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Prodrugs as drug delivery systems. XXVIII. Structural factors influencing the rate of hydrolysis of oxazolidines—a potential prodrug type *

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Summary

The hydrolysis kinetics of several oxazolidines derived from cyclohexanone and various β-aminoalcohols were studied to further explore their suitability as prodrug forms for β-aminoalcohols and for carbonyl-containing compounds. The oxazolidines were found to undergo a facile and complete hydrolysis in the pH range 2–9 at 37°C. The rates of decomposition showed a complex dependence on pH, but in most cases maximum rates occurred at neutral and basic pH. The reaction rates at these pH values were shown to decrease with increasing steric effects within the β-aminoalcohol moiety, in particular at the α-position to the nitrogen atom, and to increase with increasing electronegativity of the substituents at the β-position to the nitrogen atom. Structure–reactivity relationships on these factors are given. Along with previously derived relationships between reactivity and structural effects within the carbonyl component such relationships may be useful for the prediction of the reactivity of an oxazolidine to be designed as a prodrug derivative of a drug molecule containing a carbonyl group or a β-aminoalcohol moiety.

Introduction

In previous studies (Bundgaard and Johansen, 1982; Johansen and Bundgaard, 1983), oxazolidines were suggested to be potentially useful prodrug forms for the

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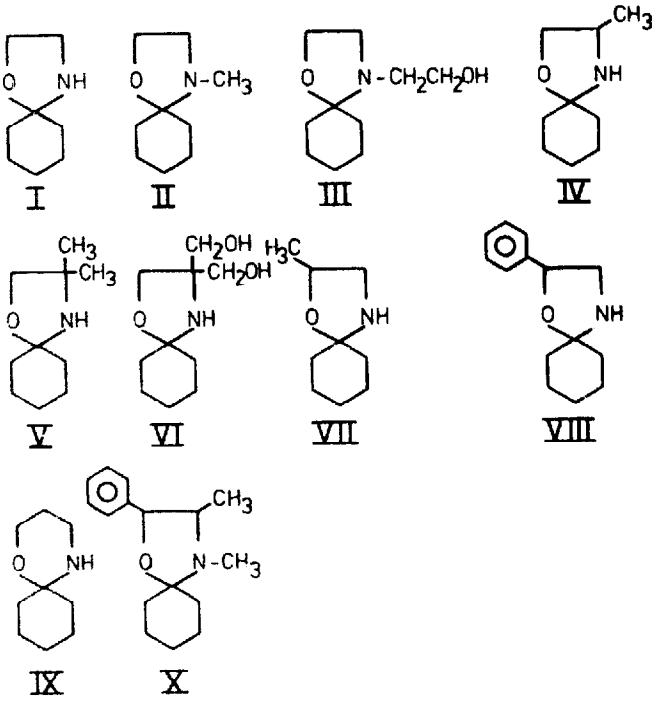


β-aminoalcohol moiety and/or carbonyl groups (aldehydes and ketones). Several drugs contain a β-aminoalcohol function, e.g. various sympathomimetic amines and β-blockers, or a carbonyl group, e.g. various steroids, and delivery problems associated with these drugs, e.g. due to unfavourable solubility or lipophilicity characteristics, may probably be overcome by using the prodrug approach. Oxazolidines are much weaker bases (pK_a 6–7) than the parent β-aminoalcohol and this results in higher lipophilicity at physiological pH (Johansen and Bundgaard, 1983). Such increased lipophilicity may become advantageous in situations where delivery problems for β-aminoalcohol-type drugs are due to low lipophilicity, e.g. in case of dermal absorption. In considering oxazolidines as prodrug candidates for carbonyl-containing substances their weak basic character may also be advantageous in that the transformation of such substances into oxazolidines introduces a readily ionizable moiety, which in turn changes the solubility characteristics of the parent compound.

In the previous studies cited the hydrolysis kinetics of several oxazolidines derived from (−)-ephedrine and various aldehydes and ketones (e.g. with cyclohexanone (X)) were determined. The oxazolidines were shown to undergo a facile and complete hydrolysis in the pH range 1–11 at 37°C. The reaction rates at physiological pH were shown to decrease with increasing steric effects of the substituents derived from the carbonyl component and to decrease with the increasing basicity of the oxazolidines.

