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Pro-drugs as drug delivery systems XX. Oxazolidines as potential pro-drug types for β-aminoalcohols, aldehydes or ketones *

Hans Bundgaard and Marianne Johansen

The Royal Danish School of Pharmacy, Departments of Pharmaceutical Chemistry A D and Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)

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Summary

The kinetics of hydrolysis of oxazolidines derived from (−)-ephedrine or (+)-pseudoephedrine and benzaldehyde or salicylaldehyde was studied at 37°C to assess their suitability as pro-drug forms for β-aminoalcohols and carbonyl-containing compounds. The oxazolidines were found to undergo a facile and complete hydrolysis in the pH range 1–11. The oxazolidines derived from benzaldehyde showed bell-shaped pH–rate profiles with rate maxima occurring at pH 4–5.5. The oxazolidine from salicylaldehyde showed a sigmoidal pH–rate profile and was 100-fold more reactive than the corresponding benzaldehyde derivative at pH > 7. At pH 7.4 and 37°C half-lives of hydrolysis from 5 s to 28 min were observed for the 3 oxazolidines studied. The oxazolidines are weaker bases than the parent β-aminoalcohols and are more lipophilic than these as determined by partition experiments in an octanol–phosphate buffer system. It is suggested that oxazolidines be considered as potentially useful pro-drug candidates for β-aminoalcohols or drugs containing carbonyl groups.

Introduction

One of the major problems for the general application of the pro-drug principle to achieve improvements in the delivery characteristics of drug substances is the limited possibilities available for making bioreversible derivatives of many drugs. Drug

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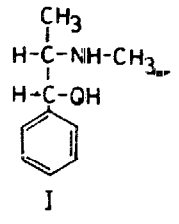
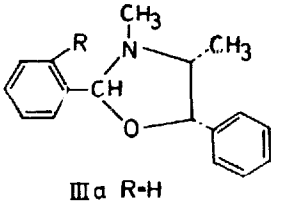
substances possessing such groups as hydroxyls or carboxyls can relatively simply be transformed to pro-drugs by suitable esterification, but for a large number of drugs no apparently readily derivatizable functional groups or entities are present in the molecules. For these compounds none or only few pro-drug forms have been explored although great activity presently occurs in this area (e.g. Bodor, 1981; Bundgaard and Larsen, 1980; Bundgaard and Johansen, 1981; Bundgaard, 1982; Pitman, 1981).

As a continuation of studies aiming to identify and evaluate potentially useful transport forms of such not easily derivatizable drug molecules, an investigation was carried out to obtain pro-drug candidates for the β-aminoalcohol moiety and or carbonyl groups (aldehydes and ketones). There are several drugs containing a β-aminoalcohol moiety, e.g. various sympathomimetic amines such as ephedrine, salbutamol, terbutaline and ethambutol, and various β-blockers such as propanol and alprenolol, which may exhibit delivery problems, e.g. due to unfavourable solubility or lipophilicity characteristics. For this moiety no pro-drug types have apparently been described and likewise, only few bioreversible derivatives have been explored of carbonyl-containing drugs. Recently, Patel and Repta (1980, 1981) reported that enol esters may be useful as pro-drugs of compounds containing enolizable carbonyl groups.

The purpose of this work is to evaluate oxazolidines as possible pro-drug models for the β-aminoalcohol moiety and/or for aldehydes or ketones. Oxazolidines are cyclic condensation products of β-aminoalcohols and aldehydes or ketones and although they have been known for several years (Bergmann, 1953) and are often used as derivatives in chromatographing β-aminoalcohols (e.g. Neelakatan and Kostenbauder, 1976; Nicholson, 1978; Munro et al., 1978), only little information is available on their stability and reactivity in aqueous solution. The hydrolysis of a series of 2-(substituted phenyl)-3-ethyloxazolidines over a restricted pH range has been studied by Fife and Hagopian (1968) while the mechanism of hydrolysis of 2-[4-(dimethylamino)styryl]-3-phenyloxazolidine has been examined (Fife and Hutchins, 1980). In the present paper the kinetics of hydrolysis of oxazolidines (IIIa, IIIb and IV) derived by condensation of (−)-ephedrine (I) or (+)-pseudoephedrine (II) with benzaldehyde or salicylaldehyde are described along with data for the lipophilicity of the compounds.

