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Oxazolidines: prodrugs for the delivery of β-aminoalcohols through human skin from aqueous solution

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Summary

Ephedrine (I) is delivered through human skin, in vitro, significantly faster from aqueous solutions of 3,4-dimethyl-5-phenyl-oxazolidine (II) with pH values between 7.0 and 10.88 than from corresponding solutions of I. The difference in penetration rates was most marked at pH 7.0 where 917 μg and < 10 μg of I was delivered through 1.3 cm^2 of skin in 24 h from 1% w/v solutions of II and I, respectively. No detectable amounts of I were delivered through 1.3 cm^2 of skin in 24 h from 1% w/v solutions of I or II in propylene glycol and delivery of I was faster from 1% w/v solutions of I in liquid paraffin than from corresponding solutions of II. Compound II has a lower pK_a value than compound I (ca. 5.5 vs 9.63) and a higher partition coefficient between water and liquid paraffin (5.6 vs 1.0). Although II rapidly hydrolyzed to I plus formaldehyde ($t_{50\%}$ < 1 min at pH values between 6 and 8), the system rapidly came to an equilibrium which increasingly favoured compound II as the initial concentration of II or the pH value was increased. Hence, it can be concluded that compound II and possibly other oxazolidines are potentially useful prodrugs for promoting the delivery through skin of I and other β-aminoalcohols from aqueous solutions with pH values close to 7.

Introduction

This paper is concerned with the relative rates at which ephedrine (I) is delivered through human skin, in vitro, from buffered aqueous solutions either of I or of 3,4-dimethyl-5-phenyloxazolidine (II). Compound II is hydrolyzed to I and formaldehyde by the reaction shown in Scheme 1 (Pfanz and Kirchner, 1958; Johansen and Bundgaard, 1983).

Compound I is a β-aminoalcohol and its cation has a pK_a value of 9.63 (Warren et al., 1971). Hence, compound I exists predominantly as a cation in aqueous solutions which have pH values compatible with human skin (pH 3–8) (Katz and Poulson, 1971) and it was expected that it would be inefficiently absorbed across the skin from such solutions. The inefficient absorption of cations, as compared to neutral molecules, has been ascribed to their low partition coefficient from water into the skin (Michaels et al., 1975) and to a tendency to bind to anionic sites in the stratum corneum (Scheuplein and Blank, 1971).

The oxazolidines formed by condensation of benzaldehyde and salicylaldehyde with I have significantly lower pK_a values than I (5.0 and 5.6, respectively) (Bundgaard and Johansen, 1982) and compound II has been ascribed a pK_a value of 6.0

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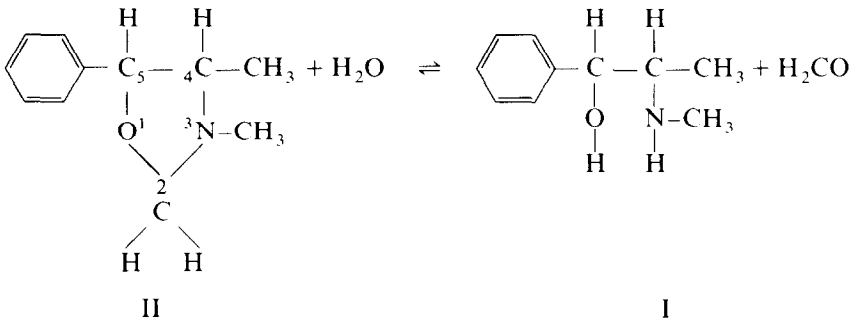

II I

Scheme 1

at 37°C (Johansen and Bundgaard, 1983). Thus, it was anticipated that II would exist as a neutral molecule to a greater extent than I in the pH range 3–8; and that II would penetrate skin more rapidly than I from aqueous solutions with pH values between 3 and 8.

When discussing the potential usefulness of oxazolidines as prodrugs, Bundgaard and Johansen (1982) concluded that they would present formulation problems, especially for parenteral solutions, on the basis of the rapid rates of which II was observed to hydrolyze in dilute solutions (ca. 5×10^{-5} M) at pH values around 7 and 37°C.

Hence, attention was given in this study to ways of preparing stable formulations of oxazolidines.

Materials and Methods

Chemicals

Ephedrine (I), ephedrine hydrochloride, formaldehyde and paraformaldehyde were used as received. Tritiated water was packed on 1/8/76 and had a radioactivity of 2.55×10 dpm $\cdot$ g^{-1}.

