllotoxin decomposition samples were also analyzed by using reversed-phase HPLC (Fig. 4).

Degradation products

The overall degradation scheme of etoposide is depicted in Fig. 5. The total of the UV absorbance

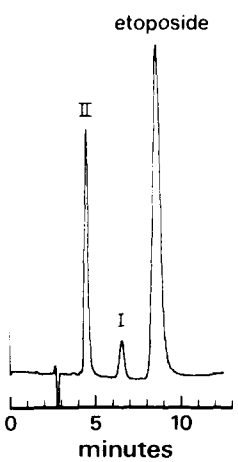

Fig. 2. HPLC chromatogram of a decomposition mixture of etoposide in acidic medium.

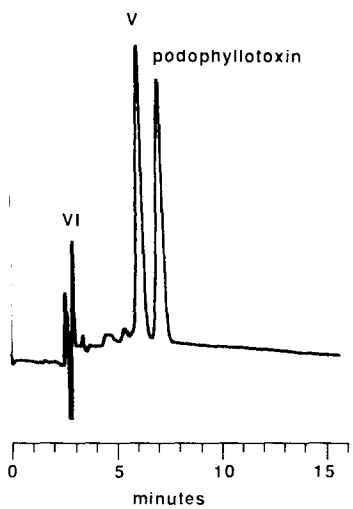

Fig. 4. HPLC chromatogram of a decomposition mixture of podophyllotoxin in alkaline medium.

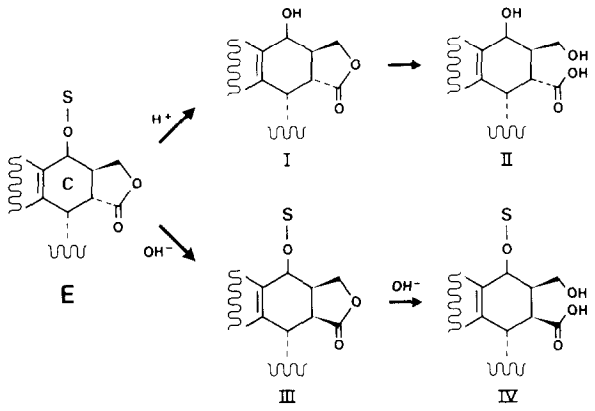

Fig. 5. Overall degradation scheme of etoposide in acidic and alkaline media. E, etoposide; **I**, the aglycone of etoposide; **II**, the *trans*-hydroxy acid of the aglycone of etoposide; **III**, *cis*-etoposide; **IV**, the *cis*-hydroxy acid of etoposide; S, D-glucopyranosyl sugar moiety.

(at 254 nm and at 280 nm) of the degradation products and residual etoposide, in terms of peak integrator units, remains constant until complete disappearance of etoposide. This again demonstrates that the degradation processes do not involve the chromophore of the etoposide molecule. Furthermore, the mass balance indicates that all degradation products are included in the HPLC assay.

Rapid scanning with the photo diode array detector on line with the HPLC system revealed that degradation products **I**, **II**, **III**, **IV** and etoposide have identical UV spectra with λ_{max} at 284 nm and λ_{min} at 263 nm. In acidic medium the glucopyranosil moiety of etoposide is cleaved yielding 4′-demethylepipodophyllotoxin (**I**). Additional evidence for the identity of this aglycone was obtained by comparison of its elution volume and that of a reference compound. The aglycone degrades further into compound **II**. The structure of **II** is not known with certainty. However, the fact that **II** is formed out of **I** proves that **II** is an aglycone. Its chromatographic behaviour, with and without a pairing ion added to the mobile phase, demonstrates that **II** has acidic properties. It is known that the *cis*-hydroxy acid derivative of etoposide can undergo lactone ring closure on acidification (Strife et al., 1980). Ring closure of a *trans*-hydroxy acid derivative is not likely on thermodynamic grounds as this chemical transformation should yield the highly strained, energetically unfavourable, *trans*-fused lactone ring. On account of the above mentioned grounds it is likely that compound **II** is the *trans*-hydroxy acid derivative of the aglycone of etoposide. Further research is warranted to prove this hypothesis.