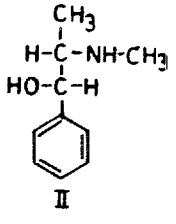


I

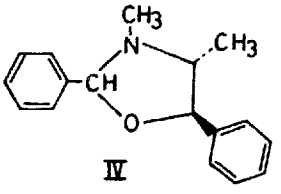


IIIa R=H

IIIb R=OH



II



IV

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Zeiss PMQ II spectrophotometer equipped with a thermostatted cell compartment and with a Perkin-Elmer 124 recording spectrophotometer, using 1 cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are corrected.

Chemicals

(−)-Ephedrine, (+)-pseudoephedrine, benzaldehyde and salicylaldehyde were purchased from AG Fluka, Switzerland. The oxazolidines were prepared according to the method (procedure B) of Soliman and El-Nenaey (1978) by stirring mixtures of the β-aminoalcohol (0.03 mol) and benzaldehyde or salicylaldehyde (0.03 mol) for 5 h at room temperature and recrystallizing the solid product obtained from ethanol, m.p. 74–75°C (erythro-2,5-diphenyl-3,4-dimethyloxazolidine (IIIa)), rep. m.p. 75–75.5°C (Soliman and El-Nenaey, 1978) and 73–74°C (Neelakantan, 1971); m.p. 66–67°C (threo-2,5-diphenyl-3,4-dimethyloxazolidine (IV)), rep. m.p. 68.0–68.5°C (Soliman et al., 1975); m.p. 118–119°C (erythro-2-phenyl-3,4-dimethyl-5-(*o*-hydroxy)phenyloxazolidine (IIIb)), rep. m.p. 118.5–119°C (Soliman et al., 1970 and 1975). The yields obtained were within 80–95%.

Buffer substances and all other chemicals or solvents used were of reagent grade.

Kinetic studies

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were hydrochloric acid, formate, acetate, phosphate, borate and carbonate solutions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of hydrolysis of the oxazolidines were followed spectrophotometrically by monitoring the increase in absorbance at 250 nm (255 nm for compound IIIb) due to liberation of benzaldehyde or salicylaldehyde. Reactions were performed in 2.5-ml aliquot portions of buffer solutions in a thermostatted quartz cuvette and were initiated by adding 20 µl of a stock solution of the oxazolidines in acetonitrile to give a final concentration of $4–6 \times 10^{-5}$ M. The pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_\infty - A_t)$ against time, where A_∞ and A_t are the absorbance readings at infinity (i.e. when the reaction is complete) and at time t, respectively.

Measurement of partition coefficients

The partition coefficients of the oxazolidine derivatives IIIa and IV were determined in an octanol-phosphate buffer (0.1 M, pH 7.40) system. The solute concentration in the octanol phase was determined spectrophotometrically at 257 nm before and after partition, the equilibrium being obtained after mixing the two phases for only 3 min (for stability reasons). The initial concentration in the octanol phase was about 3×10^{-3} M and the partitioning system consisted of 10 ml of

octanol previously saturated with buffer and 100 ml of buffer solution previously saturated with octanol. For each compound, determinations were carried out in triplicate, the log P values thereby obtained being reproducible to within ±5%.

Results and Discussion

Kinetics of hydrolysis

The kinetics of hydrolysis of the oxazolidines IIIa, IIIb and IV were studied in aqueous solution at 37°C over a wide range of pH. At constant pH and temperature the hydrolysis displayed strict first-order kinetics and in all kinetic runs, benzaldehyde or salicylaldehyde were liberated in stoichiometric amounts as determined from UV-spectrophotometry. The spectra of the reaction solutions at the conclusion of hydrolysis corresponded exactly to those of the aldehydes in equimolar concentrations and the rates of hydrolysis could easily be followed by monitoring the large increase in absorbance at 245–255 nm resulting from aldehyde formation.

