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Degradation kinetics of vinblastine sulphate in aqueous solutions

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Summary

The degradation kinetics of vinblastine sulphate (VBL) have been studied over the H_0/pH range −4 up to 14 at 80 °C. A stability indicating HPLC-system with UV-detection was used to separate the parent compound and the degradation products. The influences on the degradation rate of the buffer concentration, ionic strength and temperature have been investigated. The degradation kinetics were modeled with a non-linear least-squares curve-fitting program.

Introduction

Vinblastine (VBL) is a naturally occurring alkaloid isolated from the roots of the periwinkle plant, *Catharanthus roseus* G. Don. Structurally, VBL is a dimeric product of the indole derivate catharanthine and vindoline (Fig. 1). The drug is used in cancer chemotherapy and shows therapeutic effect against lymphomas, Hodgkin's disease and testicular carcinoma (Creasey, 1981). Vinblastine is marketed as a freeze-dried formulation (Velbe). In this solid state the compound is stable for two years when stored between 0 and 6 °C (Eli Lilly Nederland, product information). However, after reconstitution of VBL in aqueous or organic solvents the drug is susceptible to degradation reactions (Burns, 1972). This systematic study was initiated with the objective to extend the knowledge on the degradation kinetics of VBL in aqueous solutions. Apart from some fragmentary reports (Saul and Tanneberger, 1975; Keller and Ensminger, 1982; De Smet et al., 1985) no systematic stability studies of vinca alkaloids have been reported, hitherto.

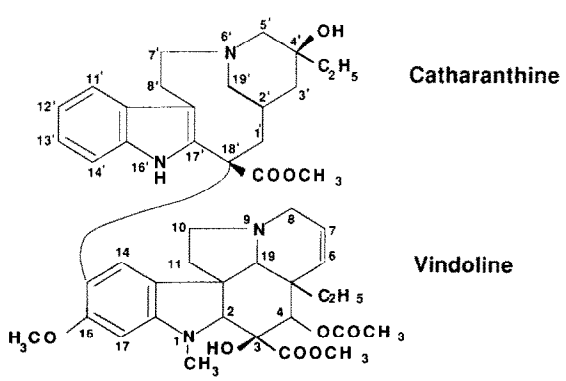

Fig. 1. Chemical structure of vinblastine.

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Experimental

Materials

Vinblastine sulphate was kindly provided by Pharmachemie (Haarlem, The Netherlands). Desacetylvinblastine sulphate was a gift from Gedeon Richter (Budapest, Hungary). All chemicals were of analytical grade and were used as received. For the preparation of aqueous solutions deionized water was filtered by a Milli-Q Water Purification System (Millipore, Bedford, MA, U.S.A.)

Buffer solutions

The following buffer solutions were used for the degradation study: H_0/pH $\leq$ 3.0: perchloric acid; pH 3.0–9.5: phosphate buffer; pH 9.5–11.0: carbonate buffer; pH $\geq$ 11.0; sodium hydroxide.

The Hammett acidity function (Bates, 1973) was used for the extension of the H_0/pH scale below 1. The Debye-Hückel equation (Bates, 1973) was used to calculate pH values between 12 and 13. The pH values between 3.0 and 11.0 were measured at 80 °C with a combined glass-reference electrode attached to a Consort P514 pH meter (Turnhout, Belgium). Calibration of the pH meter was also carried out at 80 °C. A constant ionic strength (μ) was maintained for each solution, adjusted to 0.3 with sodium chloride, except for the solutions where the hydrogen or hydroxide ion concentration exceeded 0.3 M. Sodium edetate was added to all buffer solutions in a final concentration of 5×10^{-4} M.

