# Synthesis and Enhancing Effect of Dodecyl 2-(*N,N*-Dimethylamino)propionate on the Transepidermal Delivery of Indomethacin, Clonidine, and Hydrocortisone

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The biodegradable transdermal penetration enhancer, dodecyl 2-(*N,N*-dimethylamino)propionate (II; DDAIP), was prepared by reacting dodecyl 2-bromopropionate (I), obtained by reaction of *n*-dodecanol with 2-bromopropionyl halogenide, with dimethylamine. The penetration enhancing effects of DDAIP on the transport of indomethacin, clonidine, and hydrocortisone across shed snake skin (*Elaphe obsoleta*) were evaluated. Azone and lauryl alcohol, a possible decomposition product of DDAIP, were used as standard enhancers for comparison. In terms of flux, DDAIP showed 4.7 and 7.5 times the promoting effect for indomethacin compared to azone and lauryl alcohol, respectively. With clonidine this effect was 1.7 and 3.1 times, whereas with hydrocortisone it was 2.4 and 2.8 times higher, respectively. *In vitro* biodegradability of DDAIP was demonstrated in the presence of porcine esterase. The results indicate that DDAIP increases markedly the transepidermal delivery of several types of drug substances.

KEY WORDS: dodecyl 2-(*N,N*-dimethylamino) propionate; penetration enhancer; shed snake skin; transepidermal drug delivery; indomethacin; clonidine; hydrocortisone.

## INTRODUCTION

Many attempts have been reported to improve percutaneous drug delivery (1). One approach is to use transdermal penetration enhancers. Several compounds such as acetone, propylene glycol, DMSO, *N,N*-diethyl-*m*-toluamide, azone, β-cyclodextrin, and pyrrolidones are known to enhance the penetration of drugs (1–6). In previous papers from our laboratories (7–11), new biodegradable and highly effective enhancers have been described. Biodegradable penetration enhancers potentially are less irritating and less toxic as well as showing a limited time period of activity.

The aim of the present study is to report the synthesis, biodegradability, and penetration enhancing effects of dodecyl 2-(*N,N*-dimethylamino)propionate (DDAIP) on the transport of three drug substances, indomethacin (acidic), clonidine (basic), and hydrocortisone (neutral). Shed snakeskin was used as the model membrane for the *in vitro* penetration studies. The penetration enhancement found with DDAIP was compared with azone, a typical enhancer; lauryl alcohol (12), a possible decomposition product of DDAIP; and dodecyl *N,N*-dimethylamino acetate (DDAA), an effective biodegradable penetration enhancer developed earlier in our laboratory (9).

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## MATERIALS AND METHODS

### Apparatus

IR spectra were obtained with a Perkin Elmer 1420 spectrometer. NMR spectra were recorded on a Varian 300 LX instrument. Mass spectra were obtained with a Nermag R 10-10 quadrupole mass spectrometer. HPLC was performed using a Spectroflow 783 absorbance detector and a Bio-Rad HPLC Model 1330 pump. A reversed-phase RP-CN, 4.6 × 100-mm, column (Brownlee Laboratories) was used in conjunction with the system. TLC studies were made on EM Science silica gel plates with ethyl acetate as the solvent and iodine as the visualizing agent.

### Reagents and Solvents

*N,N*-Dimethylamine was purchased from Eastman Kodak. Azone was obtained from Nelson Research and Development Co. Indomethacin, hydrocortisone, and porcine esterase were purchased from Sigma. Clonidine-HCl was a gift of Boehringer Ingelheim. Silicagel was obtained from EM Science. All of the other chemicals were from Aldrich and were used without further treatment.