NMR measurements

^{1}H-NMR spectra were recorded at 60 MHz on a Perkin Elmer R12 instrument and at 90 MHz on a Bruker WH90 instrument. ^{13}C-NMR were recorded at 22.62 MHz on a Bruker WH90 instrument operating in the pulse-FT mode.

Synthesis of II

Compound II was synthesized by the method described by Beckett et al. (1978).

Calculation of water to liquid paraffin partition coefficients

Approximately 6×10^{-5} mole of I or II were dissolved in 5.0 ml of pH 9.0 buffer that was saturated with liquid paraffin. 5.0 ml of liquid paraffin that was saturated with water was added and the mixture shaken at 25°C for 2 h. The partition coefficient, PC, was calculated from the initial and equilibrium ultraviolet absorbance of 257 nm of the compound in the aqueous phase, A_i, and from A_e respectively, using Eqn. 1.

$$PC = (A_i - A_e)/A_e \quad (1)$$

Calculation of pK_a values

The method used was based on one described by Soliman (1973). The solvent (e.g. for 40% methanol: 15 ml methanol and 30 ml water) was added to the vessel of the pH meter and nitrogen passed through to remove dissolved carbon dioxide. Compound II (2×10^{-4} mole) in 5 ml of methanol and 1×10^{-4} mole of hydrochloric acid were added and the pH value monitored as a function of time. pH values were plotted against time and, where possible, the resulting curve extrapolated back to time 0 to give the apparent pK_a value at the particular methanol concentration. The procedure was repeated in 20, 40 and 60% methanol solutions.

Source of skin

Human abdominal and leg skin, together with 0.5–3.0 cm of subcutaneous fat, was obtained at autopsy and frozen at −45°C. Before use, skin was allowed to thaw in a heat sealed plastic bag. When almost thawed the skin was removed from

the bag, placed epidermis down on a glass slab, and subcutaneous fat was removed with a scalpel.

Measurement of skin penetration

Diffusion cells were identical to those described by Coldman et al. (1969). A piece of full thickness skin was mounted between two teflon washers which were clamped onto the ground glass top of the receptor chamber. The area through which drug could penetrate was 1.3 cm^2. The receptor solution (pH 7.0 (at 25°C) Sorensons phosphate buffer) was stirred with a magnetic teflon coated stirring bar connected to a teflon sail. The donor compartment was sealed with a glass coverslip and high vacuum grease to prevent solvent evaporation. The diffusion cell was placed in a water bath at 37° and allowed to equilibrate for 30 min before 1 ml of the donor solution in a Teorell and Stenhagen citrate/phosphate/borate/hydrochloric acid buffer or in propylene glycol or liquid paraffin was added to the epidermal surface.

HPLC assay

Compound I was assayed using a strong cation exchange column (SCX), Whatman (250 × 6.4 mm) and a mobile phase consisting of 50% methanol, and 50% ammonium phosphate solution (0.03% w/v) adjusted to pH 4 with orthophosphoric acid. The column was protected with a 40 × 6.4 mm pre-column packed with 10 μm silica. The flow rate of a Constametric I LDC pump was 1 ml · min^{-1} and Compound I was detected at 257 nm using a Spectromonitor II Variable Wavelength UV-Visible Detector.

Scintillation assay

Samples from the receptor phase of the diffusion cells and from the donor solution were taken and made up to 1 ml with distilled water. Nine ml of Aqueous Counting Scintillant II (Amersham International plc, U.K.) was added and the samples counted for 10 min on a Packard Model 3390 Liquid Scintillation Counter.

Calculation of permeability constants

Compound I

Concentrations of I in the receptor phase were computed from HPLC peak heights using standard curves of peak height against concentration. Values of permeability constants, k_p values, were calculated in one of two ways. If < 10% of applied drug had penetrated the skin during the 4 days over which penetration was monitored, k_p values were calculated using Eqn. 2.

$$k_p = \frac{m \cdot V_R}{A \cdot C_D} \tag{2}$$

where; m (μg/ml/min) is the slope of plots of concentration of I in the receptor phase against time when steady state penetration had been achieved. V_R(ml) is the volume of the receptor solution A (1.3 cm^2) is the surface area of skin exposed to drug solution and C_D ($\mu g \cdot ml^{-1}$) is the initial drug concentration in the donor phase.