To further explore the potential of oxazolidines as a prodrug type information is needed on the structural factors within the β-aminoalcohol moiety which may influence the stability and reactivity of oxazolidines. This would especially be helpful for assessing the potential of oxazolidines as prodrug forms for carbonyl-containing

compounds in which case the β-aminoalcohol component would act as a transport group. The purpose of the present work is to provide such information and to this end, the kinetics of hydrolysis of a series of oxazolidines (I–VIII) derived from cyclohexanone and various β-aminoalcohols have been studied. A corresponding derivative (IX) derived from cyclohexanone and a γ-aminoalcohol has also been included.

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer and a Zeiss PMQ II spectrophotometer equipped with a thermostated cell compartment. 1-cm quartz cells were used. Readings of pH were carried out on a Radiometer Type PHM 26 meter. Nuclear magnetic resonance (NMR) data were obtained using a Varian Type EM-360 L NMR spectrometer. Melting points were taken on a capillary melting-point apparatus and are uncorrected.

Chemicals

The β-aminoalcohols and cyclohexanone were purchased from AG Fluka, Switzerland, E. Merck, G.F.R. or Sigma, U.S.A. All other chemicals used such as buffer substances and solvents were of reagent grade.

Synthesis

Most of the oxazolidines were prepared by reacting the β-aminoalcohol with cyclohexanone according to previously described procedures: 2-pentamethyleno-oxazolidine (I), b.p. 99–101°C (32 mm Hg), yield 73%, rep. b.p. 89–90°C (16 mm Hg) (Cope and Hancock, 1942); 2-pentamethyleno-3-methyloxazolidine (II), b.p. 98–101°C (28 mm Hg), yield 75%, rep. b.p. 95–97.5°C (23 mm Hg) (Bergmann et al., 1952); 3-(β-hydroxyethyl)-2-pentamethyleno-oxazolidine (III), b.p. 160–163°C (20 mm Hg), yield 68%, rep. b.p. 165–167°C (24 mm Hg) (Bergmann et al., 1952); 2-pentamethyleno-4,4-dimethyloxazolidine (V), b.p. 100–101°C (30 mm Hg), yield 25%, rep. b.p. 95–97.5°C (20 mm Hg) (Hancock and Cope, 1944); and 4,4 bis(hydroxymethyl)-2-pentamethyleno-oxazolidine (VI), m.p. 118.5–119°C, yield 50%, rep. m.p. 118–120°C (Pierce et al., 1951). 1-Oxa-5-azaspiro(5,5)-undecane (IX), m.p. 43–46°C, yield 45%, was prepared by the method described by Bergmann and Kaluszyner (1959), (rep. m.p. 44–46°C). 2-Pentamethyleno-4-methyl-oxazolidine (IV), 2-pentamethyleno-5-methyl-oxazolidine (VII) and 2-pentamethyleno-5-phenyl-oxazolidine (VIII) were prepared analogously to the above-mentioned compounds. In a typical experiment equimolar amounts of the β-aminoalcohol and cyclohexanone were dissolved in toluene and the solution was refluxed. Water was continuously removed from the reaction by means of a Dean-Stark trap. After collection of the theoretical amount of water, the toluene was removed in vacuo and the liquid residue was purified by distillation. The structure of the compounds was confirmed by NMR spectroscopy.

Kinetic studies

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were hydrochloric acid (pH ⩽ 2), phosphate (pH 2–3 and 6–7.5), acetate (pH 4–5.5) and borate (pH 8.5–10) and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The progress of the reactions was followed either by direct UV-spectrophotometry or by trapping of the cyclohexanone released. When using the direct UV-spectrophotometric method the decomposition of the oxazolidines was monitored by recording the decrease in absorbance at 215–220 nm. At these wavelengths absorption of the substrate and products differed maximally. The reactions were initiated by adding 25 μl of a stock solution of the oxazolidine in acetonitrile to 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cell, giving a final concentration of about 10^{-3} M.

Pseudo-first-order rate constants were determined from the slopes of linear plots of $\log(A_t - A_\infty)$ vs time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively. Rate constants for the slower reactions were determined using the method of Guggenheim.

