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Design of novel prodrugs for the enhancement of the transdermal penetration of indomethacin

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Abstract

A series of esters of indomethacin containing tertiary amine functional groups and designed for transdermal delivery were synthesized. The rates of chemical hydrolysis of all the esters in pH 7.4 phosphate buffer were determined. For *N,N*-diethylaminopropyl *N*-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indole acetate (ester **4**), the rates of chemical hydrolysis in pH 5.5–11.25 buffers were investigated in detail. Ester **4** was chemically stable and had a higher *n*-octanol/water partition coefficient and a greater solubility in water at pH 7.4 than indomethacin. Furthermore, the rate of transdermal penetration of ester **4** through cadaver skin in Franz cells was found to be more than 10-times faster than that of indomethacin itself. Ester **4** was found to possess surface-active properties (CMC = 0.5 mg/ml). Assuming that micelles cannot penetrate biologic membranes, a corrected permeability coefficient was calculated for ester **4** using only the monomer concentration. This value, 3.6×10^{-2} cm/h, was 100-times greater than that of indomethacin. These results suggest that prodrugs with structures similar to that of ester **4** may be useful for enhancing transdermal penetration of other carboxylic acid-containing anti-inflammatory agents.

Keywords: Indomethacin; Prodrug; Transdermal delivery

1. Introduction

The prodrug ester approach is one method of enhancing the activity of a drug by increasing its penetration rate through the skin. Since the epidermis contains many nonspecific enzymes which are capable of cleaving prodrug esters to release the active parent compounds, this approach has been widely used to improve the systemic delivery of many drugs. The enhanced penetration rate due to chemical modification is the result of modifying the drug-skin and/or drug-vehicle interactions (Sloan et al., 1984).

Milosovich and co-workers (Milosovich, 1988; Milosovich et al., 1989, 1993) showed that the transdermal penetration rates of lipophilic compounds such as testosterone, cortisone, and indomethacin can be enhanced by forming ester

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prodrugs containing a tertiary *N,N*-dialkyl (i.e., dimethyl or diethyl) functional group. This structural modification appeared to produce a unique enhancement of the transdermal penetration rates of several compounds, both in vitro, using cadaver skin and in vivo, in the hairless mouse. The results suggested that, in the case of testosterone (Milosovich, 1988), the enhancement observed for the prodrugs may be the result of one or more of the following factors: (1) increased aqueous solubility; (2) increased skin/aqueous partitioning; (3) surfactant properties of the prodrugs which may affect the skin structure; and (4) physicochemical properties of the prodrugs; i.e., they contain a tertiary amine functional group which is positively charged at physiologic pH values as a result of protonation.

The testosterone and cortisone prodrugs were chemically stable enough to be formulated into pharmaceutical dosage forms, but the most promising indomethacin prodrug appeared to be too unstable ($t_{1/2} = 3$ h in pH 7.4 buffer) for practical use. Since the indomethacin prodrug showed promise for enhanced transdermal delivery of indomethacin, the work described in this report was undertaken to design a more stable analog of indomethacin and to study its physical chemical properties as well as its transdermal penetration rate.

2. Experimental

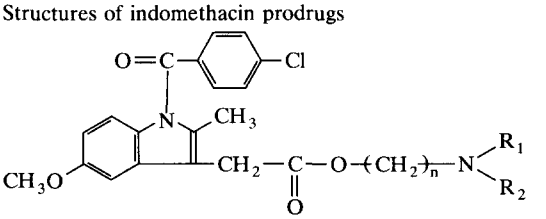
2.1. Materials

N,N-Diethylethanolamine, *N,N*-dimethylethanolamine, 3-dimethylamino-1-propanol, 3-diethylamino-1-propanol, 2-(diisopropylamino)-ethanol, 2-(dibutylamino)ethanol, 1-piperidineethanol, 4-(2-hydroxyethyl)morpholine, 1-(1-hydroxyethyl)pyrrolidine, oxalyl chloride, tetramethylsilane (TMS), and sodium acetate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Indomethacin and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Methylene chloride (ACS grade), ethyl acetate (ACS grade), diethyl ether (ACS grade), methanol (HPLC grade), ethanol (HPLC grade), acetonitrile (HPLC grade), *n*-octanol (HPLC grade) and acetone (ACS grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Human plasma was purchased from the Central Kentucky Blood Center (Lexington, KY), stored at 4° C, and used within 5 days. Sterile water for irrigation, USP, was purchased from Travenol Laboratories (Deerfield, IL). Cadaver skin, 3×8 inch strips, harvested from the posterior thigh, was purchased from St. Agnes Hospital Tissue Bank (Philadelphia, PA).