When more than 10% of drug had been absorbed during an experiment, equation 3 was used to calculate k_p values (Scheuplein, 1965)

$$\ln\left(1 - \frac{1+\alpha}{\alpha} \cdot \frac{C_R}{C_D}\right) = -\frac{(\alpha+1) \cdot A \cdot k_p \cdot t}{V_D} \tag{3}$$

where; V_D (ml) is the volume of the donor solution, α is V_D/V_R and C_R (μg/ml) is the concentration of I in the receptor phase at time t.

The value of the left hand side of Eqn. 3 was calculated at each sampling and the results plotted against time. The slopes of the linear portion of above curves, n, was used together with Eqn. 4 to calculate values of k_p.

$$k_p = -\frac{n \cdot V_D}{A(\alpha+1)} \tag{4}$$

In all experiments, C_R was taken to be the total concentration of I and II and I was the only compound detected in the receptor phase. Eqns. 3 and 4 were used to compute k_p values in all experiments except for penetration of I from aqueous buffers at pH 7 and 8 and of II from aqueous pH 7 buffer when equation 2 was used.

Tritiated water

When studying the penetration of tritiated water, the samples removed from the receptor

phase were large (0.5 ml) and were replaced with 0.5 ml of buffer. The "true" counts per minute after the i^{th} sample N_i', were calculated from the measured counts of the i^{th} sample, N_i, the volume of the receptor phase, V_R, and the volume of the sample, V_S, using Eqn. (5).

$$N_i' = N_i + \frac{V_S}{V_R}(N_1 + N_2 + N_3 + \ldots N_{i-1}) \qquad (5)$$

The slope (L) of a plot of values of N_i' against time was used to compute values of an apparent permeability constant, k_p', using Eqn. 6.

$$k_p' = \frac{L \cdot V_R \times 10^6}{A \cdot N_D} \qquad (6)$$

where N_D is the counts/min in the donor solution. The factor 10^6 was included to obtain values of manageable proportions and to make the units comparable to those reported by other workers.

Statistical analysis of data

Comparison of results was done using a two-tailed *t*-test where only two groups were involved, or multiple comparison statistics where more than two groups were involved. The level of significance used in all tests was 0.05.

Results

Stereochemistry of I and II

Comparison of the ^{1}H- and ^{13}C-NMR spectra of I and of II with spectra reported by Baudet and Gelbcke (1979a and b) revealed that both compounds were the *erythro* isomers.

Hydrolysis of II

^{1}H-NMR spectra that were measured 72 h after dissolving compound II in deuterium oxide (ca. 0.3 M) that had been adjusted to pH 12 with deutero-sodium hydroxide indicated that Compound II was the predominant species present, thereby suggesting that II is stable with respect to hydrolysis at pH 12.

At pH values of 6.7 and 7.5 (pH adjusted with sodium phosphate and deutero-hydrochloric acid) II (initially ca. 0.3 M) was rapidly hydrolyzed $t_{50\%} < 1$ min and an equilibrium between erythero isomer of I, formaldehyde and residual II established. NMR peaks corresponding to formaldehyde were apparent in the ^{13}C-NMR spectra but were hidden by the HDO peak in the H^1-NMR spectra. They were identified in the latter spectrum using an inversion recovery (partially relaxed Fourier Transform) technique.

Confirmation that II had come to equilibrium with I and formaldehyde in deuterium oxide at pH 6.7 was provided by the observation that the same equilibrium was established when equimolar amounts of formaldehyde and I (which were also equimolar with the initial concentration of II in the above experiment) were added to deuterium oxide solution and the pH value adjusted to 6.7.

The fraction of II was higher in the pH 7.5 solution than in that at pH 6.7.

At pH 1.0 (0.1 M trifluoroacetic acid) the hydrolysis reaction was very slow but the predominant species after 72 h in a 0.3 M solution were I and formaldehyde.

