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Degradation of lomustine (CCNU) in aqueous solutions

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Summary

The stability of lomustine in buffered aqueous solutions was investigated over the pH range 2.6–9.0. The degradation rate was unaffected by pH up to a pH of about 5 but increased at higher pH. The suggested unimolecular degradation mechanism in the pH-independent region of the pH-rate profile is supported by the activation parameters, the solvent isotope effect and other data obtained during this investigation. The effect of six cyclodextrin (CD) derivatives on the stability of lomustine in aqueous solutions was investigated. All the CDs increased the stability, but two, i.e., 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and 2-hydroxyethyl-β-cyclodextrin (HE-β-CD), resulted in a better stabilization than the other CDs tested. Lomustine degraded about 2 to 2.5 times slower within the HP-β-CD-complex than outside it in the solution.

Introduction

Lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea or CCNU) is an alkylating antineoplastic agent. It is sufficient stable to be administered orally and, due to its high lipid solubility, readily penetrates the blood-brain barrier. Lomustine is mainly used in the treatment of brain tumors and leukemia (Remers, 1982).

The stability of the nitrosoureas in aqueous solutions is limited and their degradation is complex, depending both on the conditions employed (e.g., pH) and the chemical structure of the nitrosourea studied (Garrett, 1960; Garrett et al., 1965; Montgomery et al., 1967; Chatterji et al., 1978). Although lomustine is rarely administered via intravenous solution a detailed knowledge of its stability in aqueous solutions is desirable. Knowledge of the effect of temperature and the dielectric constant of the medium on the rate of degradation is also needed when analytical conditions are to be designed or when desirable storage conditions are to be determined.

Cyclodextrins are cyclic oligosaccharides with hydroxyl groups on the outer surface and a void cavity in the center. They are capable of forming inclusion complexes by taking up a whole molecule, or some part of it, into the cavity. This type of encapsulation of a drug molecule will affect many of its physicochemical properties such as chemical stability and aqueous solubility (Loftsson and Bodor, 1989; Loftsson et al., 1989). It is also quite possible that many side effects associated with oral and IV administration of alkylating antineoplastic agents, for example mucosal damage in the gastrointestinal tract and pain at the injection site, can be reduced by this type of molecular encapsulation.

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Materials and Methods

Materials

Lomustine was supplied by the courtesy of H. Lundbeck A/S (Copenhagen, Denmark). The following cyclodextrins (CDs) were used as supplied, without further purification: 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, Pharmatec Inc., U.S.A.), 2-hydroxyethyl-β-cyclodextrin (HE-β-CD, Aldrich Chemical Company, U.S.A.), mixtures of glucosyl/maltosyl-α-, -β- and -γ-cyclodextrins (G/M-CD, Ensuiko, Japan), mixture of maltosyl- and dimaltosyl-β-cyclodextrin (3 : 7) (M/DM-β-CD, Ensuiko), γ-cyclodextrin (γ-CD, Sigma Chemical Co., U.S.A.) and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD, Pharmatec). All other chemicals were commercially available products of special reagent grade.

Chromatographic conditions

The quantitative determinations of lomustine was performed on high-performance liquid chromatographic (HPLC) equipment consisting of a Milton Roy ConstaMetric 3000 solvent delivery system, a Rheodyne 7125 injector, a Waters μBondapak C18 (3.9 mm × 30 cm) column and a Spectra-Physic SP8450 UV/vis detector operated at 226 nm. The mobile phase used for the quantitative determination of lomustine consisted of methanol/water (7 : 3) and the retention time was 4.0 min at 1.50 ml/min flow rate.

Buffers

Unless otherwise indicated, pure, that is formed by mixing aqueous solutions of the acid with aqueous solutions of its corresponding salt, formate (pH 2–3), acetate (pH 3–6), citrate (pH 6–7.3) phosphate (pH 6–8) and carbonate (pH 7.7–9) buffers were used. No attempts were made to maintain a constant ionic strength because it has been shown that ionic strength does not appreciably affect the rate of hydrolysis (Chatterji et al., 1978). CD was added to the buffer solutions when the effects of the various CDs had been investigated. The water used for the buffer preparation was distilled in all-glass apparatus. Water was replaced by deuterium oxide (D_2O, purity 99.8%, from Merck, F.R.G.) when the solvent isotope effect was determined. The effect of the solvent dielectric constant on the reaction rate was investigated at 50.0 °C in aqueous buffer solutions containing up to 30% (v/v) dioxane.