In neutral, weak acidic and basic solution (pH > 5) the degradation of etoposide occurs through epimerization of the *trans*-fused lactone ring moiety into the *cis*-fused lactone (**III**). Progressive degradation of this compound results in the formation of the *cis*-hydroxy acid derivative (**IV**). Contrarily to the acid-catalyzed degradative pathway the sugar part of the etoposide molecule is retained on degradation in alkaline media. Furthermore, the identities of **III** and **IV** were confirmed by comparison of elution volumes with those of reference compounds prepared by the procedures of Strife et al. (1980). No attempts were made to elucidate the structures of the podophyllotoxin (Fig. 6) degradation products. However, in analogy with etoposide degradation in basic media compound **V** may be the 2,3-*cis*-epimer. It is remarkable that in the case of etoposide its *cis*-epimer has a larger capacity factor than the precursor while for podophyllotoxin this appears to be the reverse (Buchardt et al., 1986). If the epimerization occurs through lactone ring opening, yielding a trans-hydroxy acid derivative, subsequent ring closure may result in the formation of a 2,4-lactone (neopodophyllotoxin) (Renz et al., 1965).

The chromatographic behaviour of compound

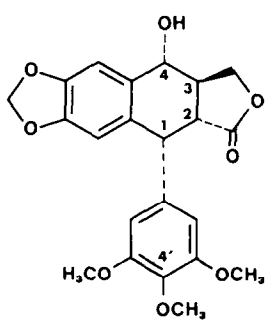

Fig. 6. Structure of podophyllotoxin.

VI suggests that this compound is a hydroxy acid derivative. Further investigations are in progress focussed on the conclusive structure elucidation of the degradation products of etoposide as well as those of podophyllotoxin.

Degradation kinetics

Order of reactions

The disappearance of etoposide in buffered media follows (pseudo) first-order kinetics over several half-lives. This is indicated by the linearity of plots of the natural logarithm of residual etoposide concentration against time at various pH values. The observed rate constants (k_{obs}) for the overall degradation have been extracted from the slopes of these plots.

Influence of pH

When etoposide degrades in unbuffered solutions pH shifts occur due to the disappearance of the phenolic parent compound with $pK_a \sim 10$ and the appearance of degradation products with an acidic carboxylic function ($pK_a \sim 4$–5). The pH shifts will result in a change of k_{obs} during the degradation process and deviations from first-order kinetics. To keep the pH constant during etoposide degradation buffered solutions were used. The overall observed rate constant (k_{obs}) for degradation of etoposide in buffers is:

$$k_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_{buffer}[buffer] \quad (1)$$

where k_0 is the (pseudo) first-order rate constant for degradation in water only, k_H and k_{OH} second-order rate constants for proton- and hydroxyl-catalyzed degradation, respectively. The term k_{buffer}[buffer] represents the sum of second-order rate constants for the degradation catalysed by each of the buffer components multiplied by its concentration. For each pH the (pseudo)first order rate constant for [buffer] = 0, ($k' = k_0 + k_H(H^+) + k_{OH}(OH^-)$) was calculated as the intercept from the linear part of the plot of k_{obs} versus [buffer] at a fixed pH.

The calculated rate constants at zero buffer concentration (k') were used in the construction of the H_0/pH profile (Fig. 7). In the pH region −1 to 3 the V-shaped graph has a slope of −1.0 and in the pH region 6 to 10 the slope is +1.0 indicating that the degradation reaction is first-order in proton and hydroxyl ion concentration, respectively, so, specific proton and specific hydroxyl catalysis apply. On Fig. 7 the points are experimental and the line theoretical using Eqn. 2.

$$k' = k_0 + k_H[H^+] + \frac{k_{OH}K_w}{[H^+]} \quad (2)$$

With the use of a non-linear least-squares curve fitting program the specific rate constants were calculated (Table 1). The pH at which the minimum occurs in the pH profile can be determined