2.2. Equipment

NMR spectra were recorded on a Varian model XL-Gemini 200 MHz spectrometer (Walnut Creek, CA). Chemical shifts were reported in ppm downfield from TMS as internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. (Atlanta, GA). Melting points were determined on a Uni-Melt Capillary Melting Point Apparatus, purchased from Arthur H. Thomas Co. (Philadelphia, PA), and are uncorrected. Franz cells (FDC = 100, 15 mm diameter) equipped with star-head magnetic stirrers were purchased from Crown Glass Co., Inc. (Sommerville, NJ). The cells were maintained at 37° C using a Haake F-Junior recirculating water-bath purchased from Cole-Parmer Instru-

Table 1
Structures of indomethacin prodrugs



Compound	*n*	R_1	R_2
1	2	$-C_2H_5$	$-C_2H_5$
2	2	$-CH_3$	$-CH_3$
3	3	$-CH_3$	$-CH_3$
4	3	$-C_2H_5$	$-C_2H_5$
5	2	$-CH(CH_3)_2$	$-CH(CH_3)_2$
6	2	$-(CH_2)_3CH_3$	$-(CH_2)_3CH_3$
7	2	$-CH_2-(CH_2)_3-CH_2-$	
8	2	$-CH_2-(CH_2)_2-CH_2-$	
9	2	$-CH_2-CH_2-O-CH_2-CH_2-$	

ment and Equipment Co. (Chicago, IL). Surface tension was measured using a monolayer balance Model 3000, purchased from KSV Instruments (Helsinki, Finland).

2.3. General method of synthesis of indomethacin esters

The esters shown in Table 1 were synthesized according to the following procedure:

Indomethacin (5 g, 14 mmol) and oxalyl chloride (8 ml, 96 mmol) were placed in a 200 ml round-bottom flask. The reaction was allowed to proceed at ambient temperature until evolution of HCl (gas) ceased. Nitrogen was bubbled through the resulting solution to remove the unreacted oxalyl chloride. The resulting acid chloride, a pale yellow solid, was not isolated but was immediately dissolved in CH_2Cl_2 (100 ml) in a round-bottom flask, and this solution was utilized in the preparation of the esters listed in Table 1. The appropriate *N,N*-dialkyaminoalkanol (R_2N-$(CH_2)_n$-OH; 7.0 mmol) was dissolved in CH_2Cl_2 (10 ml) and the solution was added dropwise to the above indomethacin acid chloride solution, with stirring, over a 10 min period. The resulting solution was then refluxed overnight. The reaction mixture was transferred to a separatory funnel, extracted with 5% Na_2CO_3 solution (3 × 50 ml), washed with distilled water (3 × 50 ml), dried over anhydrous Na_2SO_4, and filtered using suction. The CH_2Cl_2 was removed under reduced pressure at 35° C using a rotary evaporator. Anhydrous diethyl ether (400 ml) was then added and HCl gas was bubbled through the ether solution until a white precipitate resulted. The suspension was stirred for 30 min at ambient temperature and then filtered. The resulting yellow-colored hydrochloride salt was washed with ether, dried, and recrystallized from ethyl acetate-acetone or from ethanol. Overall yields were greater than 80%. The structures of the compounds prepared by this procedure were confirmed by elemental analysis and NMR spectroscopy (Jona, 1991). Purity was determined by elemental analysis, TLC, and HPLC.

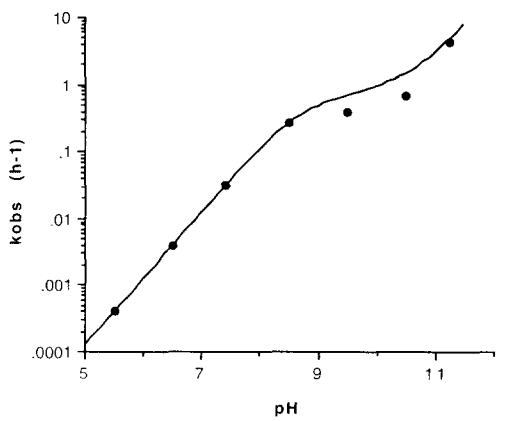


Fig. 1. pH-rate profile for ester **4**. The data points (•) were determined experimentally. The line was generated using Eq. 1.