Kinetic studies

The degradation studies of lomustine were carried out by adding stock solution (20 μl) of the drug in methanol to aqueous buffer solution (2 ml), previously equilibrated at the desired temperature in a water bath, and mixed thoroughly. The initial lomustine concentration was 8.6×10^{-5} M. All reactions were run under pseudo-first-order conditions. Aliquots (20 μl) were injected into the column at various time intervals, and the pseudo-first-order rate constants (k_{obs}) determined from the disappearance of the drug by linear regression of the natural logarithm of the peak height versus time plots. The correlation coefficient was calculated for each run.

No attempts were made to exclude light during the experiments, since preliminary investigation showed that the rate of degradation of lomustine in aqueous buffer solutions was unaffected by normal laboratory light (from 0 up to about 700 Lux).

The enthalpy of activation ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$) were determined from linear plots of $\ln(k/T)$, where k is k_{obs}, vs. $1/T$ based on the Eyring equation:

$$\ln(k/T) = \ln(k_B/h) + \Delta S^{\ddagger}/R - (\Delta H^{\ddagger}/R)1/T \quad (1)$$

where T is the absolute temperature, k_B is the Bolzmann constant, h is Planck's constant and R is the gas constant.

The stability constant (K_c) for the lomustine-CD complexes and the pseudo-first-order rate constants (k_c) for degradation of lomustine within the complexes were calculated from Lineweaver-Burk plots, assuming formation of a 1 : 1 complex (Lineweaver and Burk, 1934; Loftsson et al., 1989):

$$\frac{k_0}{k_0 - k_{obs}} = \frac{k_0}{K_c(k_0 - k_c)[CD]} + \frac{k_0}{(k_0 - k_c)} \quad (2)$$

Where k_0 represents the pseudo-first-order rate constant for the degradation of the free drug. The values of k_c and K_c for a given lomustine-CD-inclusion complex were calculated from the intercept and the slope of a linear plot obtained when $k_0/(k_0 - k_{obs})$ was plotted against the reciprocal total CD concentration (1/[CD]).

Results and Discussion

Degradation in aqueous solution

The degradation of lomustine followed first-order kinetics in aqueous buffer solutions at constant pH and temperature. The pseudo-first-order rate constants (k_{obs}) were determined by HPLC, following the disappearance of the drug as a function of time, at pH 2.66, 3.65, 4.60, 5.63, 6.02, 6.87, 7.22, 7.70, 8.21, 8.51 and 9.00 at four different buffer concentration and 55.0 °C. The k_{obs} values were plotted against the buffer concentration, and the rate constants at zero buffer concentration (k'_{obs}) were obtained by linear regression. The buffers used had little or no effect on the rate of hydrolysis of lomustine.

The pH-rate profile of lomustine shows a large plateau up to pH about 5 (Fig. 1). According to Fife (1965), such a plateau of pH-independent rate could be due to: (a) attack of both hydronium ions and hydroxide ions; (b) water attack on the substrate; or (c) spontaneous, uncatalyzed decomposition of the substrate. The first possibility seems to be highly unlikely, since if it occurred, the reaction would have a very large negative entropy of activation. Thus, the other two possibilities are left.

The relatively high enthalpies ($\Delta H^{\ddagger}$) and small negative entropies ($\Delta S^{\ddagger}$) of activation were obtained in the pH-independent plateau region (Table 1). The high enthalpies and low entropies of activation are typical for unimolecular reactions. The enhanced solvation due to the charge separation in the transition state results in a small negative entropy of activation. Bimolecular reaction involving water attack on lomustine would result much more unfavourable changes in the entropy.

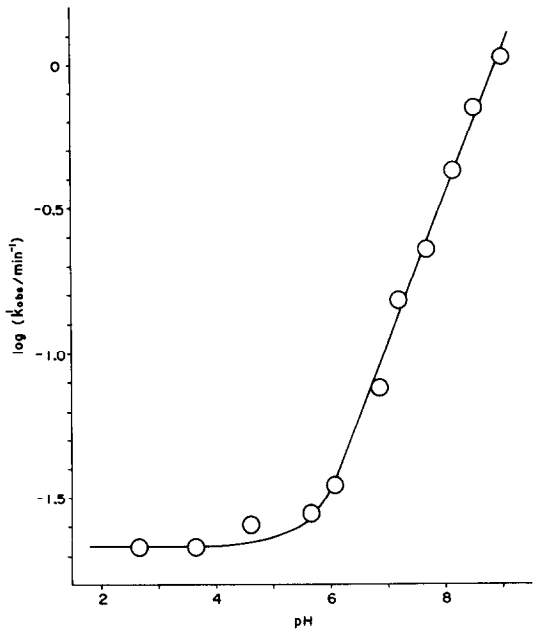


Fig. 1. The pH-rate profile for the observed first-order degradation of lomustine in aqueous buffer solutions at 55.0 °C.