### Synthesis

*Dodecyl 2-Bromo (or chloro)-propionate (I)*

A mixture of dodecanol (42.8 g, 0.23 mol), 2-bromopropionyl bromide (50 g, 0.23 mol), and triethylamine (25 g) in 300 mL of dry chloroform was stirred at room temperature for 24 hr. The reaction mixture was washed three times with water (250 mL each washing) and the organic phase was dried over anhydrous magnesium sulfate. The solvent was distilled off and the residue was chromatographed through a column of florosil using chloroform as solvent. Yield, 88%. The TLC $R_f$ value was 0.92 (ethyl acetate). IR($CHCl_3$): γ 2920, 2840 (C–H), 1730 (C=O), 1180 (C–O–C) $cm^{-1}$. $^1H$ NMR($CDCl_3$): δ 0.90 (3H, t, $CH_3$), 1.27 [23H, m, $(CH_2)_{10}$ and $CH-CH_3$], 4.18 (2H, t, $CH_2-O$), 4.42 (1H, q, $CH-CH_3$) ppm. MS: 321, $C_{15}H_{29}BrO_2$ requires 321.

*Dodecyl 2-(N,N-Dimethylamino)propionate (II; DDAIP)*

Dodecyl 2-bromopropionate (64 g, 0.2 mol) was dissolved in 100 mL diethyl ether. Triethylamine (61.2 g, 0.6 mol), liquid dimethylamine (45 g, 1 mol), and a few crystals of potasium iodide were added. The resulting mixture was refluxed 20 hr. After the filtration of the white precipitate, the solvent was evaporated *in vacuo*. The liquid residue was purified by extensive column chromatography with silica gel (130–270 mesh) using ethyl acetate as the solvent. The pure fractions were combined and the solvent removed using a rotavap under reduced pressure to give the endproduct. Yield, 85%. The TLC $R_f$ value was 0.56 (ethyl acetate). IR

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($CHCl_3$): γ 2920, 2850 (C – H), 1725 (C = O), 1100 (C – O – C) $cm^{-1}$. $^1H$ NMR($CDCl_3$): δ 0.88 (3H, t, $CH_3$), 1.26 (23H, m, $(CH_2)_{10}$ and $CH-CH_3$), 2.34 (6H, s, $N(CH_3)_2$), 3.21 (1H, q, $CH-CH_3$), 4.11 (2H, t, $CH_2-O$) ppm. MS (EI): *m/z* (% RA) 285 (10), 149 (52), 116 (28), 100 (14), 97 (32), 72 (100), $C_{17}H_{35}NO_2$ requires 285.

### Shed Snakeskins

Shed snakeskins were prepared as described previously (9). They were stored at −20°C. Before the experiment they were allowed to reach room temperature at least 10 hr before use. For one set of experiments one whole snakeskin was used.

### Assay of the Drugs

Indomethacin, clonidine, and hydrocortisone were assayed by HPLC procedures reported earlier (9,10,12).

### Penetration Study

Pieces of shed snakeskin were pretreated with 15 μL of enhancer (divided into three 5-μL applications) 2 hr before each experiment. After mounting the skin on the receptor cell, the donor cell was placed on top of the receptor cell. Suspensions of indomethacin and hydrocortisone were prepared by suspending 50 and 25 mg of the drugs, respectively, in 25 mL of pH 7.0 buffer (μ = 0.15 with NaCl) and stirring for 24 hr. A 2% clonidine-HCl solution was also prepared in the same buffer. A 0.5-mL sample of either the suspension or solution was added to the donor cell. At appropriate time intervals, samples were taken and analyzed by HPLC. The solubilities of hydrocortisone and indomethacin were obtained from previous studies (10–12).

### Esterase Hydrolysis

A 0.1-mL aliquot of porcine esterase (253 U/mg protein) was diluted to 100 mL with pH 7.0 phosphate buffer. The enhancer solution was prepared by dissolving ~12 mg (~0.042 mol) of the enhancer in 10 mL acetonitrile. One hundred microliters of this solution was transferred into a 10-mL volumetric flask; then 9.8 mL pH 7.0 buffer and 100 μL diluted esterase solution were added. The mixture was kept in a water bath at 32°C with constant stirring. The disappearance of the enhancer peak was monitored by HPLC. The absorbance wavelength was 204 nm. The solvent system was a mixture of acetonitrile and 0.02 *M* aqueous sodium hexanesulfonate (7:4) at a flow rate of 0.9 mL/min. The retention time of DDAIP was 3.9 min. The kinetic runs were done in triplicate.