The above results suggest that the equilibrium reactions shown in scheme 2 take place in deuterium oxide solutions. K_{app} is the apparent

$$\begin{array}{ccc} & \overset{II}{} & \\ D^+ \uparrow\downarrow K_a^{II} & & \\ IID^+ & & \end{array} + D_2O \overset{K_{app}}{\rightleftharpoons} \begin{array}{ccc} & \overset{I}{} & \\ D^+ \uparrow\downarrow K_a^{I} & & \\ ID^+ & & \end{array} + H_2CO$$

Scheme 2

equilibrium constant for the hydrolysis reaction, IID^+ and ID^+ are the conjugate acids of II and I, respectively, and K_a^{II} and K_a^{I} are the acid dissociation constants of II and I, respectively. The equilibrium concentrations of II and IID^+ ($[II]_T^e$) and $I + ID^+$ ($[I]_T^e$) were calculated from areas under the C–CH_3 peaks in the H^1-NMR spectra and used to calculate K_{app} values using Eqn. 7. Values of K_{app} are in Table 1.

$$K_{app} = \frac{[I]_T^e[H_2CO]^e}{[II]_T^e} \qquad (7)$$

Because of the mass balance shown in Eqn. 8,

$$[II]_o = [I]_T^e + [II]_T^e \qquad (8)$$

TABLE 1
APPARENT EQUILIBRIUM CONSTANTS FOR HYDROLYSIS OF II IN BUFFERED DEUTERIUM OXIDE SOLUTIONS AT 25°C

pH	K_{app} (M)
6.7	0.7
7.5	0.1

it is possible to calculate the fraction of the initial amount of II_0 that exists as II^e_T at equilibrium (γ) by using Eqn. 9.

$$\gamma = 1 - \frac{2}{1 + \sqrt{1 + 4[II]_o/K_{app}}} \quad (9)$$

Fig. 1 contains plots of γ against hypothetical values of $[II]_0$ at pH 6.7 and 7.5. It is evident that the fraction of II^e_T increases with increasing pH value at particular values of $[II]_0$ and with increasing $[II]_0$ at each pH value.

pK_a value of II

The pH value of a half neutralized solution of II (4×10^{-3} M) in 60% methanol in water increased rapidly from approximately 5.2 to 8.8. The pH change was complete within 4 min in 60% v/v methanol in water and earlier in solutions that contained less methanol. Typical plots in 40% and 60% v/v methanol are in Fig. 2.

Based on the NMR spectral changes that

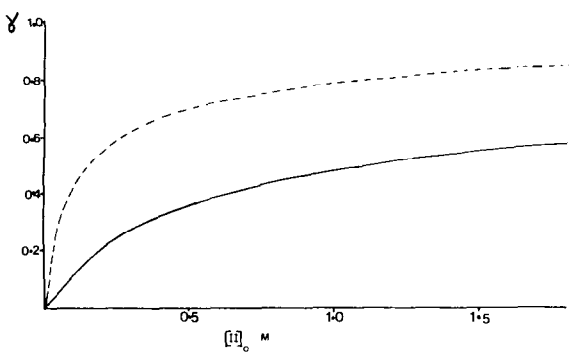

Fig. 1. Plots against initial concentration of II ($[II]_0$) of calculated values of the fraction of $[II]_0$ that would exist in equilibrium mixtures as II (γ) at pH 6.7 (———) and 7.5 (— — —) in deuterium oxide.

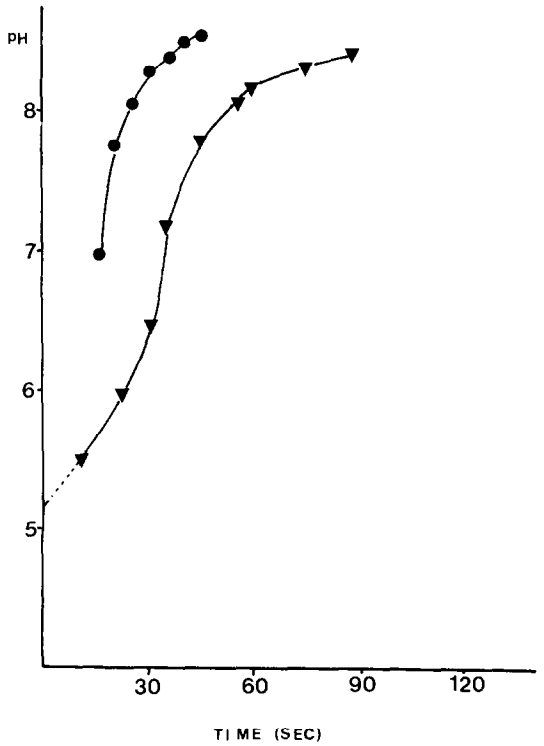

Fig. 2. Plots against time of the pH value of half-neutralized solutions of II in 40% v/v (●) and 60% v/v (▲) methanol in water at 25°C.