The deuterium solvent isotope effects on the hydrolysis of lomustine in buffer solutions were measured at 55.0 °C. The hydrolysis rate constant (k_D) in buffered deuterium oxide solution at pH 3.69 was 1.80×10^{-2} min^{-1} compared to (k_H) 2.07×10^{-2} min^{-1} in water, which results in an isotope effect (k_H/k_D) of 1.15. At pH 6.90 the K_D was determined to be 5.80×10^{-2} min^{-1} and the k_H to be 6.87×10^{-2} min^{-1}, which also results in $k_H/k_D = 1.18$. This small deuterium solvent isotope effect can also be associated with an unimolecular reaction mechanism.

The effect of solvent dielectric constant (ϵ) on the hydrolysis of lomustine in buffer solutions was

TABLE 1

Enthalpies and entropies of activation for the hydrolysis of lomustine aqueous buffer solution

pH	$\Delta H^{\ddagger}$ (kJ/mol)	$\Delta S^{\ddagger}$ (J/mol per K)
2.66	97.0	−26.1
3.65	93.5	−8.1
5.63	98.7	−8.1
6.86	130	93.5

TABLE 2

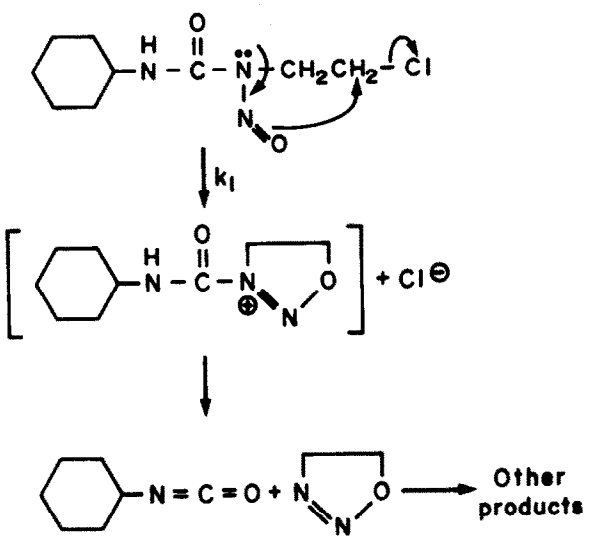
Observed pseudo-first-order rate constants (k_{obs}) for the degradation of lomustine in various aqueous buffer-dioxane mixtures at pH 2.52 and 50.0 °C

Dioxane % (v/v)	ε [a]	$k_{obs} \times 10^2$ (min^{-1})
0	69.60	1.42
10	61.43	1.05
20	53.30	0.67
30	45.27	0.39

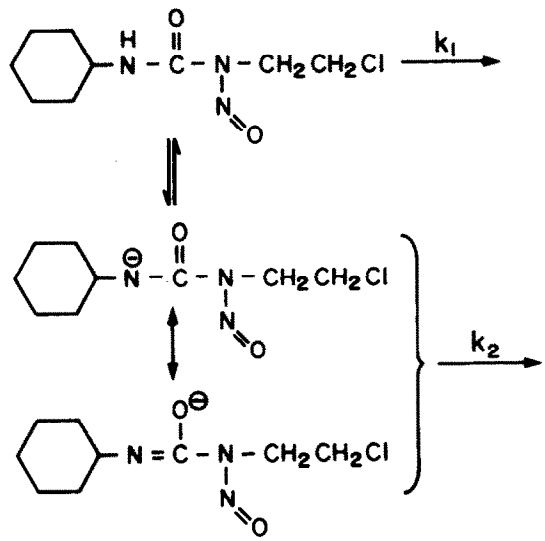
[a] The dielectric constant (ε) of each mixture was calculated at 50 °C according to Owen and Harris (1958).

studied in water/dioxane mixtures at 55.0 °C. The dielectric constant of each water/dioxane mixture was calculated at 50.0 °C (Table 2). The decrease in the solvent dielectric constant resulted in a decrease in the rate constant, which can be associated with unimolecular decomposition of lomustine under formation of highly polar transition state.