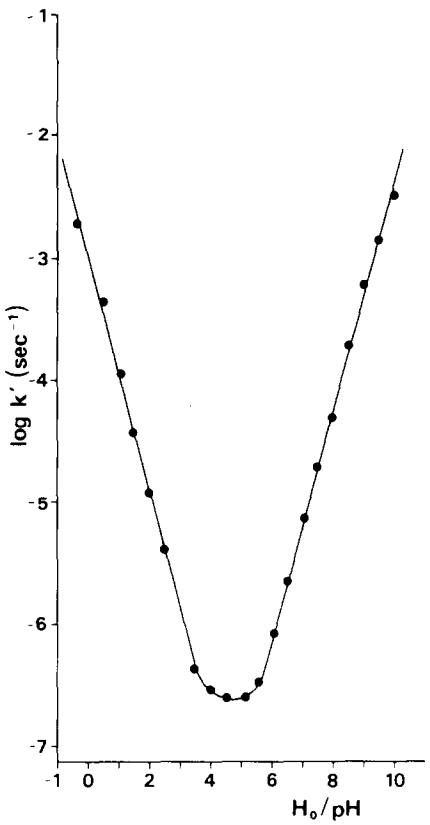

Fig. 7. log k'–pH profile for the degradation of etoposide at 50 °C.

TABLE 1

Rate constants for catalysed degradation reactions of etoposide and cis-etoposide

	50 °C		25 °C
Etoposide			
k_H	1.1×10^{-3} M^{-1} s^{-1}	C_3	1.8×10^{-14} Ms^{-1}
k_0	2.5×10^{-7} s^{-1}	C_4	2×10^{-25} M^2 s^{-1}
k_{OH}	6.15 M^{-1} s^{-1}		
cis-Etoposide			
C_3			4×10^{-13} Ms^{-1}
C_4			1.6×10^{-25} M^2 s^{-1}

graphically or be calculated from the first derivative of Eqn. 2,

$$\frac{dk'}{d[H^+]} = k_H - \frac{k_{OH}K_w}{[H^+]^2} = 0 \qquad (3)$$

or:

$$pH_{min} = \tfrac{1}{2}pK_w + \tfrac{1}{2}\log\frac{k_H}{k_{OH}} \qquad (4)$$

At 50 °C $pK_w = 13.26$. So pH_{min} is calculated to be 4.8 which is in agreement with the graphically derived value. At pH_{min} the half-life of etoposide at 50 °C is 780 h.

Kinetic experiments at pH values > 9 were performed at 25 °C as the degradation reactions were too fast in this pH region to be monitored at 50 °C with the techniques used in this study. A plot of log k' vs pH in the pH region 9–11 (Fig. 8) shows an inflexion which can be attributed to an acid–base equilibrium of etoposide involving the phenolic function. The log k'–pH graph for podophyllotoxin (Fig. 8), possessing a nondissociable C4′ methoxygroup, is a straight line applying to Eqn. 5:

$$k' = \frac{k_{OH}\cdot K_w}{[H^+]} \qquad (5)$$

The value for k_{OH} is 0.278 M^{-1} s^{-1}.

Due to the concomitant existence of neutral etoposide and deprotonated, negatively charged,

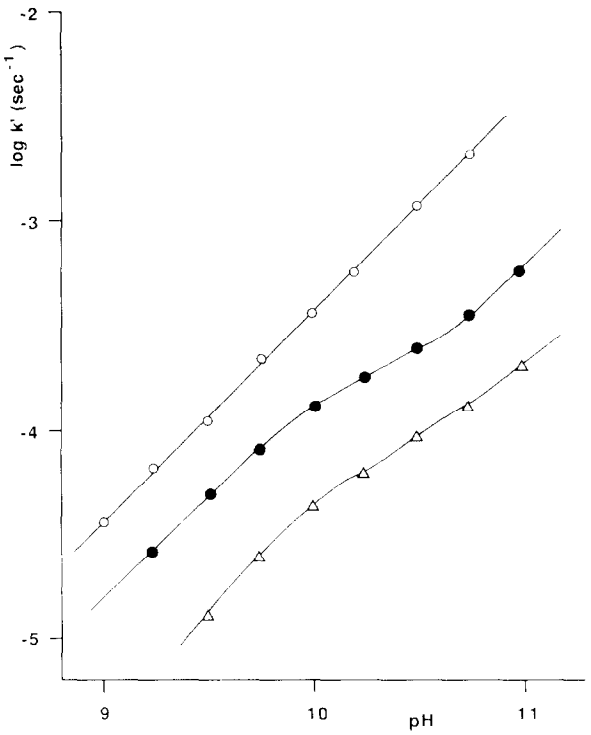

Fig. 8. log k'–pH profiles for the degradation of etoposide (●——●), podophyllotoxin (○——○) and *cis*-etoposide (△——△) at 25 °C.