The rates of hydrolysis of the oxazolidines V and VI were measured by trapping the cyclohexanone formed with thiosemicarbazide (pH < 4) or semicarbazide (pH 4–7) and following the increase in absorbance of the corresponding carbazone derivative at 274 and 235 nm, respectively, as described previously (Bundgaard and Johansen, 1980; Johansen and Bundgaard, 1983). The carbonyl trapping reagent was included in the buffer solutions at a concentration of 5×10^{-3} M. It was controlled that trapping of the cyclohexanone was fast relative to its formation and that the trapping agent had no effect on the reaction rate in the concentration used. The initial oxazolidine concentration was about 2×10^{-4} M and the reactions were performed either directly in a thermostated cuvette or in flasks kept in a water bath. Pseudo-first-order rate constants were determined from plots of $\log(A_\infty - A_t)$ against time.

Results and Discussion

Kinetics of hydrolysis

The kinetics of hydrolysis of the oxazolidines I–VIII and the tetrahydro-1,3-oxazine IX were studied in aqueous buffer solutions at 37°C over the pH range 2–7.5. Except for an initial short period (see below) the hydrolysis displayed strict pseudo-first-order kinetics as determined by following the reaction progress by direct UV-spectrophotometry or by measuring the cyclohexanone formed with a trapping agent as described above. At the experimental conditions studied the hydrolysis of all the compounds went to completion as evidenced by measurement of cyclohexanone in trapped form. The hydrolysis of the compounds was found to be subject to significant buffer catalysis. The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis showed in all cases a linear dependence on buffer con-

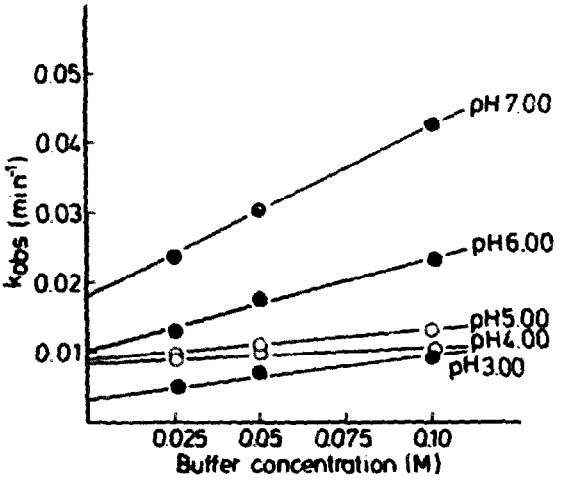


Fig. 1. Effect of phosphate (●) and acetate (○) buffer concentration on the observed pseudo-first-order rate constants for the degradation of the oxazolidine V (37°C; $\mu = 0.5$).

centration as illustrated in Fig. 1 for the decomposition of oxazolidine V in acetate and phosphate buffers.

The influence of pH on the hydrolysis rate is shown in Figs. 2 and 3, where the logarithms of the k_{obs} values at zero buffer concentration (k_0, obtained by extrapolation of plots such as those in Fig. 1 to zero buffer concentration) are plotted against pH.