accompanied the dissolution of II in deuterium oxide, it was concluded that the pH value at 0 s was the acid pK_a value of II (pK^{II}_a) and that the pH change resulted from the hydrolysis of II to the more strongly basic compound I. The pK_a value of II in 60% solutions of methanol in water (pK'_a) was calculated, by extrapolating plots such as that shown in Fig. 2 back to time = 0 s, to be 5.2. The hydrolysis reaction in 20% v/v and 40% v/v methanol in water proceeded too rapidly for plots of pH against time that could be extrapolated back to zero time to be constructed. The pK_a value of II in water was estimated to be 5.5 (i.e. pK'_a in 60% v/v methanol + 0.3). Soliman (1973) has shown that similar oxazolidines to II are weaker bases by approximately 0.3 units in 60% methanol than in water. The estimated pK_a value of II (pK^{II}_a) in water at 25°C is similar to the values of 6.0 which was calculated from kinetic data for hydrolysis of II at 37°C (Johansen and Bundgaard, 1983).

Extrapolation of pK'_a values for I that were calculated from the pH values of half-neutralized aqueous methanolic solutions of I back to the value in 0% methanol yielded a value of 9.5 (cf. the previously reported value of 9.63, Warren et al., 1971).

The partition coefficient of I and II between water at pH 9 and liquid paraffin

NMR studies indicated that the equilibrium between compounds II and I plus formaldehyde (Scheme 2) favoured II at pH 9.0 in approximately 1% w/v solutions. Hence, the partition coefficient determinations were carried out between a pH 9.0 aqueous buffer and liquid paraffin.

The observed partition coefficients of I and II at pH 9.0 were 0.2 and 5.6, respectively.

Compound II has a pK_a value in water that is < 7 and hence 5.6 is the partition coefficient of the neutral molecule of II. However, Compound I, which has a pK_a value of 9.63 (Warren et al., 1971) would be > 50% ionized at pH 9.0. The parition coefficient of the neutral molecule I (PC_I) was calculated from the observed partition coefficient at pH 9.0 (PC_I^{obs}) using Eqn. 10.

$$PC_I = PC_I^{obs}\left(1 + 10^{pK_a^I - pH}\right) \qquad (10)$$

PC_I was calculated to be 1.0, a value identical to that previously reported by Shaw et al. (1975).

In vitro skin penetration by I

Table 2 contains calculated permeability constants, $k_{p,obs}^I$ values, for delivery of I through human skin from aqueous buffers, from propylene glycol and from liquid paraffin. Permeability constants are the rate constants for the steady state penetration of molecules through a barrier. Hence, the larger the permeability constant, the larger the penetration rate. Also included are values of the amount of I that was delivered through 1.3 cm^2 of skin in 24 h.

Values of $k_{p,obs}^I$ for delivery of I through human skin from aqueous buffers were pH dependent in the pH range 7–10.88. Because compound I has a pK_a value of 9.63 (Warren et al., 1971) the increased delivery rate with increasing pH value is consistent with the postulate that the neutral molecules of I are delivered through skin faster than the cation. Support for this postulate comes from the constancy (at the 0.05 level) of values of the permeability constant for ephedrine base, k_p^I, which were calculated using equation 11.

$$k_p^I = k_{p,obs}^I\left(1 + 10^{pK_a^I - pH}\right) \qquad (11)$$

The average value of k_p^I in Table 2 (175 $\mu \cdot cm \cdot min^{-1}$) is close to the value calculated by Shaw et al. (1975) (148 $\mu \cdot cm \cdot min^{-1}$).

In vitro delivery of I through human skin from solutions of II

No compound II was detected in the receptor solution in any experiment following the application of II or I plus formaldehyde to the skin.