The hydrolysis of the oxazolidines IIIa and IV was found to be subject to significant buffer catalysis while the rate of hydrolysis of the oxazolidine derived from salicylaldehyde (IIIb) was only slightly affected by the buffers used to maintain constant pH as illustrated by some representative data given in Table I. The hydrolysis rates showed a linear dependence on buffer concentration as shown in Figs. 1 and 2 in which the observed pseudo-first-order rate constants (k_{obs}) are

TABLE I

EFFECT OF BUFFER CONCENTRATION ON THE RATES OF HYDROLYSIS OF SOME OXAZOLIDINES AT 37°C (μ=0.5)

pH (buffer)	Buffer concentration (M)	Pseudo-first-order rate constants (min^{-1})		
		IIIa	IIIb	IV
3.01 (formate)	0.025	0.129		
	0.05	0.146	0.188	0.453
	0.10	0.176	0.195	0.502
4.53 (acetate)	0.025	0.592	1.63	0.534
	0.05	0.643	1.74	0.610
	0.10	0.688		
	0.20	0.835	1.87	0.791
9.25 (borate)	0.025	0.098	9.12	
	0.05	0.109		
	0.10	0.121	9.30	
	0.20	0.141		
6.50 (phosphate)	0.025	0.411		0.063
	0.05	0.483		0.069
	0.10	0.663		0.094
	0.20	0.975		0.131

TABLE II

CATALYTIC RATE CONSTANTS OF VARIOUS BUFFER SPECIES FOR THE HYDROLYSIS OF OXAZOLIDINES IN AQUEOUS SOLUTION AT 37°C (μ=0.5)

Oxazolidine	k^{a}_{HAc} ($M^{-1}\cdot min^{-1}$)	$K^{a}_{Ac^-}$ ($M^{-1}\cdot min^{-1}$)	$k_{H_2PO_4^-}$ ($M^{-1}\cdot min^{-1}$)	$k_{HPO_4^{2-}}$ ($M^{-1}\cdot min^{-1}$)
IIIa	~0	3.0	5.4	0.9
IIIb	2.1	0.7	0.7	~0

[a] HAc = acetic acid; Ac^- = acetate ion.

plotted against the total buffer concentration. From such plots treated in the usual manner (e.g. Jencks, 1969) the specific catalytic rate constants for hydrolysis of IIIa and IV by acetate and phosphate buffer species were derived and are listed in Table II.

The influence of pH on the hydrolysis rate for the oxazolidines is shown in Figs. 3 and 4, where the logarithms of the k_{obs} values at zero buffer concentration (k_0, obtained by extrapolation of plots such as those in Figs. 1 and 2 to zero buffer concentration) are plotted against pH. The k_0 values at pH <2.2 were obtained directly from runs in hydrochloric acid solutions.