etoposide species in the pH range under investigation, Eqn. 2 must be extended to:

$$k' = \left(k_0 + k_H[H^+] + \frac{k_{OH}K_w}{[H^+]}\right)f + \left(k_0^- + k_H^-[H^+] + \frac{k_{OH}^-K_w}{[H^+]}\right)(1-f) \qquad (6)$$

The superscript minuses of the rate constants refer to the deprotonated etoposide molecule and when no superscript is used it implies that the rate constant is related to the neutral etoposide form; f is the fraction of neutral etoposide species and, consequently, $(1-f)$ is the fraction of deprotonated etoposide species. The K_a value of etoposide is then defined as:

$$K_a = \frac{(1-f)[H^+]}{f} \qquad (7)$$

Combination of Eqns. 6 and 7 yields:

$$k' = \frac{C_1[H^+]^2 + C_2[H^+] + C_3 + C_4[H^+]^{-1}}{C_5 + [H^+]} \quad (8)$$

where:

$C_1 = k_H$

$C_2 = k_0 + k_H^- K_a$

$C_3 = k_{OH} K_w + k_0^- K_a$

$C_4 = k_{OH}^- K_w K_a$

$C_5 = K_a$

As the solvent-catalyzed degradation reaction of etoposide and the proton-catalyzed degradation reaction of deprotonated etoposide are kinetically indistinguishable the rate constants k_0 and k_H^- are combined in C_2. The same holds for k_{OH} and k_0^- which are also coupled rate constants and therefore combined in C_3. Comparison of the experimentally determined stability curve with computer simulation experiments based upon a model according to Eqn. 8 indicate that between pH 9 and 11 the constants C_1 and C_2 provide no significant contribution to the pH-rate profile. Therefore the theoretical Eqn. 8 could be reduced to:

$$k' = \frac{C_3 + C_4[H^+]^{-1}}{C_5 + [H^+]} \quad (9)$$

Using the non-linear curve-fitting program C_3, C_4 and C_5 were calculated. The results are included in Table 1. The kinetically determined pK_a of etoposide (10.1) is in reasonable agreement with the spectrophotometrically determined value (9.8). At pH > 6 etoposide, through epimerization, is converted into *cis*-etoposide, which in turn degrades through intramolecular ester hydrolysis into the cis-hydroxy acid derivative, visualized by the scheme:

etoposide $\xrightarrow{k_1}$ **III** $\xrightarrow{k_2}$ **IV**

where k_1 and k_2 are the (pseudo) first-order rate constants for these conversions. The courses of the concentration of **III** and **IV** during etoposide degradation are described by Eqn. 10 and Eqn. 11, respectively:

$$[III]_t = [\text{etoposide}]_0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (10)$$

$$[IV]_t = [\text{etoposide}]_0 \times \left(1 - \frac{k_2}{k_2 - k_1} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t}\right) \quad (11)$$

where $[III]_t$ is the concentration of *cis*-etoposide

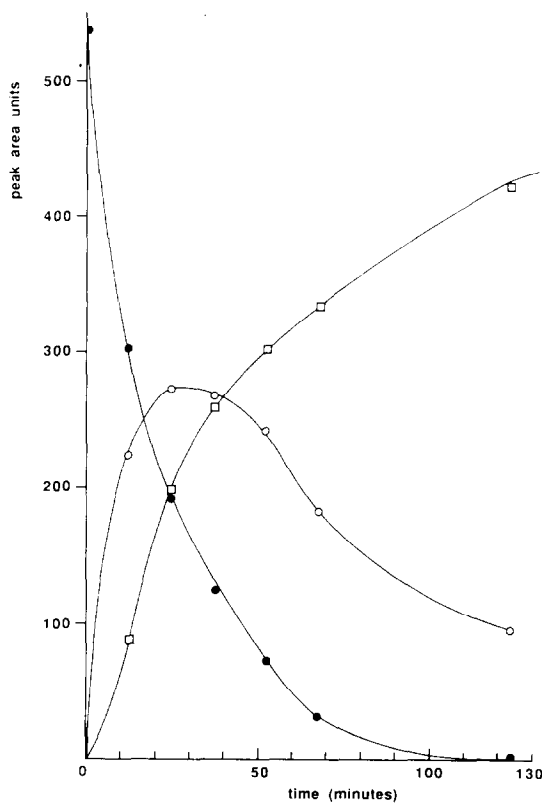

Fig. 9. Reactant concentrations. (●———●), Etoposide; (○———○), III; (□———□), IV; in terms of peak area integrator units vs time for the conversions: etoposide → **III** → **IV**, at pH 11.01.