Kinetic measurements

The degradation kinetics of VBL were studied at a temperature of 80 $\pm$ 0.5 °C. The reactions were initiated by adding 10 μl of a methanolic stock solution of VBL (1.5×10^{-2} M) to 3.0 ml of buffer solution in a glass container of 10 ml. The initial VBL concentration was about 5×10^{-5} M. After closing the vial with a butylrubber septum and an aluminium cap the sample was mixed on a vortex mixer for 5 s. The container was placed in a thermostatically controlled waterbath, and protected from light. At appropriate time intervals, 100 μl samples were taken from the reaction solution, by using a polypropylene syringe, and stored in conical polypropylene tubes (capacity 1.5 ml). After the sample was drawn it was cooled directly in ice-water and stored at −20 °C until analysis. Samples at a pH above 9.5 were analyzed immediately after cooling. The pH of samples with extreme low pH values ($\leq$ 0.5) was adjusted with some grains of solid sodium phosphate or sodium carbonate to acceptable pH values directly before storage at −20 °C. Under these storage conditions no further degradation was observed for at least 2 months.

Apparatus and analytical procedures

The chromatographic system consisted of a M6000 solvent delivery system (Waters Assoc., Milford, MA, U.S.A.), equipped with a Spectroflow 773 absorbance detector (Kratos Analytical Instruments, Ramsey, U.S.A.), operating at 259 or 298 nm. Samples of 20 μl were injected with a Waters Intelligent Sample Processor (model 710) automatic injector. The analytical column (100 × 3.9 mm; i.d.; 5 μm) was packed at our laboratory with Hypersil ODS material (Shandon, Runcorn, U.K.) and thermostated at 25 °C. The mobile phase consisted of methanol and 10 mM phosphate buffer pH 7.0 (60 : 40 w/w). The solvents were filtered with a Millipore filter (0.2 μm) and were, after mixing, degassed by sonification for 10 min. The flow rate was 1.0 ml/min for all experiments. The chromatograms were recorded on a flat-bed recorder (Kipp and Zonen, Delft, The Netherlands). Quantification of undegraded VBL was based on peak area measurement using a SP 4000 Central Processor Integrator (Spectra Physics, Santa Clara, CA, U.S.A.) or on peak height measurement. Standard calibration graphs of VBL solutions in methanol (20 μl injected) exhibited linearity in the concentration range of interest, 5×10^{-5} M to 3×10^{-6} M.

Results and Discussion

Chromatography

The separation of VBL and its degradation products can be obtained by reversed-phase HPLC (Görög et al., 1977; Thimmaiah and Sethi, 1985). The HPLC system we already used to determine VBL in plasma and urine samples (Vendrig et al.,

1987) also proved to be suitable to study the degradation kinetics of VBL. Good separation between VBL and a major degradation product of VBL, desacetylvinblastine (DVBL) was obtained. Typical HPLC chromatograms of undegraded and degraded samples, at different pH values, are shown in Fig. 2. The HPLC system used is stability-indicating. This was investigated by applying thin-layer chromatography to combined elution volumes of VBL after repetitive injection of the same mixture. The methanol of the mobile phase was evaporated under nitrogen at room temperature. The aqueous phases of six 20 μl injections were combined and were freeze-dried. The extract was dissolved in methanol. This solution and a methanolic reference solution of VBL were spotted on a silica gel thin-layer plate (20 × 10 cm). The plate was developed with a mobile phase consisting of acetonitrile and methanol (1 : 1, v/v). After drying the plate with a warm stream of air it was sprayed with the Dragendorff reagent (Munier modification) (Kirchner, 1978). The extract contained only one spot with the same R_f value as VBL (R_f value is 0.5). Furthermore, the ratio of