TABLE 2

PERMEABILITY CONSTANTS OF I AND MASS OF I DELIVERED THROUGH 1.3 cm^2 OF HUMAN SKIN IN 24 h FROM SOLUTIONS OF I [a]

Solvent	No. of experiments	$k_{p,obs}^I$ (±S.D.) [b] ($\mu \cdot cm \cdot min^{-1}$)	k_p^I (±S.D.) [c] ($\mu \cdot cm \cdot min^{-1}$)	Mass penetrating in 24 h (±S.D.) (μg)
Aqueous buffer, pH 7.00	4	< 1		
Aqueous buffer, pH 8.00	5	6 (8)	245 (327)	211 (69)
Aqueous buffer, pH 9.00	6	39 (23)	194 (114)	653 (275)
Aqueous buffer, pH 10.00	4	93 (15)	130 (21)	958 (234)
Aqueous buffer, pH 10.88	8	114 (47)	120 (49)	1924 (707)
Liquid paraffin	3	220 (40)		2955 (583)
Propylene glycol	6	< 1		< 1

[a] 1% w/v solutions of I (0.06 M).

[b] ± standard deviation.

[c] calculated permeability constant for neutral molecules.

TABLE 3
APPARENT PERMEABILITY CONSTANTS OF II AND MASS OF I DELIVERED THROUGH 1.3 cm^2 OF HUMAN SKIN IN 24 h FROM SOLUTIONS OF II [a]

Solvent	No. of experiments	$k^{II}_{p,obs}$ (±S.D.) ($\mu \cdot cm \cdot min^{-1}$)	Mass delivered in 24 h (±S.D.) [b] (μg)
Aqueous buffer, pH 7.00	6	60 (13)	917 (55)
Aqueous buffer, pH 8.00	6	253 (44)	3081 (497)
Aqueous buffer, pH 9.00	5	370 (142)	4373 (1539)
Aqueous buffer, pH 10.00	5	445 (74)	5149 (804)
Aqueous buffer, pH 10.88	5	510 (193)	2694 (633)
Liquid paraffin	4	33 (13)	350 (227)
Propylene glycol	2	< 1	< 1

[a] 1% w/v solutions of II (0.056 M).
[b] ± standard deviation.

However, I was observed in the receptor solution following application of II in aqueous buffers with pH values between 7.0 and 10.88, or of I plus an excess of formaldehyde (1 : 1.67) at pH values between 7 and 10.88, or of I plus varying concentrations of formaldehyde. Values of $k^{II}_{p,obs}$, which were calculated on the basis that each mole of I in the receptor solution represented delivery through the skin of a mole of II, are in Table 3 (from solutions of II) and 4 (from solutions of I plus formaldehyde). It will be argued that molecules of II which enter the skin are rapidly converted to I plus formaldehyde in an aqueous environment after passing the rate controlling barrier. This aqueous environment may be within the epidermis or dermis of the skin or it may be the receptor solution.

The results in Tables 2 and 3 reveal that:
(1) compound I is delivered through skin faster from liquid paraffin solutions of I than from similar solutions of II;
(2) no significant delivery of I occurred from solutions of I or II in propylene glycol;
(3) compound I is delivered through skin faster from aqueous buffers pH (7.00–10.88) which initially contained II than from approximately equimolar solutions which initially contained I. The effect is most marked at pH 7.00 but is still significant at pH 10.88; and
(4) although the pH dependence of $k^{II}_{p,obs}$ values was less pronounced than that observed for $k^{I}_{p,obs}$ values, values at pH 10.88 were significantly larger than the values at pH 7.00.

Values of $k^{II}_{p,obs}$ that were calculated for de-

TABLE 4
APPARENT PERMEABILITY CONSTANTS OF II AND MASS OF I DELIVERED THROUGH HUMAN SKIN IN 24 h FROM AQUEOUS SOLUTIONS OF I [a] AND FORMALDEHYDE

$[H_2CO]$ (M)	pH	No. of experiments	$k^{II}_{p,obs}$ (±S.D.) [b] ($\mu \cdot cm \cdot min^{-1}$)	Mass delivered in 24 h (±S.D.) [b] (μg)
0.10	7.00	7	115 (50)	3445 (1109)
0.10	8.00	7	279 (107)	3526 (1135)
0.10	9.00	7	290 (31)	3656 (282)
0.10	10.00	6	413 (236)	4269 (1614)
0.10	10.88	6	611 (129)	5835 (640)
0.00	8.00	5	6 (8)	127 (125)
0.06	8.00	11	268 (105)	3530 (712)
0.10	8.00	7	279 (107)	3526 (1135)
0.30	8.00	9	373 (124)	4685 (634)

[a] 1% w/v solutions of I (0.06 M).
[b] ± standard deviation.