at time t, $[IV]_t$ the concentration of the *cis*-hydroxy acid derivative at time t and $[etoposide]_0$ the initial concentration of etoposide. The reactant concentration vs time for the consecutive first-order reactions: etoposide → **III** → **IV** at pH 11.01, serving here as an example, are plotted on Fig. 9.

Applying Eqns. 10 and 11, in which $[III]_t$, $[IV]_t$ and $[etoposide]_0$ are expressed as peak area integrator units, k_1 and k_2 can be determined by using the non-linear curve fitting computer programs. k_1 is in good agreement with the k_{obs} values calculated from the first-order plots of overall etoposide degradation. k_2 is the observed (pseudo) first-order rate constant for *cis*-etoposide degradation. These calculated values have been incorporated on Fig. 8 and have been used in the same manner as the etoposide data for calculation of the pK_a, C_3, C_4 and C_5, by using Eqn. 9 (Table 1). The pK_a of *cis*-etoposide (10.8) appeared to be substantially higher than the value for etoposide. On account of the structural resemblances of etoposide and its *cis*-congener the difference was unexpected. It must be noted that the kinetic determination of the pK_a of *cis*-etoposide is less accurate than that of etoposide because k_2 values are less accurate than the k_{obs} values of etoposide.

Influence of buffers

The degradation of etoposide is strongly affected by phosphate, acetate and carbonate buffer components. This appears from experiments where k_{obs} is measured at constant pH, ionic strength and temperature but at different buffer concentrations. In the low buffer concentration region a linear relationship between k_{obs} and [buffer] exists. At higher buffer concentrations the curve levels. This has been observed, more or less, in the entire pH region studied. An example is given on

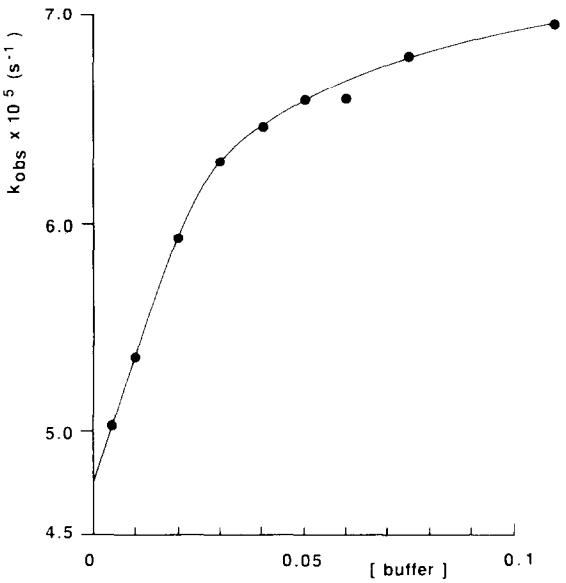

Fig. 10. The effect of carbonate concentration at pH = 9.5 on k_{obs} for the degradation of etoposide at 25 °C.

TABLE 2

k_{obs} Values (in s^{-1}) for the degradation of etoposide in various buffer solutions at 50 °C

pH	[buffer]	k_{obs}	pH	[buffer]	k_{obs}
3.3	0.005	4.1×10^{-7}	7.8	0.005	4.8×10^{-5}
	0.01	4.3×10^{-7}		0.01	5.0×10^{-5}
	0.05	5.0×10^{-7}		0.02	5.3×10^{-5}
	0.10	5.6×10^{-7}		0.03	5.5×10^{-5}
	0.20	5.7×10^{-7}		0.04	5.5×10^{-5}
6.5	0.005	2.2×10^{-6}		0.06	5.7×10^{-5}
	0.01	2.3×10^{-6}		0.10	6.1×10^{-5}
	0.05	2.4×10^{-6}	8.55	0.01	1.4×10^{-4}
	0.10	2.4×10^{-6}		0.05	2.1×10^{-4}
	0.20	2.4×10^{-6}		0.10	2.2×10^{-4}
				0.20	2.4×10^{-4}

pH 3.3, acetate; pH 6.5, 7.8 and 8.55, phosphate; [buffer] in M.