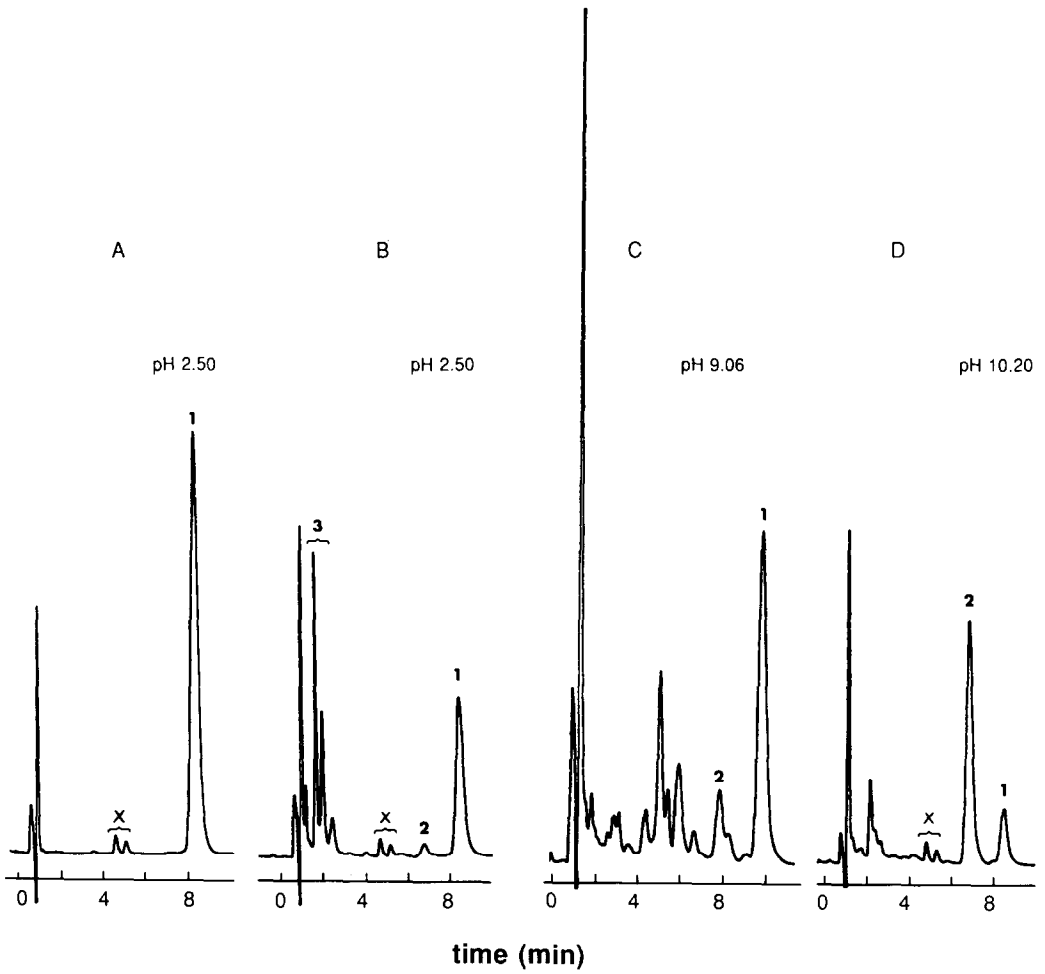

Fig. 2. Chromatograms of VBL before degradation and after some time at 80 °C at different pH values. A: 0.03 M perchloric acid, $\mu = 0.3$, $t = 0$ min. B: 0.03 M perchloric acid, $\mu = 0.3$, $t = 115.5$ h. C: 0.035 M phosphate buffer pH 9.0, $\mu = 0.3$, $t = 62$ h. D: 0.035 M carbonate buffer pH 10.5, $\mu = 0.3$, $t = 6$ h. Peak 1 = VBL; peak 2 = DVBL; peaks x = unknown compounds, also present in blank solutions; peaks 3 = unidentified degradation products of VBL.

peak heights of VBL determined with the HPLC system at 259 nm and at 298 nm did not change during the course of degradation.

Degradation products

Below pH 1.5 mainly desacetylvinblastine (DVBL) was formed, which was identified by comparing the elution volume with the reference compound. Desacetylvinblastine appeared to be stable at 80 °C for several days. For example, at pH 1.22 DVBL is stable for more than 90 h. At pH values above 1.5 DVBL was formed to a lesser extent. Besides, 3 other peaks eluting near the solvent front were observed (Fig. 2B, peak 3). Between pH 2.5 and 7.5 the amount of DVBL formed was negligible. Up to pH 5.0, only 3 peaks eluting near the solvent front were detected. At higher pH values an array of different products was formed (Fig. 2C). Above pH 10.5 DVBL was again the main product formed (Fig. 2D). Also some small peaks eluting near the solvent front were noticed in chromatograms of samples at these high pH values.