2.4. Analytical methods

High-performance liquid chromatographic (HPLC) analyses were performed on a system consisting of a Beckman Model 112 pump (Berkeley, CA) and a Kratos Spectroflow 773 Absorbance Detector (Westwood, NJ). Samples were introduced with a 100 μl Hamilton syringe (Reno, NV) into a Rheodyne Model 7125 loop injector (Cotati, CA) equipped with a 20 μl loop. The column employed was an Alltech 10 μm Lichrosorb RP8 column, 250 × 4.6 mm (Deerfield, IL). The mobile phase consisted of 50% acetonitrile and 50% 0.1 M acetate buffer, pH 4.0 (pH adjusted with sulfuric acid). All samples were run at ambient temperature. The flow rate was 2.0 ml/min, and UV detection was carried out at 242 nm.

2.5. Stability studies in aqueous buffers

The reactions were initiated by adding 250 μl of a stock solution of the specific ester, 2 mg/ml in either phosphate buffer (esters **4**, **7**, and **9**) or acetonitrile (esters **1**, **2**, **3**, **5**, and **8**) to 10 ml of a pH 7.4, 0.05 or 0.5 M phosphate buffer solution (the ionic strength of the 0.05 M buffer was adjusted to 0.5 μ with NaCl), preheated to 37° C, in a screw-top vial. The solutions were kept in a water bath at 37° C. At appropriate time inter-

vals, 100 μl samples were removed and injected into the HPLC system. The rate constant for the hydrolysis of each ester was determined by log-linear regression of semilog plots of percent remaining vs time ($r > 0.98$). The experiments were run in triplicate for each ester. The half-lives did not vary more than ±10%. The pH was measured at the beginning and end of each experiment and did not vary by more than ±0.05 units.

The procedure above was repeated for ester **4** in buffer solutions at seven pH values between 5.5 and 11.25 (see Fig. 1). Phosphate buffers were used for pH values from 5.5 to 8.5, and borate buffers were used for pH values from 9.5 to 11.25. No significant buffer catalysis was observed at any pH value.

2.6. In vitro plasma hydrolysis studies

The reactions were initiated by adding 500 μl of stock solution containing either ester **1**, **2**, **3**, **4**, **5**, or **9** (2 mg/ml in 0.2 M phosphate buffer, pH 7.4) to 5 ml of human plasma, preheated to 37° C, in screw-top vials. The solutions were kept in a water bath at 37° C, and at appropriate time intervals, 250 μl samples were withdrawn and added to 500 μl of methanol to precipitate the plasma proteins. The samples were vortexed for 30 s, centrifuged for 5 min in an Eppendorf centrifuge, and analyzed by HPLC. The rates of degradation were determined by log-linear regression of semilog plots of amount remaining vs time ($r > 0.97$). Each experiment was run in triplicate. The half-lives did not vary more than ±10%. The pH was measured at the beginning and end of each experiment and did not vary by more than ±0.05 units.

2.7. Solubility studies

An excess of indomethacin or ester **4** was equilibrated with pH 7.4 phosphate buffer (0.5 M) at 25° C in a screw-top vial with frequent shaking, vortexing, and sonicating for about 1 h. The saturated solution was filtered through a 0.2 μm Acro-disc filter, and the filtrate was analyzed by HPLC.

2.8. Partitioning studies (n-octanol / buffer)

A known concentration of indomethacin in pH 7.4 phosphate buffer (0.5 M) was vortexed for 15 min with an equal volume of *n*-octanol in a screw-top vial. The equilibrium concentrations of indomethacin in the buffer and *n*-octanol layers were determined using HPLC. Solutions containing various concentrations (0.1–8.0 mg/ml) of ester **4** were vortexed with an equal volume of *n*-octanol for 15 min in screw-top vials. The concentrations of ester **4** in the buffer and *n*-octanol layers were determined by HPLC. All experiments were conducted in triplicate. The calculated partition coefficients did not vary by more than 10%.

2.9. Surface tension measurements

Solutions containing several different concentrations of ester **4** in pH 7.4 phosphate buffer (0.5 M) were freshly prepared, and their surface tensions were measured in triplicate using the standard Wilhelmy plate technique.