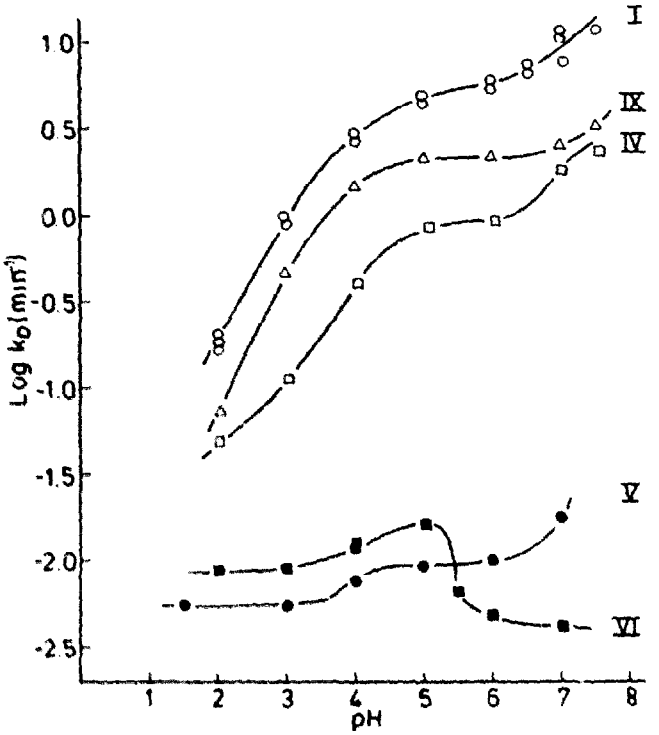


Fig. 2. pH–rate profiles for the hydrolysis of various oxazolidines at 37°C ($\mu = 0.5$).

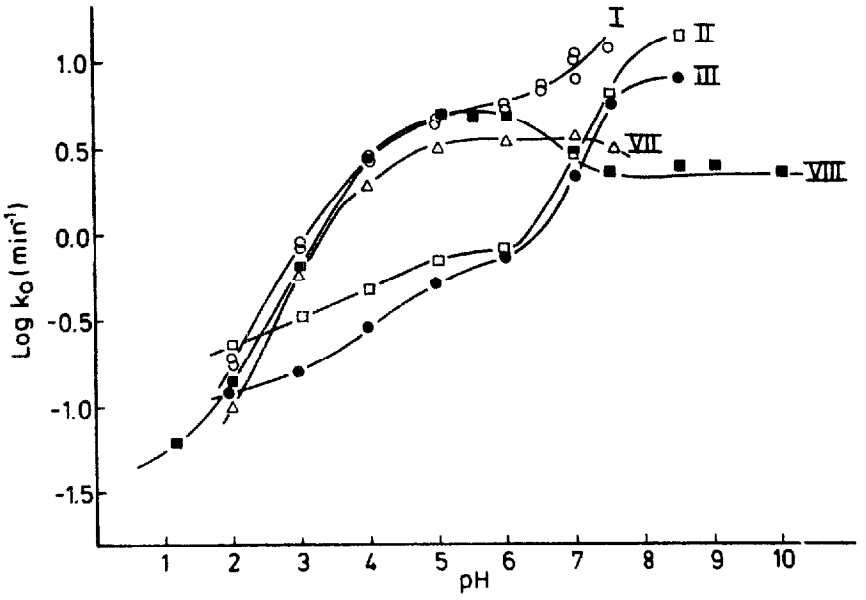
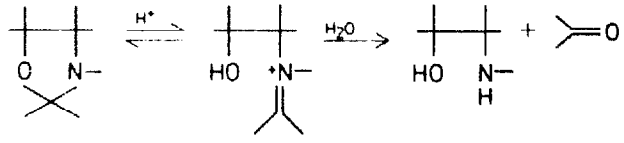


Fig. 3. pH–rate profiles for the hydrolysis of various oxazolidines at 37°C (μ = 0.5). ○, I; □, II; ●, III; △, VII; ■, VIII.

These pH–rate profiles are not easily interpretable but most likely, the unusual variation of hydrolysis rate with pH indicates the involvement of a kinetically significant intermediate in the reaction pathway and a change of the rate-determining step in the overall hydrolysis of the compounds with pH. Previous studies (Fife and Hagopian, 1968; Fife and Hutchins, 1980; McClelland and Somani, 1981) on oxazolidine hydrolysis have shown that the hydrolysis occurs in two separate reaction stages: reversible ring opening to a cationic Schiff base followed by hydrolysis of this intermediate to give the β-aminoalcohol and the carbonyl component (Scheme 1). In these studies involving oxazolidines derived from aromatic aldehydes or ketones the ring-opening reaction proceeding with C–O bond breaking was found to be subject to hydrogenium ion catalysis as well as to involve a unimolecular C–O bond breaking or a water-catalyzed reaction. The cationic Schiff base intermediate formed was further shown to be in equilibrium with the oxazolidine and to undergo both spontaneous and hydroxide ion-catalyzed hydrolysis to yield the parent aldehyde or ketone and β-aminoalcohol.