Kinetics

Temperature. The degradation kinetics were studied at 80 °C because of the low reaction rates at lower temperatures. For example, at 20 °C at pH 5.06 the half-life time of VBL, calculated from the Arrhenius plot, is 6400 h.

Order of reaction. The decay of the VBL concentration follows pseudo-first-order kinetics over at least 3 half-lifes at constant pH (Fig. 3). The observed pseudo-first-order rate constant (k_{obs}) for the overall degradation was calculated by least-squares linear regression analysis as the slope of a plot of the natural logarithm of the remaining VBL concentration ($[VBL]_t$) versus time as depicted by Eqn. 1

$$\ln[VBL]_t = \ln[VBL]_0 - k_{obs} \cdot t \quad (1)$$

where $[VBL]_0$ is the initial VBL concentration.

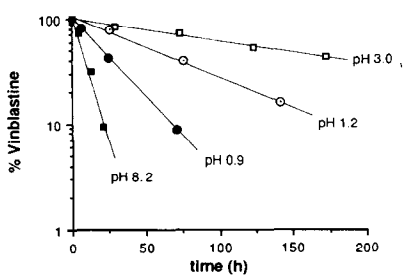

Fig. 3. Semilogarithmic first-order plots for the degradation of VBL at 80 °C at different pH values.

TABLE 1

The effect of phosphate buffer concentration on k_{obs} for the degradation of VBL at different pH values

Temperature 80 °C; $\mu = 0.3$.

pH	[buffer] (M)	k_{obs} (s^{-1})	pH	[buffer] (M)	k_{obs} (s^{-1})
3.3	0.005	1.9×10^{-6}	5.5	0.005	3.8×10^{-6}
3.3	0.015	1.5×10^{-6}	5.5	0.015	5.2×10^{-6}
3.3	0.025	1.1×10^{-6}	5.5	0.025	6.6×10^{-6}
3.3	0.035	1.1×10^{-6}	5.5	0.035	6.5×10^{-6}
3.3	0.050	0.6×10^{-7}	5.5	0.050	5.0×10^{-6}
6.2	0.005	6.5×10^{-2}	9.1	0.005	2.9×10^{-5}
6.2	0.015	5.9×10^{-2}	9.1	0.015	2.5×10^{-5}
6.2	0.025	5.4×10^{-2}	9.1	0.025	2.7×10^{-5}
6.2	0.035	5.9×10^{-2}	9.1	0.035	2.8×10^{-5}
6.2	0.050	5.9×10^{-2}	9.1	0.050	2.9×10^{-5}

Standard deviation in k_{obs}. The standard deviation (S.D.) in k_{obs} was determined at pH 0.5 (perchloric acid, $\mu = 0.3$) and at pH 7.0 at a buffer concentration of 0.035 M ($\mu = 0.1$). The mean $k_{obs} \pm$ S.D. are $8.2 \pm 0.6 \times 10^{-2}$ s^{-1} ($n = 8$) and $8.3 \pm 0.7 \times 10^{-2}$ s^{-1} ($n = 7$), respectively. Other rate constants are determined in duplicate.

Influence of buffers. The influence on the degradation rate of different concentrations of buffer ions was investigated. During the degradation course the pH did not change. In the range of 0.005–0.050 M buffer no effect was found. Representative data are listed in Table 1. Since DVBL was a major degradation product, we also investigated the influence of different concentrations of acetate buffer. The k_{obs} did not differ significantly from those obtained with phosphate buffers at the same pH.