2.10. In vitro transdermal studies

Cadaver skin specimens were kept frozen until ready for use. The specimens were thawed by immersion in a 37° C water bath for 4 min and then placed in a dish containing normal saline overnight to ensure complete hydration. Each skin specimen was cut into approx. 1 inch squares, and each square was mounted between the donor and receptor compartments of a Franz cell with the dermal side facing down. Each skin specimen was examined for holes and tears. After securely clamping the Franz cell assembly together, the receptor compartment was filled with 2% bovine serum albumin/normal saline solution equilibrated to 37° C. Care was taken to ensure that no air bubbles remained in the receptor compartment. The receptor fluid was stirred continuously at 600 rpm, and 500 μl of a 10% suspension of indomethacin or ester **4** in pH 7.4 phosphate buffer (0.5 M) was applied to the upper surface of the skin ($t = 0$). The cell system was occluded

Table 2
Observed half-lives for hydrolysis of the indomethacin prodrug esters (pH 7.4 phosphate buffer, 0.05 M, $\mu = 0.5$, 37° C, $n = 3$)

Ester	Half-life ± S.D. (h)
1	2.8 ± 0.2
2	1.2 ± 0.1
3	9.5 ± 0.8
4	22.7 ± 1.8
5	5.0 ± 0.3
6	41.3 ± 1.9
7	1.8 ± 0.1
8	2.8 ± 0.2
9	10.2 ± 0.5

by securely placing a rubber stopper in the cell cap. At the following time points: 0, 2, 4, 6, 8, 10, 12, and 24 h, the entire contents of the receptor compartment were removed using a 10 ml syringe fitted with a 3 inch length of small-bore silicone rubber tubing. The receptor compartment was refilled with fresh receptor fluid immediately after sampling. A 500 μl aliquot of the receptor fluid was added to 1.0 ml methanol in a test tube to precipitate the albumin. The test tube was then vortexed for 30 s and centrifuged at 10000 rpm for 5 min. The resulting supernatant was analyzed for indomethacin and its ester using HPLC.

3. Results and discussion

The hydrolysis of all the esters in aqueous solution at 37° C and pH 7.4 followed first-order kinetics for at least four half-lives. The half-lives for hydrolysis of all the esters under the same conditions are listed in Table 2. Although each experiment was performed in triplicate, inter-experiment variation was less than 10%. It is apparent from Table 2 that esters **4** and **6** are chemically the most stable prodrugs of indomethacin, whereas esters **2** and **7** are the least stable.

The influence of pH on the hydrolysis of ester **4** at 37° C is shown in Fig. 1. The shape of this pH-rate profile indicates that the hydrolytic processes within the pH range studied can be described in terms of specific base-catalyzed hydrolysis of the protonated and neutral species of the prodrug ester (Hussain and Schurman, 1969; Martin et al., 1993).

The results of the hydrolysis studies with the other eight prodrug esters (Table 2) showed that both the length of the alkyl chain of the ester moiety and the size of the substituent on the nitrogen have significant effects upon the rates of hydrolysis of these esters. These results are consistent with the intramolecular involvement of the protonated form of the tertiary amine stabilizes the initial transition state in the esterolysis mechanism, and shown in Structure 10. It is evident from the above that the stability of such a transition state would be significantly influenced by the length of the alkyl chain, as well as the molecular volume of the substituents on the tertiary amino group. Alkyl chains longer than $-(CH_2)_3-$ appear to form thermodynamically less stable transition states than shorter chains, and this results in a slower rate of hydrolysis.

The effect of the lengths of the side chains on intra-molecular hydrolyses has been observed in transacylation reactions of hemiesters of salicylamide (Tawfiq et al., 1990) and in the hydrolyses of hemiesters of *p*-nitrophenol (Gaetjens and Morawetz, 1960). Increasing the length of the chain by two $-(CH_2)-$ groups greatly reduced the rates of both of these reactions. This was not attributable to the slight differences in the pK_a values of the carboxylic groups, but to the involvement of a transition state intermediate stabilized by a hydrogen bond (Tawfiq et al., 1990).

In the current study, esters with bulky N-substituents were found to have slower hydrolysis rates, and this is attributable to decreased stability of the transition state via a sheer steric effect. These data suggest that more chemically stable analogs of indomethacin prodrug esters with structures similar to that of ester **4** could be prepared by careful structural modification of the amino alcohol moiety.