## RESULTS

DDAIP can be prepared by two pathways. In the first pathway *n*-dodecanol was reacted with 2-bromopropionyl halogenide to produce dodecyl 2-bromopropionate (I), which further reacts with liquid *N*,*N*-dimethylamine to give the enhancer. The use of 2-bromopropionyl bromide instead of 2-bromopropionyl chloride gave a higher quantity of I. In the second pathway *n*-dodecanol was condensed with *N*,*N*-dimethylalanine (13) in the presence of 1,1′-carbonyldiimidazole to form II (Scheme I). Since, by the second pathway, more impurities are formed and the yield is lower, the first pathway was preferred. The structures of I and II were verified by TLC, IR, $^1H$-NMR, and MS.

The evaluation of the penetration results was accomplished using graphs obtained by plotting the cumulative amount of penetrated drug against time. Permeabilities and fluxes were obtained using the straight portions of the curves according to the following equation:

$$P = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{A} \cdot \frac{1}{C_d}$$

where $P$ is the permeability, $\Delta Q/\Delta t$ is the slope of the straight portion of the curve, $A$ is the surface area, and $C_d$ is the initial drug concentration in the donor compartment.

To reduce variability among runs, shed skin from the same snake was used for each set of experiments. Under the same conditions the penetration enhancements of the drugs were compared with that of azone and lauryl alcohol and were calculated by dividing the steady-state slope of DDAIP or lauryl alcohol by the steady-state slope of the control. By this comparison the penetration enhancement of the drug due to control was taken as 1.0. Since the area of skin was kept constant at 1.8 $cm^2$, the comparison of penetration enhancement of drug by DDAIP, azone, and lauryl alcohol can be done by comparing the slopes of the amount of drug penetrating versus time.

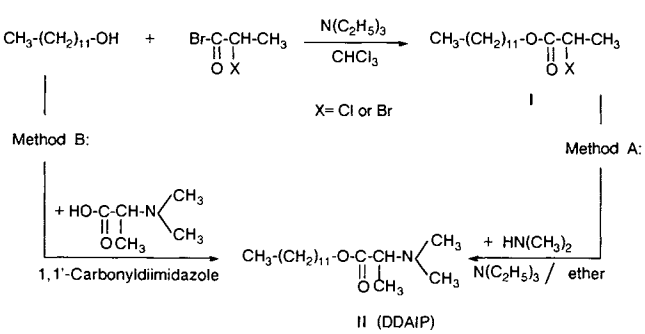
### Effect of DDAIP on *in Vitro* Penetration of Indomethacin

Due to the low solubility of indomethacin (2.35 μg/mL at pH 2.9 and 168.3 μg/mL at pH 5.7) and low enhancement obtained at these pH values (10), the experiments were done at pH 7.0. Figure 1 shows a typical time-course profile of indomethacin for DDAIP, azone, and lauryl alcohol. Each data point represents the mean value from 10 to 20 trials. The flux of the control was always close to zero. Calculated permeabilities and fluxes are given in Table I.

### Effect of DDAIP on *in Vitro* Penetration of Clonidine

*In vitro* transdermal penetration of clonidine through DDAIP-, azone-, and lauryl alcohol-pretreated skin is shown in Fig. 2. Calculated permeabilities and fluxes and relative enhancements are shown in Table II.

To determine the role of pH and drug concentration on clonidine-HCl penetration in the presence of DDAIP, 0.5, 1,




Scheme I. Synthesis of DDAIP [dodecyl 2-(*N*,*N*-dimethylamino)propionate].