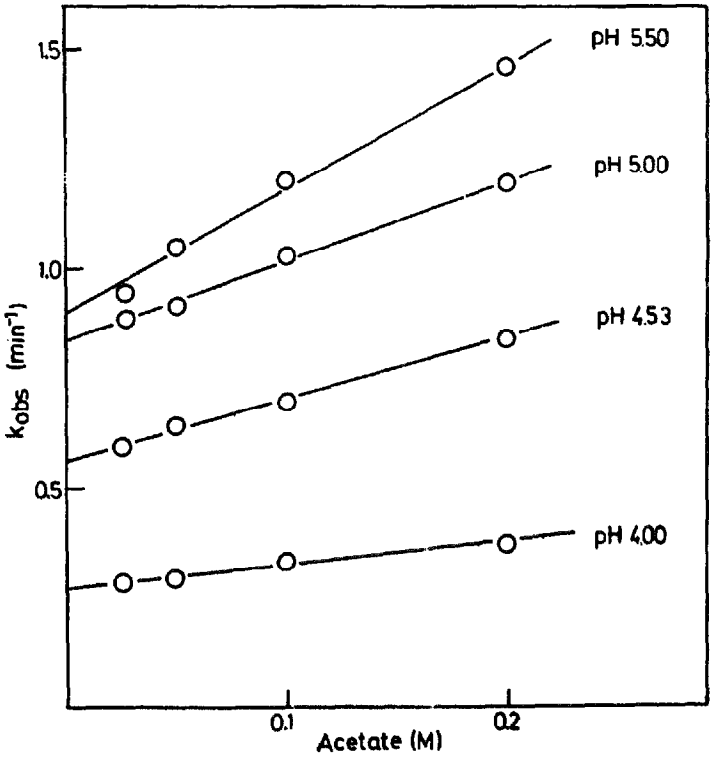


Fig. 1. Effect of acetate buffer concentration on the pseudo-first-order rate constant for the hydrolysis of oxazolidine IIIa at various pH values (37°C, μ=0.5).

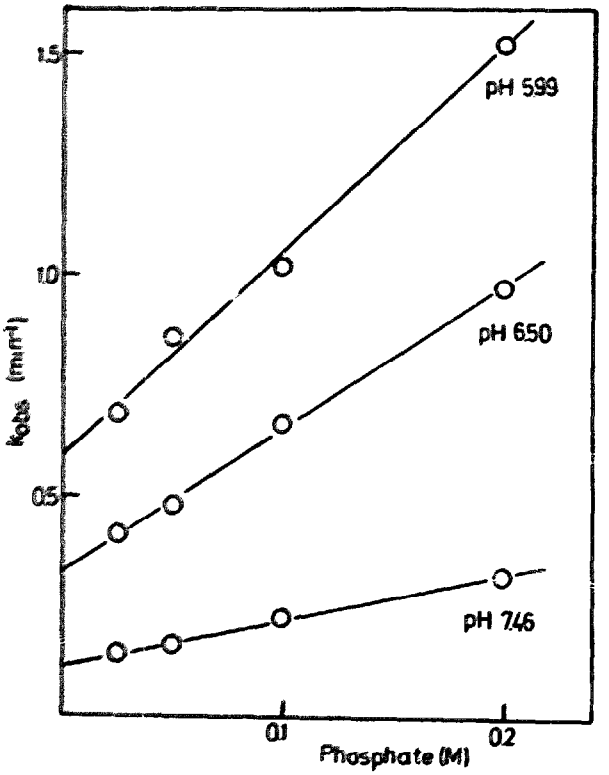


Fig. 2. Effect of phosphate buffer concentration on the pseudo-first-order rate constant for the hydrolysis of oxazolidine IIIa at various pH values (37°C, $\mu = 0.5$).