Influence of ionic strength. The influence of the ionic strength was investigated for each pH by adding different amounts of sodium chloride to the solutions, while buffer concentration and tem-

TABLE 2

The effect of the ionic strength on k_{obs} for the degradation of VBL at different pH values

Temperature 80 °C; buffer concentration 0.035 M.

pH	μ	k_{obs} (s^{-1})	pH	μ	k_{obs} (s^{-1})
3.3	0.1	1.7×10^{-6}	4.3	0.1	1.0×10^{-2}
3.3	0.2	1.2×10^{-6}	4.3	0.2	1.2×10^{-2}
3.3	0.3	1.6×10^{-6}	4.3	0.3	1.2×10^{-2}
3.3	0.4	1.9×10^{-6}	4.3	0.4	1.5×10^{-2}
5.5	0.1	4.5×10^{-6}	9.1	0.1	2.5×10^{-5}
5.5	0.2	6.5×10^{-6}	9.1	0.2	2.8×10^{-5}
5.5	0.3	5.6×10^{-6}	9.1	0.3	3.9×10^{-5}
5.5	0.4	5.4×10^{-6}	9.1	0.4	2.1×10^{-5}

perature were kept constant. Within the range investigated ($\mu = 0.1$–0.4) the ionic strength had no influence on the degradation rate (Table 2).

Influence of temperature. The effect of the temperature on the degradation rate was determined at pH 0.9, 3.0, 5.1, 6.7 and 8.7 (buffer concentration 0.035 M; $\mu = 0.3$) over the range 60–80 °C. The Arrhenius relationship was obeyed. From the plots of the $\ln(k_{obs})$ and the reciprocal of the absolute temperature the activation energy (E_a) and frequency factor (A) have been calculated (Table 3).

Influence of the pH. The kinetic studies were performed in buffered solutions to maintain the pH at a constant value during the experiment. The k_{obs} was found to be independent of the buffer concentration. Also the ionic strength had no influence on the rate constant so corrections were not necessary for this parameter. The k_{obs} values calculated for the pH–rate profile are the mean of all experiments ($n = 4$ or $n = 8$) at a specific pH.

TABLE 3

Activation energy (E_a) and frequency factor (A) for the degradation of VBL at various pH values (80 °C)

pH	E_a (kJ·mol^{-1})	A (s^{-1})
0.9	66	6×10^{4}
3.0	60	1×10^{3}
5.1	71	1×10^{5}
6.9	115	3×10^{12}
8.7	106	2×10^{11}

Regression analysis of the pH–rate profile of vinblastine

The shape of the measured pH–rate profile indicates that VBL has pK_a values of about −1, 4 and 7. Vinblastine possesses 4 possible prototropic N-functions ($N_{6'}$, $N_{16'}$, N_1 and N_9; Fig. 1). The "indole nitrogen" ($N_{16'}$) from the catharanthine part of the molecule is probably responsible for the first pK_a value. The pK_a of a real indole nitrogen is −3.6 (25 °C) (Perrin, 1972a). The pK_a of 4 can be ascribed to the "3-methoxyaniline" nitrogen (N_1). This value is in good agreement with the theoretical pK_a value of such a nitrogen (4.2, 20 °C; 3.8, 50 °C) (Perrin, 1972b). The two other nitrogen atoms ($N_{6'}$, and N_9) have approximately the same pK_a value. They are probably responsible for the inflection point at pH 7. Because the shape of the pH–rate profile indicates at least 3 pK_a values and the chemical structure of VBL points to 4 pK_a values, regression analysis was performed to both a 3 and 4 pK_a model.