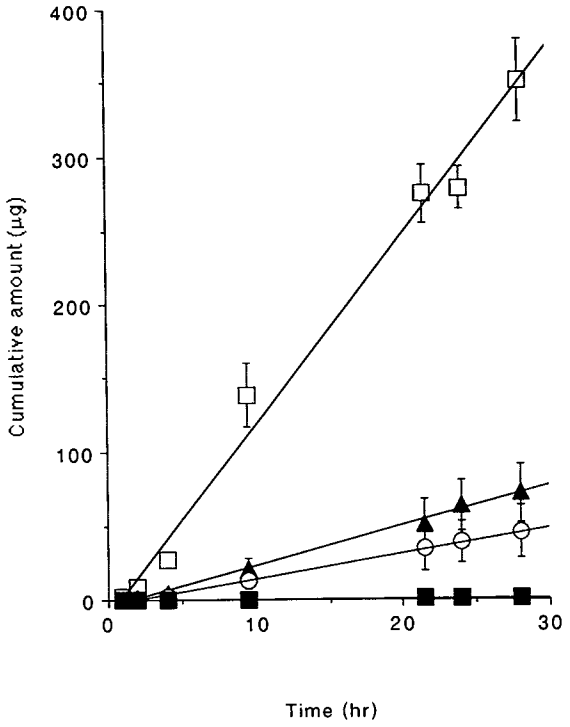



Fig. 1. The penetration profiles of indomethacin in the presence of DDAIP (□), azone (▲), lauryl alcohol (○), and control (■). Each point and bar shows the mean and SD of 20 experiments performed in four different shed snakeskins.

and 2% clonidine-HCl solutions at pH 4.65 and 7.0 were applied to the donor cells. The following flux values were obtained, respectively: 2.7, 6.5, and 13.5 and 24.0, 31.1, and 40.2 μg/hr · $cm^2$, whereas with controls $J$ values were 0.11 and 0.32 μg/hr · $cm^2$.

### Effect of DDAIP on *in Vitro* Penetration of Hydrocortisone

Significant penetration of hydrocortisone was observed in the presence of DDAIP at pH 7.0 as shown in Figure. 3. The flux of hydrocortisone alone was always close to zero. Calculated permeability data for hydrocortisone are given in Table III.

### Biodegradability of the Enhancer

DDAIP was designed to be biodegradable. A diminution of peak heights versus time was obtained in the presence of porcine esterase in pH 7.0 phosphate buffer. Due to the low UV absorbance of DDAIP the detector wavelength was set at 204 nm. The disappearance of the compound was followed by plotting the logarithm of peak heights versus time.

## DISCUSSION

Enhancers are generally reported as substances which could temporarily diminish the barrier of the stratum corneum (2,4). Dodecyl *N,N*-dimethylaminoacetate (DDAA), which was prepared in this laboratory, has been extensively studied. It enhances the transdermal penetration of indomethacin (9) about two times as much as azone.

As a continuation of our work on new active and biodegradable enhancers, some new alkyl *N,N*-dimethylaminoalkanoate derivatives were synthesized. Starting from the DDAA structure, the substituted aminoacetate moiety was replaced with branched- and long-chain alkyl groups. The substitution of one hydrogen atom of the acetate moiety in DDAA with a methyl group gives dodecyl 2-(*N,N*-dimethylamino)propionate (DDAIP).

In the present study we report the synthesis, biodegradability, and enhancements obtained with model acidic, basic, and neutral drugs, i.e., indomethacin, clonidine, and hydrocortisone, by DDAIP. Compared to azone, as a typical enhancer, the absorption promoting effect of DDAIP was substantial. To demonstrate that its enhancing effect is due to the molecule, and not primarily to the decomposition product, lauryl alcohol, which shows some penetration enhancement for various drugs (12), lauryl alcohol was also included in the penetration experiments. Compared to DDAIP, the absorption promoting effect of lauryl alcohol is very small, suggesting that for significant penetration enhancement, the