A similar mechanism may be involved in the hydrolysis of the oxazolidines investigated in this study. Using UV-spectrophotometry it was possible to observe at certain pH values an initial rapid formation of an absorption band with λ_{max} at



Scheme 1

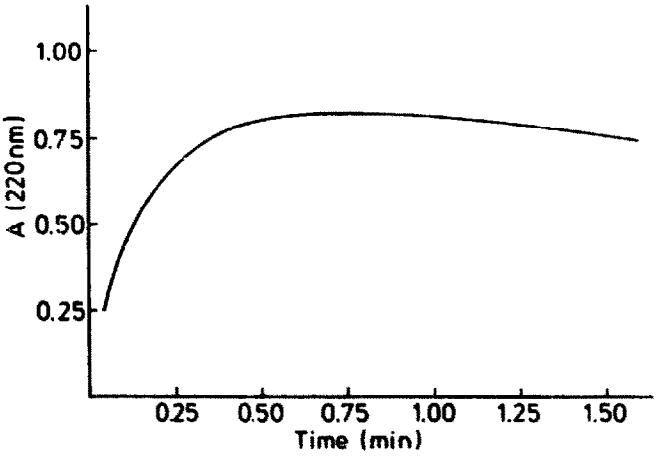


Fig. 4. Plot showing the initial rapid formation and subsequent slower disappearance of an intermediate with λ_{max} at about 220 nm in the degradation of the oxazolidine II in aqueous solution at pH 3.0 and 20°C.

about 220 nm for all oxazolidines except for the more stable compounds V and VI (Fig. 4). The absorption maxima of Schiff bases derived from cyclohexanone and aliphatic amines are expected to occur at 220–230 nm (Hine and Yeh, 1967) and therefore, the intermediate detected is most likely the corresponding Schiff base. In all cases the apparent formation of the Schiff base intermediate (i.e. the ring-opening reaction) was found to proceed much faster than its subsequent hydrolysis. It should be noted that the observed rate constants for the disappearance of the intermediate do not necessarily represent the true constants for breakdown since the intermediate is in rapid equilibrium with the starting oxazolidine.

Structural effects on reaction rate

The rate data obtained show that the structure of the β-aminoalcohol moiety of the oxazolidines has a pronounced effect on the rate of oxazolidine hydrolysis in

TABLE 1

HALF-LIVES OF OVERALL HYDROLYSIS OF VARIOUS OXAZOLIDINES IN ACIDIC AND NEUTRAL AQUEOUS SOLUTION AT 37°C

Oxazolidine	$t_{1/2}$ (min)	
	pH 2.0	pH 7.0
I	3.8	0.07
II	3.1	0.2
III	5.8	0.3
IV	14.4	0.4
V	12.6	42
VI	77.9	158
VII	6.3	0.2
VIII	5.0	0.2

Fig. 5.

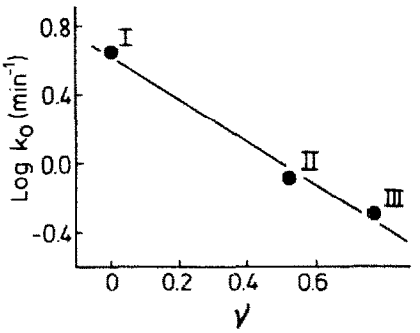


Fig. 6.

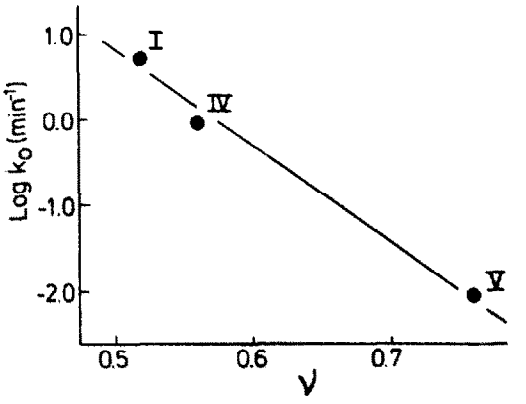


Fig. 5. Plot of log k_0 (at pH 6.0) against the steric substituent parameter (ν) for the substituents on the nitrogen atom of the oxazolidines I, II and III.