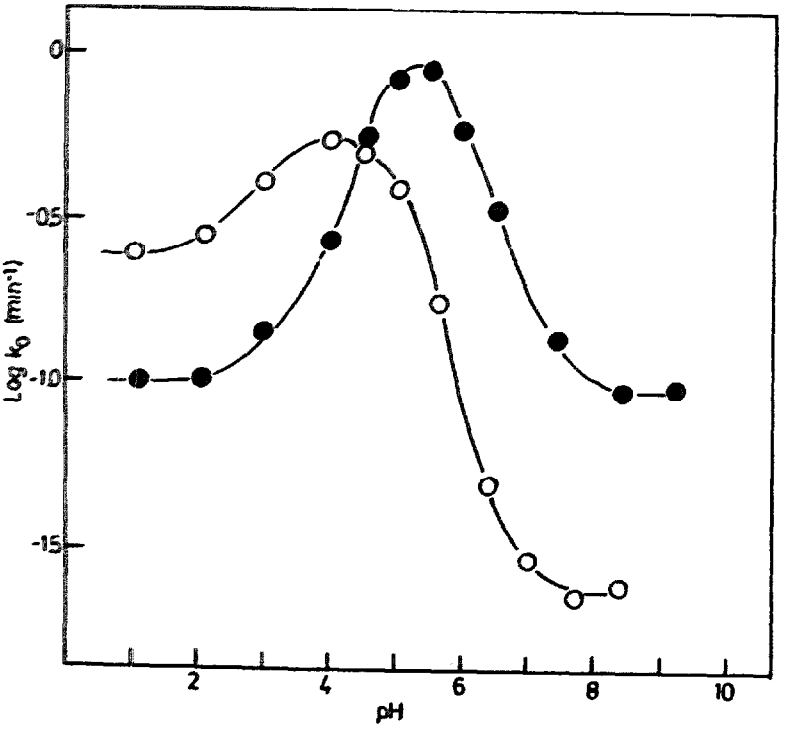


Fig. 3. pH–rate profiles for the hydrolysis of the oxazolidines IIIa (●) and IV (○) at 37°C ($\mu = 0.5$).

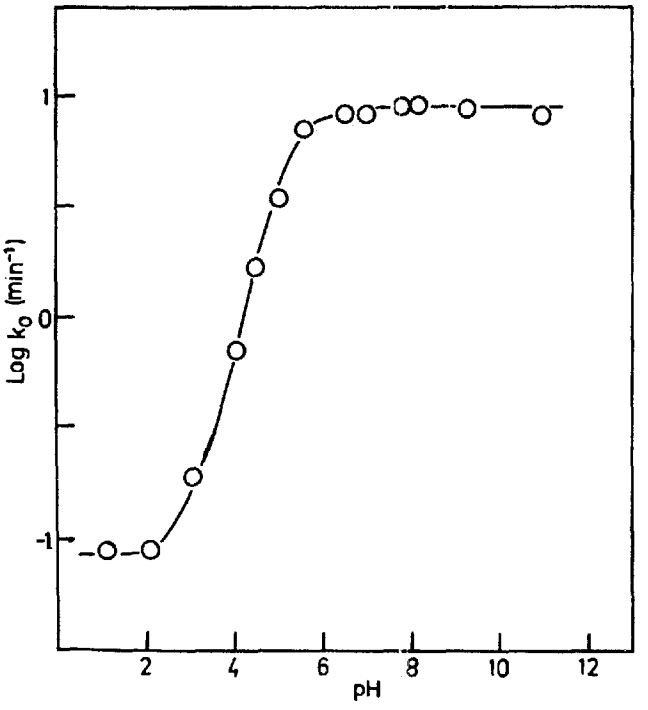


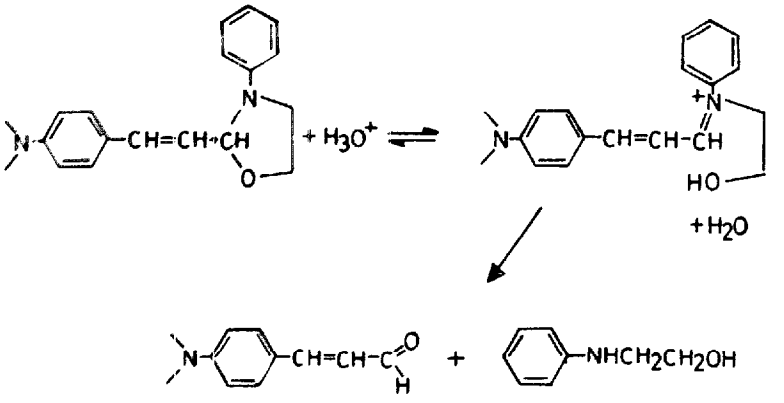
Fig. 4. pH–rate profile for the hydrolysis of the oxazolidine IIIb at 37°C (μ=0.5).