TABLE 3

k_{obs} Values (in s^{-1}) for the degradation of etoposide in 0.01 M buffer solutions at 25 °C as function of the ionic strength (μ)

pH	μ	k_{obs}	pH	μ	k_{obs}
8.60	0.03	5.8×10^{-6}	10.36	0.02	2.2×10^{-4}
	0.05	6.1×10^{-6}		0.04	2.4×10^{-4}
	0.13	6.6×10^{-6}		0.12	2.6×10^{-4}
	0.53	7.5×10^{-6}		0.52	3.2×10^{-4}

Fig. 10. Other representative data are listed in Table 2.

Influence of ionic strength

The influence of ionic strength (μ) on the degradation of etoposide has been studied by adding various amounts of sodium chloride to solutions of fixed pH and total buffer concentration. In the acid region of the pH profile the effects were negligible. Results from experiments at pH = 8.60 and pH = 10.36 are listed in Table 3.

Influence of temperature

The influence of temperature on the degradation of etoposide was investigated in 0.01 M buffer solutions and perchloric acid solutions in the temperature range 5–60 °C. The Arrhenius relationship between the natural logarithm of k_{obs} and the reciprocal of absolute temperature holds. Results, in terms of activation energies and frequency factors, are listed in Table 4.

Degradation mechanism

Like other glycosides, such as doxorubicin (Wassermann and Bundgaard, 1983; Beijnen et al., 1985), etoposide undergoes hydrolytic degradation in acidic media with the formation of an aglycone (Dow et al., 1983). Consecutive degradative reactions may involve acid-catalyzed ester hydrolysis of the lactone ring system, initiated by protonation of the lactone carbonyl function followed by nucleophilic attack by water molecules at C13. Epimerization of etoposide at pH values > 4 occurs through enolization (Fig. 11) (Strife et al., 1980). The enolization step and subsequent conversion of the enol into *cis*-etoposide requires proton transfers which are facilitated by bases such as hydroxide ions, water molecules or anions of the acid used as buffer. This explains the observed general base catalysis as well as the specific base catalysis at zero buffer concentration on degradation of etoposide at pH > 5. The action of the dibasic phosphate as a proton conductor is illustrated in Fig. 11. *cis*-Etoposide further degrades into the *cis*-hydroxy acid derivative (**IV**), which may proceed according to generally accepted mechanisms of alkaline ester hydrolysis (Sykes, 1968).

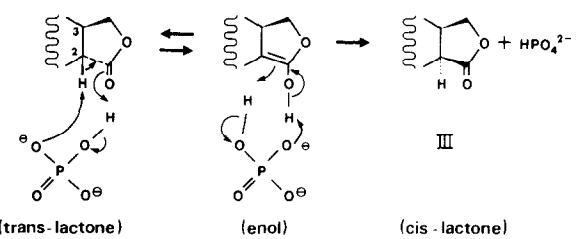

Fig. 11. Epimerization of etoposide and the action of dibasic phosphate as a proton-conductive catalyst.

TABLE 4

Activation energies (E_a) and frequency factors (A) for the degradation of etoposide in 0.01 M buffers as function of pH

pH	E_a (kJmol^{-1})	A(s^{-1})
0.96	103	7.4×10^{12}
7.99	115	2.4×10^{14}
11.04	76	1.3×10^{10}
11.47	71	3.8×10^{9}

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References

Bates, R.G., *Determination of pH; Theory and Practice*, Wiley, New York, 1973, pp. 165–169.

Beijnen, J.H., Wiese, G. and Underberg, W.J.M., Aspects of the chemical stability of doxorubicin and seven others anthracyclines in acidic solution. *Pharm. Weekbl. Sci. Ed.*, 7 (1985) 109–116.

Buchardt, O., Jensen, R.B., Hansen, H.F., Nielsen, P.E., Andersen, D. and Chinoin, I., Thermal chemistry of podophyllotoxin in ethanol and a comparison of the cytostatic activity on the thermolysis products. *J. Pharm. Sci.*, 75 (1986) 1076–1080.