**Table I.** Effects of DDAIP, Azone, and Lauryl Alcohol on the Penetration of Indomethacin Through Shed Snakeskin at 32°C and pH 7.0 ($n$ = 10–20)

| Compound | Slope[a] | Intercept | $r^b$ | RE[c] | Flux (μg/hr · $cm^2$) | Permeability (cm/hr) |
|---|---|---|---|---|---|---|
| DDAIP | 12.9 (0.4)[d] | −10.75 | 0.989 | 430 | 7.1 | $7.8 \times 10^{-3}$ |
| Azone | 2.7 (0.3) | −4.76 | 0.995 | 90 | 1.5 | $1.7 \times 10^{-3}$ |
| Lauryl alcohol | 1.7 (0.2) | −3.16 | 0.997 | 57 | 1.0 | $1.1 \times 10^{-3}$ |
| Control | 0.03 (0.003) | −0.019 | 0.997 | 1 | 0.02 | $1.8 \times 10^{-5}$ |

[a] Slope of the regression line.
[b] Correlation coefficient of the regression line.
[c] Relative enhancement compared to control.
[d] Standard deviation in parentheses.

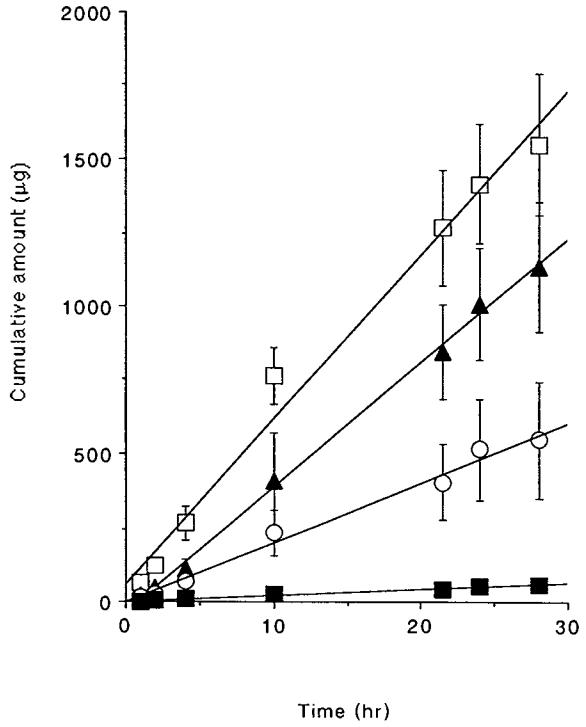



Fig. 2. The penetration profiles of clonidine in the presence of DDAIP (□), azone (▲), lauryl alcohol (○), and control (■). Each point and bar shows the mean and SD of 20 experiments performed in four different shed snakeskins.

intact enhancer is important. The lag times for the penetration profiles of all three drugs were between 0.5 and 1.5 hr.

After 28 hr of the experiment ~70% of indomethacin penetrated through the skin. For azone ~13% of the applied amount was transported to the receptor cell. DDAIP also showed about two times the enhancement of DDAA, an effective biodegradable enhancer developed in our laboratories (9). As the solubility and flux of indomethacin are very low under acidic conditions (10), the experiments were conducted at pH 7.0.