Fig. 6. Plot of log k_0 (at pH 6.0) against the steric substituent parameter (ν) for the oxazolidines I, IV and V. The ν values used refer to the moiety of the oxazolidines derived from the α-substituent to the nitrogen including the ring carbon atom in the oxazolidines; e.g. for the compound I the ν value is that for methyl.

both acidic and neutral solutions. In Table 1 are given the half-lives of decomposition of the various compounds at pH 2 and 7 (37°C).

The structural effects influencing the reaction rate appear to involve predominantly steric effects. Thus, the oxazolidine I is almost 500-fold more reactive than the compound V bearing two methyl groups in the α-position to the nitrogen atom. It has previously been noted (Srivastava et al., 1967) that oxazolidines are stabilized by such a gem-dimethyl group α to nitrogen.

The oxazolidines I, II and III differ only in their substituents at the nitrogen atom and the variation of the rates of hydrolysis of these derivatives at pH 6.0 can be accounted for in terms of different steric properties of the amine substituent. As seen in Fig. 5, a linear correlation exists between log k_0 (at pH 6.0) and the steric substituent parameter ν (Charton, 1977). The regression equation between log k_0 and ν for these oxazolidines is given by Eqn. 1:

$$\log k_0 = -1.2\,\nu + 0.63 \quad (r = 0.991;\ n = 3) \tag{1}$$

Similarly, the difference in the rates of hydrolysis of the oxazolidines I, V, VI, which only differ structurally by the substituents in the α-position to the nitrogen, can be attributed to the different steric properties of these substituents. A plot of log k_0 (at pH 6.0) against ν for these compounds is shown in Fig. 6, the regression equation being:

$$\log k_0 = -10.9\,\nu + 6.2 \quad (r = 0.995;\ n = 3) \tag{2}$$

Considering the oxazolidines I, VII and VIII their difference in reactivity can be attributed primarily to an electrical effect. This is seen from Fig. 7 in which log k_0 for these oxazolidines is plotted against the Taft substituent parameter σ^* for the

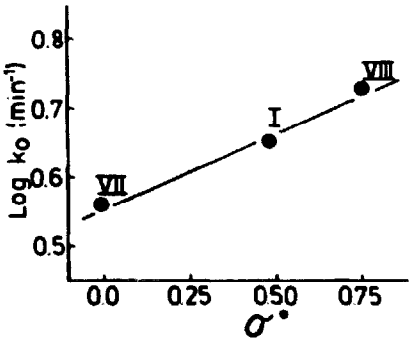


Fig. 7. Plot of log k_0 (at pH 6.0) against the Taft substituent parameter σ^* for the substituents H, CH_3 and C_6H_5 in the oxazolidines I, VII and VIII.

substituents H, CH_3 and C_6H_5 (Perrin et al., 1981). The regression equation for the relationship between log k_0 (at pH 6.0) and σ^* is:

$$\log k_0 = 0.22\,\sigma^* + 0.56 \quad (r = 0.992;\ n = 3) \tag{3}$$

These structure–reactivity relationships imply that the stability of oxazolidines in neutral aqueous solutions is markedly increased with increasing steric effects within the β-aminoalcohol moiety, in particular at the α-position to the nitrogen atom, and is decreased by the introduction of electron-withdrawing groups at the β-position to the nitrogen atom in the β-aminoalcohol moiety. Along with the previous findings on the influence of the carbonyl component on the reactivity of oxazolidines (Johansen and Bundgaard, 1983) these relationships may be useful for the prediction of the reactivity of an oxazolidine to be designed as a prodrug derivative of a drug molecule containing a carbonyl group or a β-aminoalcohol moiety.

To test the predictability of the relationships derived, it may be worthwhile to consider the oxazolidine X derived from ephedrine and cyclohexanone. At pH > 7 and 37°C the pseudo-first-order rate constant for its hydrolysis is 0.13 min^{-1} (Johansen and Bundgaard, 1983). In this pH range the corresponding rate constant for the oxazolidine VIII is 2.2 min^{-1} (cf. Fig. 3). Using Eqns. 1 and 2 it can be calculated that by introducing a methyl group in the 3- and 4-position of VIII (i.e. VIII → X) the reactivity decreases with a factor of 11.5. Thus, the predicted rate constant for the oxazolidine X is $2.2/11.5 = 0.19$ min^{-1} which is close to the observed value of 0.13 min^{-1}.

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