Since a prodrug ester of testosterone with an amino alcohol side chain structurally similar to that of ester **4** was found to penetrate cadaver skin at a much faster rate than testosterone itself (Milosovich et al., 1993) and since ester **4** is chemically stable and has desirable aqueous and

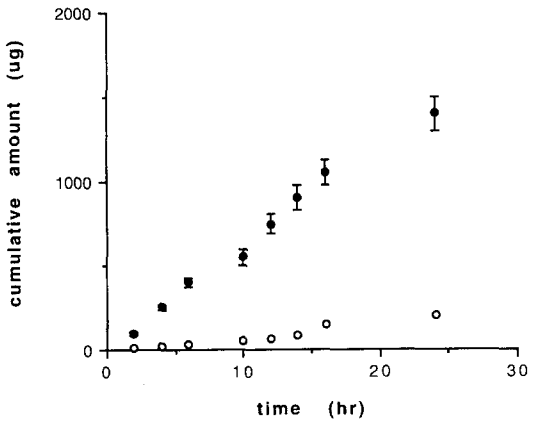


Fig. 2. Cumulative amounts of indomethacin (○) and ester **4** (●) found in the receptor compartments of Franz cells with allograft cadaver skin.

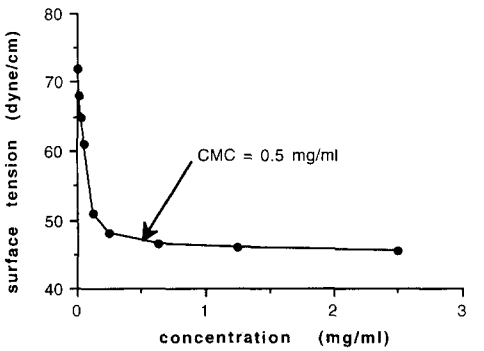


Fig. 3. Plot of surface tension vs concentration for ester **4** in pH 7.4 buffer.

non-aqueous solubilities, it was decided to examine its transdermal penetration characteristics, in vitro, using human cadaver skin.

It was found that ester **4** penetrated cadaver skin at a considerably faster rate than indomethacin, as shown in Fig. 2. Furthermore, the indomethacin prodrug esters described above were found to hydrolyze rapidly in human plasma (see Table 3). The results suggest that these prodrugs would generate indomethacin rapidly following transdermal delivery to the blood.

To gain an insight into the physical chemical properties that might cause the observed enhancement of the skin penetration rate of ester **4**, its solubility in water at pH 7.4, its *n*-octanol/pH 7.4 buffer partition coefficient, and its surface-active properties were studied. The data in Table 4 show that both the solubility and the partition coefficient of ester **4** are greater than those of indomethacin. On the other hand, ester **4** was found to possess marked surface-active properties, as shown in Fig. 3. The CMC of ester **4** was found to be 0.5 mg/ml, a value close to that of sodium lauryl sulfate (Ong and Kostenbauder, 1976).

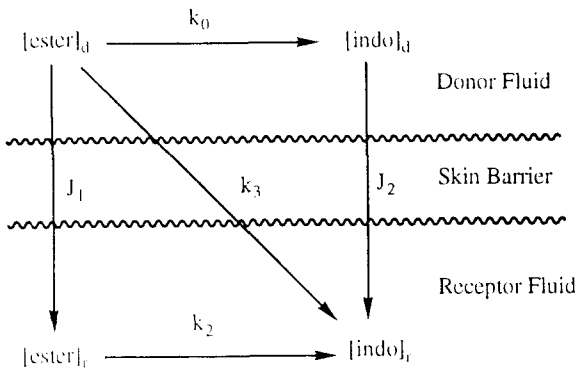
To accurately assess the influence of the permeability coefficient of the intact ester upon the overall skin penetration rate of ester **4**, it is

Table 3
Observed half-lives for hydrolysis of the indomethacin prodrug esters in human plasma (37° C) ($n = 3$)

Ester	Half-life ± S.D. (min)
1	8.7 ± 0.7
2	28.1 ± 1.7
3	3.2 ± 0.2
4	6.9 ± 0.5
5	8.3 ± 0.5
9	36.6 ± 1.8

Table 4
Physicochemical parameters for indomethacin and ester **4** (25° C)

Parameter	Indomethacin	Ester **4**	*n*
Measured			
Solubility (mg/ml) in pH 7.4 phosphate buffer (0.5 M)	2.9 ± 0.05	13.8 ± 0.1	5
Partition coefficient (octanol/pH 7.4 buffer)	18.3 ± 1.1	113 ± 2.5	5
CMC (mg/ml)	–	0.5 ± 0.02	3
Calculated			
Diffusion coefficient (cm^2/s)	2×10^{-11}	2×10^{-11}	
Permeability coefficient (cm/h)	3.2×10^{-4}	3.6×10^{-2} (micelle) 1.3×10^{-3} (non-micelle)	