Due to the ionogenic nature of clonidine the flux of each species in solution is the product of its permeability coefficient and its concentration. Using simultaneous equations for $K_B$ and $K_{BH}$ + (10), the permeability coefficients of the nonionized and ionized species were calculated as $1.9 \times 10^{-2}$ and $1.3 \times 10^{-3}$ cm/hr. The premeability coefficient of the nonionized species is about 15 times higher than the ionized species. The fluxes calculated for the nonionized and ionized species are as follows: for pH 4.65, 0.17 and 13.3; and for pH 7.0, 29.1 and 11.1 μg/hr · cm$^2$. At pH 4.65 the contribution to the total flux is due mostly to the flux of protonated species, whereas at pH 7.0 the flux of the nonionized form is 2.6 times higher than the flux of the ionized form. At pH 4.65 the permeability of clonidine is at least three times less than at pH 7.0. This is probably due to the larger concentration of the nonionized form of clonidine at pH 7.0. The role of the initial concentration of clonidine on transdermal delivery was also examined at pH 4.65 and 7.0. It was found that $J_{total}$ values were concentration dependent and they follow Fick's first law. For initial concentrations of 2, 1, and 0.5%, calculated $K_{BH+}$ values were $1.3 \times 10^{-3}$, $1.3 \times 10^{-3}$, and $1.0 \times 10^{-3}$, respectively.

About 30% of the applied hydrocortisone penetrated into the receptor cells with DDAIP pretreated skin, while with azone ~15% was transferred.

The existence of esterase activity has been reported in shed snakeskin (14). To confirm the biodegradability of DDAIP, its fragmentation in the presence of porcine esterase was examined. The plot of the logarithms of the peak heights versus time as shown in Fig. 4 indicates that the degradation follows pseudo-first-order kinetics, with a $k_{obs}$ of 0.038 min$^{-1}$ and a $t_{1/2}$ of 18.5 min. The $k_{obs}$ values is the sum of the hydrolysis due to the esterase and also to the phosphate buffer. Under the same experimental conditions the hydrolysis rate in the buffer was found to be 0.0041 min$^{-1}$. Esterase-catalyzed biodegradability of the enhancer is therefore confirmed. The isolated hydrolysis products, lauryl alcohol and *N*,*N*-dimethylalanine, were verified by comparison with authentic samples. Esterase activity in the skin is probably much lower than that observed for the porcine esterase used in this study. Therefore the apparent $t_{1/2}$ value of the degradation of DDAIP may be much longer in

Table II. Effects of DDAIP, Azone, and Lauryl Alcohol on the Penetration of Clonidine Through Shed Snakeskin at 32°C and pH 7.0 ($n$ = 10–20)

| Compound | Slope[a] | Intercept | $r$[b] | RE[c] | Flux (μg/hr · cm$^2$) | Permeability (cm/hr) |
|---|---|---|---|---|---|---|
| DDAIP | 78.9 (5.8)[d] | −30.66 | 0.998 | 28 | 43.8 | $2.2 \times 10^{-3}$ |
| Azone | 44.0 (4.5) | −37.47 | 0.990 | 16 | 24.4 | $1.2 \times 10^{-3}$ |
| Lauryl alcohol | 25.0 (3.6) | −19.52 | 0.992 | 9 | 13.9 | $0.7 \times 10^{-3}$ |
| Control | 2.8 (0.75) | −1.87 | 0.997 | 1 | 1.5 | $0.8 \times 10^{-4}$ |

[a] Slope of the regression line.
[b] Correlation coefficient of the regression line.
[c] Relative enhancement compared to control.
[d] Standard deviation in parentheses.