TABLE 5
APPARENT PERMEABILITY CONSTANTS (k'_p) FOR PENETRATION OF HUMAN SKIN BY TRITIATED WATER FROM WATER AND FROM AQUEOUS FORMALDEHYDE SOLUTIONS

$[H_2CO]$ (M)	pH	No. of experiments	k'_p (±S.D.) ($\mu \cdot cm \cdot min^{-1}$)
0	7.0	4	31 (6)
0.1	7.0	4	47 (10)
0	8.0	7	42 (12)
0.1	8.0	4	35 (13)
0.3	8.0	3	44 (9)
0	9.0	4	30 (11)
0.1	9.0	4	31 (5)
0	10.0	6	33 (10)
0.1	10.0	4	29 (11)

livery of I from aqueous solutions of I plus formaldehyde (Table 4) were very similar to the $k^{II}_{p,obs}$ values calculated for delivery from solutions which initially contained compound II. The results at pH 8.0, in which the initial formaldehyde concentration was varied from 0.06 M (i.e. equimolar with I) to 0.3 M, reveal that $k^{II}_{p,obs}$ values only increase slightly with the addition of excess formaldehyde.

The effect of formaldehyde on skin penetration by tritiated water

Table 5 contains apparent permeability constants (k'_p) for delivery of tritiated water through human skin from buffers at pH 7.0 with or without added formaldehyde.

There was no significant difference in skin permeability to tritiated water except in the results at pH 7 where the slightly larger k'_p value in the presence of 0.1 M formaldehyde was only significant at the 0.05 level. The values of k'_p were similar to values of 33.3 $\mu m \cdot min^{-1}$ and 16.7 $\mu m \cdot min^{-1}$ reported by Scheuplein and Blank (1971) and Scheuplein (1965), respectively.

Discussion

An ideal prodrug for enhancing delivery of drugs through skin should:

(1) remain stable with respect to degradation in its formulation up to the time it is absorbed;
(2) penetrate the skin more rapidly than the parent drug;
(3) be rapidly transformed to the parent drug following its absorption; and
(4) possess no independent pharmacological properties and be non-toxic.

The present study suggests that, at least on the basis of points 1–3 above, compound II is a potentially useful prodrug for enhancing delivery of I through human skin from aqueous solutions. It will also be argued that oxazolidines may have general application as prodrugs for enhancing delivery of β-aminoalcohols through skin and other membranes.

Although II is rapidly hydrolysed in water to yield I and formaldehyde ($t_{50\%} \ll 1$ min in water between pH 6–8) (Johansen and Bundgaard, 1983), the position of equilibrium is influenced by both the initial concentration of II and by the pH value of the solution (Fig. 1). The fraction of the initial amount of II that exists as II at equilibrium (γ) increases as either the initial concentration of II or the pH value is increased at pH values around 7. The proposed mechanism of the reaction would require that the pH effect would become insignificant when a substantial proportion of I existed in the neutral form (i.e. at pH values $> pK^I_a + 2$ or 11.63). Thus, a pharmaceutical formulator can adjust the fraction of II relative to I in the equilibrated solution by adjustment of pH or of initial concentration of II. The values of K_{app} and γ that were calculated from NMR data cannot be applied directly to calculate the fractions of various species in water because they may be effected by a deuterium isotope effect. However, it is expected that the fraction of II at equilibrium in water will vary in much the same way as illustrated in Fig. 1.

The permeability constants and data on amount of I being delivered through 1.3 cm^2 of human skin in 24 h from aqueous solutions of I in Table 2, indicate that it is essentially the neutral molecules of I rather than the cations that cross the skin. This is consistant with the findings of Michaels et al. (1975). Thus, no detectable amounts of I were delivered through the skin at pH 7.0. However, the results in Table 3 reveal that signifi-

cant amounts of I were delivered through the skin from solutions of II at pH 7.0 and that the rate of delivery increased with increasing pH. Comparison of the data for penetration at pH 8 in Table 3 with the data in Table 4 for delivery of I from a solution that was initially equimolar in I and formaldehyde at pH 8 reveals that both solutions led to the same delivery rates for I. This is consistent with the proposal that the same equilibrium mixture of II, I and formaldehyde is achieved by dissolving either II or I and formaldehyde in water and that neutral molecules of II as well as I penetrate the skin. The possibility that the formaldehyde in the equilibrium mixture increased the permeability of the skin is discounted by examination of the results in Table 5 which indicate that formaldehyde, up to concentrations of 0.1 M, does not significantly affect the permeability of skin to tritiated water.