Scheme 1.

necessary to isolate the flux value for penetration of the intact ester from the other rate processes responsible for disappearance of the ester from the donor compartments of Franz cells. Specifically, the rates of hydrolysis of the ester in the donor and receptor compartments and its rate of hydrolysis during residence in the skin barrier must be considered. The rate processes responsible for the disappearance of ester **4** from the donor compartment are summarized in Scheme 1. If ester hydrolysis occurs at a significant rate in the donor fluid, $[ester]_d$ will disappear at a more rapid rate than would be expected if only transdermal penetration of the intact ester were occurring. If the rate of appearance of the ester in the receptor fluid is followed, hydrolysis in the receptor compartment will result in smaller values of $[ester]_r$ than would be expected.

The appearance of the ester in the receptor fluid can be expressed mathematically as follows:

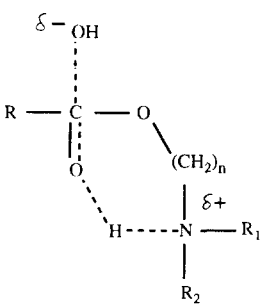
$$\frac{d[ester]_r}{dt} = D_S \cdot K_{SV} \cdot S \cdot \frac{[ester]_d}{h} - k_2[ester]_r - k_0[ester]_d \quad (1)$$

where $d[ester]_r/dt$ is the overall rate of appearance of ester **4** in the receptor fluid, D_S denotes the diffusion coefficient (cm^2/s), K_{SV} is the partition coefficient, S represents the surface area of the skin segment (cm^2), $[ester]_d$ is the concentration of the ester in the donor fluid, h denotes the thickness of the skin (cm), k_2 corresponds to the first-order rate constant for hydrolysis of the ester in the receptor fluid, $[ester]_r$ represents the concentration of intact ester in the receptor fluid, and k_0 is the first-order rate constant for hydrolysis of the ester in the donor fluid.

Although the in vitro stability studies suggested that the rate of ester hydrolysis in the donor solution ($k_0 \cdot [ester]_d$) could be significant, experimentally the donor fluid was kept saturated with respect to the ester at all times; therefore, $[ester]_d$ remained constant throughout the experiment and the first term on the right-hand side of Eq. 1 is a constant.

Ester **4** was found to hydrolyse relatively rapidly in the receptor fluid (2% bovine serum albumin in pH 7.4 buffer) with a first-order rate constant (k_2) of 5.8×10^{-3} h^{-1} ($t_{1/2} = 119$ h), and this value was used in calculations involving Eq. 1.

Recently, it was reported (Roy and Manoukian, 1994) that significant esterase activity (vs ketorolac prodrug esters) can be demonstrated in cadaver skin specimens that are obtained within 24–48 h postmortem, preserved in Ringer's solution, and studied within 24 h of collection. On the other hand, the enzymatic activity of the skin specimens used in this report was probably destroyed by their handling; i.e., frozen storage, thawing, washing with normal saline, etc. Also, the ester passes through the skin barrier rapidly, resulting in a short residence time for exposure to the hydrolytic enzymes that are found in viable epidermis (Holland et al., 1984). For these reasons, k_3 in Scheme 1 was considered to be negli-



Structure **10**.

gible and no term involving k_3 was included in Eq. 1.

Taking into consideration these assumptions, the apparent corrected flux value (J) at steady state can be calculated using the following equation (Jona, 1991):

$$J = D_S \cdot K_{SV} \cdot S \cdot \frac{[\text{ester}]_d}{h} = \frac{k_2[\text{ester}]_r}{(1 - e^{-k_2 \cdot t})} \qquad (2)$$

In order to determine which of the interrelated physicochemical parameters shown in Eq. 2 and Table 4 best correlates with the observed enhancement in the rate of penetration of the prodrug, the values of the diffusion coefficients (D_S) and the permeability coefficients were calculated using Eq. 2 and compared.