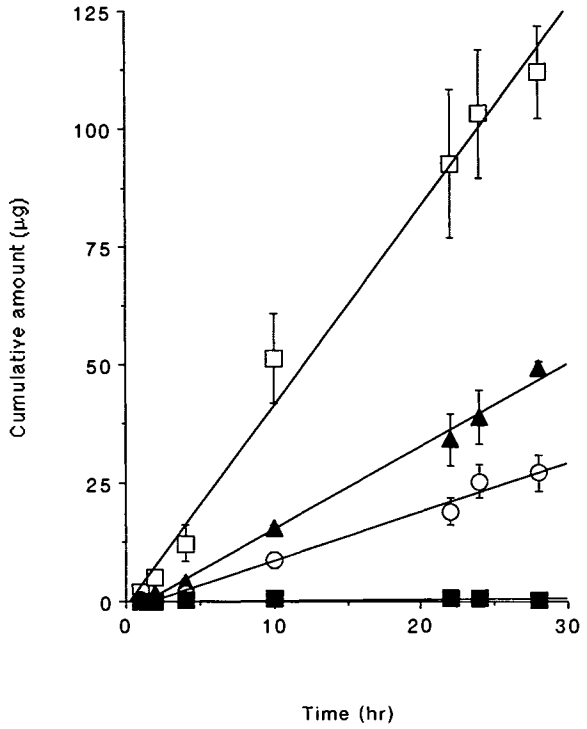



Fig. 3. The penetration profiles of hydrocortisone in the presence of DDAIP (□), azone (▲), lauryl alcohol (○), and control (■). Each point and bar shows the mean and SD of 10 experiments performed in four different shed snakeskins.

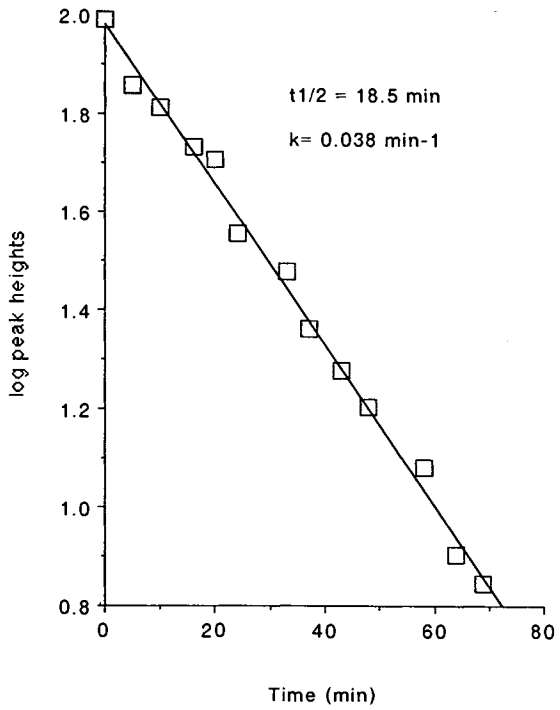



Fig. 4. The biodegradability of DDAIP in the presence of porcine esterase at 32°C and pH 7.0.

the skin. In a transdermal study with propranolol using DDAA as enhancer, where $t_{1/2}$ = 6.8 min in the presence of porcine esterase (15), Hirvonen *et al.* (16) showed that the delivery is no longer enhanced after 4 days, whereas with azone enhancement still existed after 7 days.

The relative enhancement obtained in this study was especially marked for indomethacin; relatively lower enhancements of hydrocortisone and clonidine are probably related to their different lipophilicities and p$K_a$ values. The results show that DDAIP is a biodegradable compound, has excellent transepidermal penetration enhancing properties, and can be used as an enhancer for several classes of drug substances.

## ACKNOWLEDGMENTS

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Table III. Effects of DDAIP, Azone, and Lauryl Alcohol on the Penetration of Hydrocortisone Through Shed Snakeskin at 32°C and pH 7.0 ($n$ = 10–20)

| Compound | Slope[a] | Intercept | $r^b$ | RE[c] | Flux (μg/hr · cm²) | Permeability (cm/hr) |
|---|---|---|---|---|---|---|
| DDAIP | 4.24 (0.58)[d] | −0.887 | 0.986 | 265 | 2.3 | $6.4 \times 10^{-2}$ |
| Azone | 1.75 (0.32) | −2.207 | 0.993 | 109 | 0.9 | $2.6 \times 10^{-3}$ |
| Lauryl alcohol | 1.03 (0.29) | −1.528 | 0.990 | 64 | 0.5 | $1.5 \times 10^{-3}$ |
| Control | 0.016 (0.009) | 0.119 | 0.992 | 1 | 0.009 | $2.5 \times 10^{-5}$ |

[a] Slope of the regression line.
[b] Correlation coefficient of the regression line.
[c] Relative enhancement.
[d] Standard deviation in parentheses.

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