The mathematical equation for k_{obs} for the 3 pK_a model is as given below.

$$k_{obs} = \left(M_1[H^+] + M_2 + \frac{M_3}{[H^+]} + \frac{M_4}{[H^+]^2} + \frac{M_5}{[H^+]^3} + \frac{M_6}{[H^+]^4} \right) \left(1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2} + \frac{K_1K_2K_3}{[H^+]^3} \right)^{-1} \quad (2)$$

where

$$M_1 = k_{0,H};$$

$$M_2 = k_{1,H}K_1 + k_{0,S};$$

$$M_3 = k_{2,H}K_1K_2 + k_{1,S}K_1 + k_{0,OH}K_w;$$

$$M_4 = K_1\left(k^1_{3,H}K_2K_3 + k_{2,S}K_2 + k_{1,OH}K_w\right);$$

$$M_5 = K_1K_2\left(k_{3,S}K_3 + k_{2,OH}K_w\right);$$

$$M_6 = k_{3,OH}K_1K_2K_3K_w.$$

The equation for the 4 pK_a model is given by:

$$k_{obs} = \left(M_1[H^+] + M_2 + \frac{M_3}{[H^+]} + \frac{M_4}{[H^+]^2} + \frac{M_5}{[H^+]^3} + \frac{M_6}{[H^+]^4} + \frac{M_7}{[H^+]^5} \right) \left(1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2} + \frac{K_1K_2K_3}{[H^+]^3} + \frac{K_1K_2K_3K_4}{[H^+]^4} \right)^{-1} \quad (3)$$

where

$M_1 = k_{0,H}$;

$M_2 = k_{1,H}K_1 + k_{0,S}$;

$M_3 = k_{2,H}K_1K_2 + k_{1,S}K_1 + k_{0,OH}K_w$;

$M_4 = K_1\left(k^I_{3,H}K_2K_I + k^{II}_{3,H}K_2K_{II} + k_{2,S}K_2 + k_{1,OH}K_w\right)$;

$M_5 = K_1K_2\left(k_{4,H}K_3K_4 + k^I_{3,S}K_I + k^{II}_{3,S}K_{II} + k_{2,OH}K_w\right)$;

$M_6 = K_1K_2\left(k_{4,S}K_3K_4 + k^I_{3,OH}K_1K_w + k^{II}_{3,OH}K_{II}K_w\right)$;

$M_7 = k_{4,OH}K_1K_2K_3K_4K_w$.

M depicts the macro-reaction constant of the different species; K represents the dissociation constant; $k_{i,H}$, $k_{i,OH}$ and $k_{i,S}$ represent the reaction constant of the H^+, OH^- and solvent (water)-catalyzed degradation reactions of the i^{th} species. The index i indicates the number of protons dissociated starting to calculate from the fully protonated form ($VBLH_4^{4+}$, $i = 0$). The third and fourth protolytic equilibrium overlap. Therefore two species VBL^+ have been included in the 4 pK_a model. The superscripts I and II refer to these two different forms.

A detailed derivation of the above equations and the meaning of the symbols used, will be described by Van Der Houwen et al. (1988).

The measurements at pH values lower than −1.5 are not involved in the calculation. The constants $M_1 - M_6/M_7$ and the pK_a values were calculated with a non-linear least-squares curve-fitting program, using an Olivetti microcomputer. In Table 4 the results of the optimal fits are listed. The precision of the calculated values is limited. This is partially due to the reproducibility in the kinetic measurements themselves and partially because the inflection points corresponding to the pK_a values are not pronounced. This is particularly a problem with the two highest values of the 4 pK_a model.

In Figs. 4 and 5 the experimental values of k_{obs} are indicated by squares. The solid lines are the model values calculated using Eqns. 2 and 3, respectively, and the constants listed in Table 4. The contribution of the separate macro-reaction constants to k_{obs} is indicated with dotted lines.