All these results agree well with the mechanism suggested by Chatterji et al. (1978) for spontaneous, uncatalyzed decomposition of (2-haloethyl)nitrosoureas in aqueous buffer solutions (see Scheme 1). This reaction can be regarded as a S_N1 reaction, where the nucleophile is the oxygen



Scheme 1. Spontaneous, uncatalyzed decomposition of lomustine in the platcau region of the pH-rate profile.



Scheme 2. Ionisation of lomustine in aqueous solutions.

on the nitroso-moiety of the molecule and the leaving group is the chloride ion.

The sharp increase in the rate of degradation of pH about 6 is probably due to ionisation of the drug (Scheme 2). The pK_a of the N-3 proton of nitrosoureas has been estimated to be between 8 and 9 (Garrett et al., 1965; Chatterji et al., 1978). The ionised form of the (2-haloethyl)nitrosoureas is very unstable and undergoes rapid decomposition in aqueous solutions, through at least two different routes, under formation of several different products (Chatterji et al., 1978).

Effect of CDs on rate of degradation

The effects of several CDs on the stability of lomustine in aqueous buffer solutions was investigated at 55.0 °C (Table 3). The CD concentration was kept at 2.5% (w/v) and the initial lomustine concentration was in all cases 8.6×10^{-5} M. Comparison of the rate constants in Table 2 with the rate constants obtained under the same conditions but without the CDs (k_0 in Table 4) shows that all the CDs have stabilizing effect on lomustine. Generally, addition of the β-CDs to the reaction medium results in a better stabilization of the drug than when the γ-CDs were added to the medium. Two of the β-CDs, i.e., HP-β-CD and HE-β-CD, possess better stabilizing abilities than

TABLE 3

The first-order rate constants for the degradation of lomustine in aqueous buffer solutions containing lomustine or 2.5%(w/v) 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), 2-hydroxyethyl-β-cyclodextrin (HE-β-CD), mixtures of glucosyl/maltosyl-α-, -β- and -γ-cyclodextrins (G/M-CD), mixture of maltosyl- and dimaltosyl-β-cyclodextrin (3:7) (M/DM-β-CD), γ-cyclodextrin (γ-CD) or 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD) at 55.0°C

pH	$k_{obs} \times 10^2$ (min^{-1})					
	HP-β-CD	HE-β-CD	G/M-CD	M/DM-β-CD	γ-CD	HP-γ-CD
2.59	1.29	1.01	1.75	1.50	2.26	1.93
3.67	1.14	1.12	1.38	1.38	2.07	1.68
5.90	1.64	1.55	2.26	1.98	3.06	2.26
6.99	4.58	4.20	6.19	6.78	8.25	6.51

the other cDs and one of those, HP-β-CD, was selected for further study. The stability constant (K_c) for the lomustine-HP-β-CD-complex and the pseudo-first-order rate constant (k_c) for degradation of lomustine within the complex were determined in various aqueous buffer solutions from Lineweaver-Burk plots assuming formation of 1 : 1 complex (Loftsson et al., 1989). The pseudo-first-order rate constant for the degradation of the free drug (k_0) was determined under the same conditions, i.e., in the same buffer solutions without CD (Table 4). Lomustine was degraded about 2 to 2.5 times slower within the lomustine-HP-β-CD-complex than outside it in the solution (the k_0/k_c ratio is 2 to 2.5). This is much lower than what has been determined for both melphalan ($k_0/k_c \approx 30$) and chlorambucil ($k_0/k_c \approx 20$) in neutral aqueous buffer solution at 60 and 50°C, respectively (Loftsson et al., 1989).

TABLE 4

The first-order rate constants and the stability constants for the degradation of lomustine in aqueous HP-β-CD buffer solutions at 55.0°C

pH	$k_0 \times 10^2$ (min^{-1})	$k_c \times 10^2$ (min^{-1})	K_c (M^{-1})
2.59	2.33	0.97	514.4
3.67	2.07	0.95	465.3
5.90	3.04	1.27	771.4
6.99	6.87	3.35	36.1

Acknowledgement

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