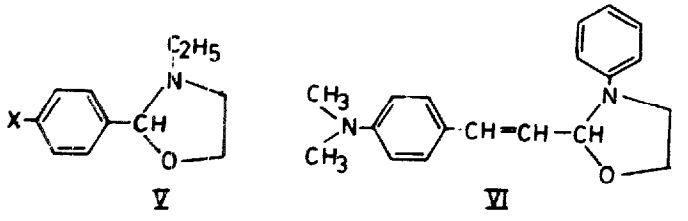
The bell-shaped pH–rate profiles for compounds IIIa and IV show a maximum rate at about pH 5.5 and 4.0, respectively, and an independence of rate upon pH at pH values below 2 and above 7.5. These pH–rate profiles are not easily interpretable but the unusual variation of hydrolysis rate with pH most certainly indicates the involvement of a kinetically significant intermediate in the reaction pathway and a change of the rate-determining step in the overall reaction with pH. The oxazolidines IIIa and IV are weak bases with pK_a values of 5.6 and 5.0, respectively, as estimated from half-neutralization potentials in non-aqueous solvents (Soliman, 1973) and accordingly, the pH–rate profiles cannot be interpreted solely in terms of different reactivities of protonated and free base species. It is interesting to note, however, that the rate maxima occur at pH values around the pK_a values.

Previous studies by Fife and co-workers (Fife and Hagopian, 1968; Fife and Hutchins, 1980) on oxazolidine hydrolysis have shown that for various 2-(substituted phenyl)-3-ethyloxazolidines (V) two reactions could be detected by UV-spectrophotometry in moderately concentrated hydrochloric acid solutions, a very rapid formation of a cationic Schiff-base intermediate by ring opening and the much slower hydrolysis of this intermediate to give the aldehyde product (Fife and Hagopian, 1968). Recently, the formation of a similar cationic Schiff-base intermediate was demonstrated in the hydrolysis of 2-[4-(dimethylamino)styryl]-3-phenyloxazolidine (VI) at pH < 10 (Fife and Hutchins, 1980). For compound VI hydrolysis of the Schiff-base intermediate was found to be rate-determining in the overall decomposition at all pH values up to 10 and a reaction scheme as shown in

Scheme 1 was proposed. Ring opening proceeds with C–O bond breaking and is subject to hydronium ion catalysis. The cationic Schiff-base intermediate formed is in equilibrium with the oxazolidine and undergoes spontaneous and hydroxide-ion catalyzed hydrolysis to yield the parent aldehyde and β-aminoalcohol (Fife and Hutchins, 1980).

Scheme 1





V VI

A similar mechanism may be involved in the hydrolysis of the oxazolidines IIIa, IIIb and IV. Using UV-spectrophotometry no Schiff-base intermediate could be detected for these compounds in the pH-range studied (1–11) which is in accord with the failure of detecting an intermediate in the hydrolysis of the structurally related oxazolidine V (X = H) at similar pH values (Fife and Hagopian, 1968). The rate of appearance of benzaldehyde or salicylaldehyde from IIIa, IIIb or IV exhibited strict first-order kinetics and no lag time was observed. Thus, if formed, the Schiff-base intermediate must be present in small steady-state concentrations.

Comparing the relative reactivities of the oxazolidines studied it is of interest to note that the erythro-ephedrine oxazolidine (IIIa) is more reactive than the threo-isomer (IV) at neutral and alkaline pH but less reactive in acidic solutions. This difference may be a reflection of the different pK_a values of the oxazolidines and different steric interactions in the molecules. The reaction of (−)-ephedrine and (+)-pseudoephedrine with benzaldehyde and other aromatic aldehydes has been shown to be a stereospecific reaction resulting in an asymmetric synthesis (Neelakantan, 1971; Neelakantan and Molin-Case, 1971). The oxazolidines formed are optically pure and the conformation of ephedrine is retained in the ring structure. Thus, a greater steric interaction between the 4-methyl and 5-phenyl groups occurs

in the oxazolidine IIIa where these groups are *cis*-oriented than in IV where the groups are *trans*-oriented. The diastereoisomeric oxazolidines also differ significantly in their general acid-base catalyzed hydrolysis (Table II).