Both models show a high correlation with the experimental data. The sum of the squared relative deviations is almost equal for both fits (1.95 and 1.94, respectively). This indicates that fitting

TABLE 4

Reaction constants for the degradation of VBL, ionization constants and pK values at a temperature of 80 °C, calculated according to a 3 and a 4 pK_a model

	3 pK_a model	4 pK_a model
M_1	– [a]	– [a]
M_2	3.9×10^{-4} s^{-1}	3.9×10^{-4} s^{-1}
M_3	6.5×10^{-6} M·s^{-1}	6.4×10^{-6} M·s^{-1}
M_4	4.3×10^{-9} M^2·s^{-1}	4.5×10^{-9} M^2·s^{-1}
M_5	7.4×10^{-15} M^3·s^{-1}	1.3×10^{-14} M^3·s^{-1}
M_6	7.6×10^{-24} M^4·s^{-1}	2.2×10^{-21} M^4·s^{-1}
M_7		2.1×10^{-30} M^5·s^{-1}
K_1	7.1	7.0
K_2	1.6×10^{-4}	1.8×10^{-4}
K_3	2.7×10^{-7}	7.4×10^{-7}
K_4		9.1×10^{-8}
pK_1	−0.8	−0.8
pK_2	3.8	3.8
pK_3	6.6	6.1
pK_4		7.0

[a] Contribution not significant.

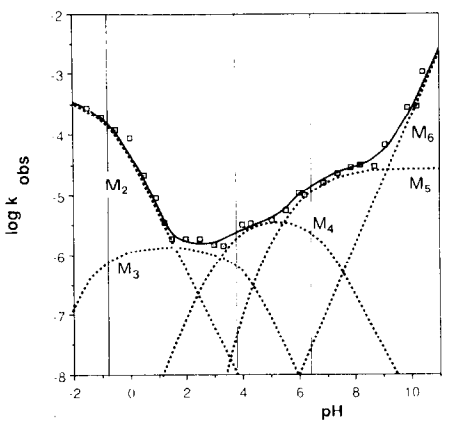

Fig. 4. Log k_{obs}–pH rate profile for the degradation of VBL at 80 °C, fitted according to a 3 pK_a model.

of the pH–rate profile of VBL can be carried out equally well using a 3 pK_a and a 4 pK_a model. The value of pK_3 in the 3 pK_a model is the mean of the values of pK_3 and pK_4 in the 4 pK_a model. According to the molecular structure of VBL the 4 pK_a model is to be preferred.

The slope in the pH region −0.5 to 1.5 is −1. Between pH 2.5 and 3.3 the degradation rate is independent of the pH. At pH values above 4 no slope of +1 is visible. The shape of the pH–rate profile in this region suggests the overall degradation is strongly influenced by dissociation equilibria.

The macro-reaction constants M_2–M_7 can be linked to the formation of different products

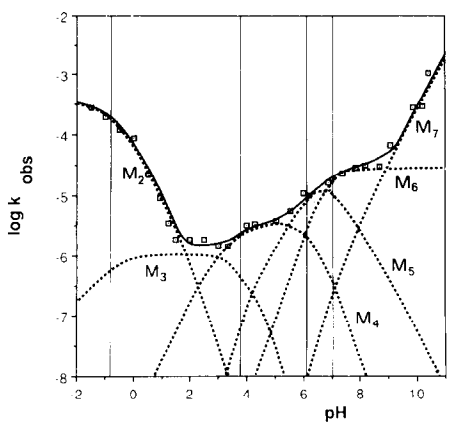

Fig. 5. Log k_{obs}–pH rate profile for the degradation of VBL at 80 °C, fitted according to a 4 pK_a model.

shown in Fig. 2. M_2 (Fig. 5) belongs to the formation of DVBL by probably hydrogen-catalyzed hydrolysis. M_3, M_4, and M_5 go together with the formation of the 3 peaks (Fig. 2B, peak 3). M_6 belongs to the formation of an array of peaks (Fig. 2C). M_7 belongs to the probably hydroxyl-catalyzed hydrolysis of VBL into DVBL.

Conclusion

Vinblastine is most stable in aqueous solution between pH 2.0 and 4.0. A solution of VBL at pH 3.0 still contains 90% VBL after 39 days at room temperature (20 °C). The concentration of phosphate buffer in the range 0.005–0.050 M has no influence on the degradation rate. Also sodium chloride added to an ionic strength of 0.4 does not accelerate the degradation process.

The degradation kinetics of two other vinca alkaloids, vincristine and vindesine, are under current investigation.

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