Cavalli, F., VP 16-213 (Etoposide). *Cancer Chemother. Pharmacol.*, 7 (1982) 81–85.

Danigel, H., Schmidt, L., Jungclas, H. and Pflüger, K.-H., Combined thin layer chromatography/mass spectrometry: an application of ^{252}Cf plasma desorption mass spectrometry for drug monitoring. *Biomed. Mass Spectrom.*, 12 (1985a) 542–544.

Danigel, H., Pflüger, K.-H., Jungclas, H., Schmidt, L. and Dellbrügge, J., Drug monitoring of etoposide (VP 16-213) I. A combined method of liquid chromatography and mass spectrometry. *Cancer Chemother. Pharmacol.*, 15 (1985b) 121–124.

Dow, L.W., Sinkule, J.A., Look, A.T., Horvath, A. and Evans, W.E., Comparative cytotoxic and cytokinetic effects of the epipodophyllotoxins 4′-demethylepipodophyllotoxin-9-(4,6-*O*-2-ethylidene-β-D-glycopyranoside) and 4′-demethylepipodophyllotoxin-9-(4,6-*O*-2-thenylidene-β-D-glycopyranoside) and their metabolites on human leukemic lymphoblasts. *Cancer Res.*, 43 (1983) 5699–5706.

Duncan, G.F., Farmen, R.H., Movahhed, H.S. and Pittman, K.A., High-performance liquid chromatographic method for the determination of etoposide in plasma using electrochemical detection. *J. Chromatogr.*, 380 (1986) 357–365.

Floor, B.J., Klein, A.E., Muhammad, N. and Ross, D., Stability indicating liquid chromatographic determinations of etoposide and benzyl alcohol in injectable formulations. *J. Pharm. Sci.*, 74 (1985) 197–200.

Hersch, M.R. and Ludden, T.M., High-performance liquid chromatographic assay for etoposide in human plasma. *J. Pharm. Sci.*, 75 (1986) 815–817.

Ho, D.H., Kannellopoulos, K.S., Brown, N.S., Issell, B.F. and Bodey, G.F., Radioimmunoassay for etoposide and teniposide. *J. Immunol. Methods*, 85 (1985) 5–15.

Holthuis, J.J.M., *Bioanalysis, Electrochemistry and Pharmacokinetics of Etoposide, Teniposide and Metabolites*, Thesis, State University of Utrecht, The Netherlands, 1985.

Issell, B.F., Muggia, F.M., and Carter, S.K. (Eds.), *Etoposide (VP16). Current Status and New Developments*, Academic, London, 1984.

Phillips, N.C. and Lauper, R.D., Review of etoposide. *Clin. Pharm.*, 2 (1983) 112–119.

Renz, J., Kuhb, M. and Von Wartburg, A., Umesterungsreactionen und Podophyllum Lignanen. *Liebigs Ann. Chem. Bd.*, 681 (1965) 207–224.

Schmoll, H., Review of etoposide single agent activity. *Cancer Treat. Rev.*, 9 (1982) 21–35.

Sinha, B.K. and Myers, C.E., Irreversible binding of etoposide (VP16-213) to deoxyribonucleic acid and proteins. *Biochem. Pharmacol.*, 33 (1984) 3725–3728.

Strife, R.J., Jardine, I. and Colvin, M., Analysis of the anticancer drugs VP16-213 and VM 26 and their metabolites by high performance liquid chromatography, *J. Chromatogr.*, 182 (1980) 211–220.

Sykes, P., *A Guide Book to Mechanism in Organic Chemistry*, 2nd edn., Longmans, London, 1968, pp. 184.

Van Maanen, J.M.S., Van Oort, W.J. and Pinedo, H.M., In vitro and in vivo metabolism of VP 16-213 in the rat. *Eur. J. Cancer Clin. Oncol.*, 18 (1982) 885–890.

Wassermann, K. and Bundgaard, H. Kinetics of the acid-catalyzed hydrolysis of doxorubicin. *Int. J. Pharm.*, 14 (1983) 73–78.

Yamashita, K., Watanabe, K., Takayama, H., Ishibashi, M. and Miyazaki, H., Measurement of plasma etoposide by radioimmunoassay. *J. Pharm. Biomed. Anal.*, 5 (1987) 11–20.