The oxazolidine derived from salicylaldehyde (IIIb) behaves quite differently from the corresponding oxazolidine from benzaldehyde (IIIa). As seen from Fig. 4 the pH–rate profile is sigmoidal, the rate increasing up to pH 6 and then becoming invariable with pH. The reactivities of IIIa and IIIb are similar at pH 1–2 but at pH 5–6 oxazolidine IIIb is about 10-fold more reactive, increasing to 100-fold at pH > 7.5. Evidently, the ortho-situated hydroxyl group in IIIb greatly accelerate the oxazolidine hydrolysis rate, probably by some kind of intramolecular catalysis. The invariability of the rate upon pH in the range 6–11 may indicate that the pK_a of the hydroxyl group is greater than 11. This could possibly be the case as a result of intramolecular hydrogen bonding between the hydroxyl group and the neighbouring N-3 atom.

Compared with compounds IIIa and IV the oxazolidine V (X = H) shows a higher reactivity in acidic and alkaline solutions. Fife and Hagopian (1968) have reported that this oxazolidine undergoes hydrolysis at 30°C with rate constants of 1.0 ± 0.2 min^{-1} within the pH ranges 1.1–3.0 and 7.5–12.1. The pH–rate profile may be similar to those in Fig. 3 but no rate data were reported at pH values between 3 and 7.5.

The lipophilicity of the oxazolidines

The apparent partition coefficients ($P = C_{octanol}/C_{aqueous}$) for the oxazolidines IIIa and IV were measured using an octanol–aqueous buffer system, the buffer being 0.1 M phosphate of pH 7.40. The values found for log P were 1.58 (IIIa) and 1.64 (IV). The log P value for ephedrine in a similar octanol–phosphate buffer (pH 7.4) system has been reported to be -1.35 (Wang and Lien, 1980). Ephedrine occurs predominantly on the protonated form at pH 7.4 (the pK_a being 9.6) and the log P value for ephedrine free base is 1.02 (Wang and Lien, 1980). The results show that the oxazolidines prepared from benzaldehyde are more lipophilic than the parent ephedrine, especially at physiological pH where the oxazolidines are in the free base form and ephedrine is largely protonated.

Consideration of oxazolidines as pro-drug types

The present study shows that, in principle, oxazolidines should be regarded as potentially useful pro-drug types for β-aminoalcohols or carbonyl-containing compounds. The oxazolidines of the two ephedrine isomers are hydrolyzed quantitatively in the pH range 1–11, the half-lives of hydrolysis at pH 7.4 and 37°C being 5 min (IIIa), 28 min (IV) and 5 s (IIIb). These rates might not be expected to change much in vivo. Considering the pH–rate profiles for the oxazolidines it appears difficult to ensure adequate in vitro stability of the oxazolidines in solution regardless of pH and this may be a severe drawback in terms of usefulness for parenteral administration. However, structural factors both within the aldehyde or ketone part and the aminoalcohol moiety may certainly influence the stability as indicated by the data for IIIa and IIIb but this remains to be investigated more thoroughly. The oxazoli-

dines are much weaker bases than the parent β-aminoalcohols and this is a major contributing factor to the higher lipophilicity observed for the oxazolidines at physiological pH. This increased lipophilicity may become advantageous in situations where delivery problems for β-aminoalcohols are due to low lipophilicity.

When considering oxazolidines as possible pro-drug types for carbonyl-containing compounds instead of for β-aminoalcohols it is worth mentioning that whereas benzaldehyde is an easily oxidizable liquid with a pungent odor, its masked oxazolidine derivatives are crystalline solids with a high solid-state stability. Benzaldehyde has recently attracted considerable attention as an anti-tumor agent (Takeuchi et al., 1978; Miyakawa et al., 1979; Watanuki and Sakaguchi, 1980; Kochi et al., 1980; Nambata et al., 1981) and its physical properties create obvious formulation problems (Takeuchi et al., 1978). A peroral dosage form of benzaldehyde in the form of a crystalline oxazolidine pro-drug of the type described may possibly the useful to overcome such problems. After administration benzaldehyde will be released in the gastrointestinal tract and/or in the blood after absorption of the pro-drug, depending on the stability in the stomach and intestine.

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