Thus, the enhancement of rate of delivery of I that is produced by applying aqueous solutions of II or of I plus formaldehyde can be largely ascribed to penetration through the rate determining barrier in the skin of II, which is a weaker base than I ($pK_a^{II} \simeq 5.5$ vs $pK_a^{I} = 9.63$) and exists to a greater extent as neutral molecules in the pH range 7–10.88. The relatively small variation in delivery rates of I from solutions of I plus formaldehyde when the initial formaldehyde concentration was varied from 1 to 5 times the initial concentration of I (Table 4) suggests that the equilibrium favours II to a substantial extent throughout the range of experimental conditions employed.

Thus, it is proposed that II would be the major species in the donor solution at pH 10.88 in the presence of an excess of formaldehyde (Table 4) and that the resultant $k_{p,obs}^{II}$ value (611) would be close to the absolute value for delivery through the skin of neutral molecules of II from water. Comparison of this value to the permeability constant for neutral molecules of I from water ($k_{p,}^{I}$ values in Table 2; 172 ± 59) indicates that II has a smaller affinity for water and a greater tendency to leave water and penetrate skin than does I. This is the opposite to what was found from a comparison of the permeability constants for skin penetration of I and II from liquid paraffin ($k_{p,obs}^{I}$ 220 ± 40; $k_{p.obs}^{II}$ 33 ± 13). This result suggests that II has a smaller tendency than I to leave liquid paraffin and enter the skin.

These results are consistent with the calculated partition coefficients for I and II between water and liquid paraffin (1.0 and 5.6), respectively. Thus, I has a greater affinity than II for water (and a smaller tendency to escape) and II has a greater affinity than I for liquid paraffin (and a smaller tendency to escape).

The failure to observe penetration of I from solutions of either I or II in propylene glycol is surprising as this solvent has a polarity between those of water and liquid paraffin. One possible explanation is that the hydroscopicity of the propylene glycol causes water to flow from the receptor to the donor. The water gradient so established would inhibit penetration of I into the receptor.

The failure to detect II in the receptor solutions following application to the skin of concentrated solutions of II or I plus formaldehyde is consistent with the observations that:

(1) the half left for hydrolysis of II to I plus formaldehyde is < 1 min at pH 7.4 and 37°C (Johansen and Bundgaard, 1983); and
(2) the equilibrium reaction shown in Scheme 2 favours I and formaldehyde in dilute aqueous solutions such as those that exist in the receptor solutions whereas it favours I in the concentrated donor solutions.

It is possible that appreciable hydrolysis of II to I and formaldehyde occurs within the skin (e.g. in aqueous environments in the epidermis and dermis) but such hydrolysis must take place after the rate determining barrier to penetration (presumably the stratum corneum).

One further question that will need to be addressed before prodrugs such as II can be employed concerns the acceptability and possible toxicity of the formaldehyde that will result when the prodrug is transformed to the parent drug. Thus although some workers recommend that the concentration of formaldehyde in cosmetics should not exceed 0.2% (Elder, 1984), Epstein and Maibach (1966) have reported that the incidence of allergic reactions to topically applied formaldehyde is low and the threshold irritation concentration on intact skin has been recorded as 2% (Frosch and Kligman, 1978). In view of the fact

that the formulator will be aiming to have most of the formaldehyde in the formulation covalently bonded to the β-aminoalcohol, it is probable that aqueous formulations of prodrugs such as II would be pharmaceutically acceptable for topical use on intact skins. However, questions related to systemic toxicity and possible mutagenicity of formaldehyde will have to be addressed before employing II or other oxazolidines formed by formaldehyde.

The studies reported in this paper were exclusively concerned with the skin penetration of II. However, it is believed that the principles developed should be more widely applicable to drug delivery. In particular, an oxazolidine has been shown to penetrate a biological membrane from water faster than a β-aminoalcohol at pH values around 7. Although the oxazolidine hydrolyzes rapidly in water, the position of equilibrium can be controlled by adjustment of initial concentration and pH. It is suggested that these principles are likely to apply to the formulation of other β-aminoalcohols using other aldehydes, which are perhaps less toxic than formaldehyde, and for promoting penetration of other biological membranes (e.g. following parenteral or oral administration).

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