The enhancement in the flux of ester **4** compared with that of indomethacin cannot be attributed to differences in the diffusion coefficients of the two compounds through the skin. The apparent diffusion coefficients (D_S) of indomethacin and ester **4**, as calculated using the Daynes and Barrer lag time equation (Scheuplein and Blank, 1971), were both approx. 2×10^{-11} cm^2/s. This value is extremely close to that found for other compounds with similar physicochemical properties, such as the narcotic antagonist, fentanyl (Plezia et al., 1986).

For ester **4**, two values were calculated for the permeability coefficient. One value, 1.3×10^{-3} cm/h (listed under non-micelle in Table 4), was obtained by assuming that the driving force for diffusion was the total concentration of ester **4** in solution at pH 7.4 (i.e., 13.8 mg/ml). This permeability coefficient is about 4-times greater than that of indomethacin.

However, if it is assumed that, at the concentration used in the diffusion study, the ester exists largely as micelles (see Fig. 3), and since it may be assumed that micelles are not able to cross biological membranes (Wilson and Dietschy, 1972), a second value for the permeability coefficient can be calculated using the critical micelle concentration as the driving force. This value (listed under micelle in Table 4) was found to be 3.6×10^{-2} cm/h. If this value is the true permeability coefficient of the ester, then human skin is about 100-times more permeable to ester **4** than to indomethacin. The permeability coefficient of indomethacin at pH 7.2 and 32° C through cadaver skin was reported to be 3.6×10^{-4} cm/h (Hirvonen et al., 1991), which is remarkably close to the value of 3.2×10^{-4} cm/h shown in Table 4.

Based on the ratio of their partition coefficients, one would expect the permeability coefficient of ester **4** to be 6-times greater than that of indomethacin. If one assumes that the driving force for diffusion of ester **4** through the skin is its total concentration in solution at pH 7.4, then the difference in the permeability coefficients for indomethacin and ester **4** would be determined only by the difference in their respective partition coefficients. If, however, one assumes that only the monomer can penetrate through the skin and the micelles cannot, then such large differences in the two permeability coefficients cannot be rationalized based solely upon the differences in their partition coefficients. In this case, enhancement in the permeability coefficient may be due to one or more of the following phenomena:

(a) The prodrug, being positively charged, diffuses by virtue of a charge interaction with the negatively charged skin components (Burnette, 1989).

(b) Ester **4** and indomethacin penetrate the skin via different pathways. Based on previous reports of the diffusion characteristics of compounds with structural features similar to those of ester **4** and with *n*-octanol/water partition coefficients greater than 40 (e.g., fentanyl and sufentanil), it appears that the diffusion of these compounds are aqueous strata controlled, whereas the diffusion of compounds with *n*-octanol/water partition coefficients in the range of that of indomethacin (i.e., approx. 18) are controlled by the stratum corneum (Chien, 1982; Scheuplein and Bronaugh, 1983; Flynn, 1985).

(c) Ester **4** exhibits surface activity, and hence enhances its own penetration. Ester **4** was found to exhibit surface activity as determined by a reduction in the surface tension of its solution. Its CMC was found to be 0.5 mg/ml. This value is in the range observed for surfactants, such as sodium lauryl sulfate (Ong and Kostenbauder, 1976). It

has been shown that the most effective transdermal enhancer for indomethacin is dodecyl *N,N*-dimethylamino acetate (Hirvonen et al., 1991). Upon pretreating cadaver skin with this pure enhancer for 3.5 h, the permeability coefficient of indomethacin at pH 7.2 was increased from 3.6×10^{-4} to 1.3×10^{-2} cm/h, representing an enhancement in the total flux of about 36-fold. Although the mechanism of its action is not known, dodecyl *N,N*-dimethylamino acetate accelerates the penetration of both lipophilic and hydrophilic compounds through human and animal skin (Hirvonen et al., 1991). The permeability coefficient of prodrug ester **4** and its total flux is very similar to that found for indomethacin in the presence of the enhancer. In view of the similarity between the structure of ester **4** and dodecyl *N,N*-dimethylamino acetate, it is possible that the prodrug enhances its own absorption by a mechanism similar to that of the enhancer. It is conceivable that the observed enhancement is partially due to the surface activity of ester **4**.

The results of this study along with the observations of Milosovich and co-wokers with a prodrug of testosterone (Milosovich, 1988; Milosovich et al., 1993) suggest that structural modifications similar to those used for indomethacin could be applied to other anti-inflammatory agents containing a carboxylic group, as well as to other lipophilic compounds that can be esterified with tertiary aminoalkanols or tertiary amino acids.

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