Prodrugs of Peptides. 18. Synthesis and Evaluation of Various Esters of Desmopressin (dDAVP)

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Various aliphatic carboxylic acid esters and a carbonate ester of the tyrosine phenolic group in desmopressin were synthesized to assess their suitability as prodrugs with improved bioavailability compared to the parent peptide. The chemical stability of the esters in aqueous solution was similar to that of simple phenol esters. The derivatives were quantitatively converted to desmopressin by enzymatic hydrolysis in human plasma and rabbit liver homogenate. The esters with a straight side chain were rapidly hydrolyzed by α-chymotrypsin, but the sterically hindered pivalate ester proved more stable than desmopressin itself toward this proteolytic enzyme. All the esters were more lipophilic than desmopressin in terms of octanol–buffer partition coefficients. The transport of the compounds across confluent monolayers of Caco-2 cells was examined. No correlation between permeability and lipophilicity was found but the pivalate ester showed a markedly higher flux relative to desmopressin. It is concluded that appropriate esterification of desmopressin at its tyrosine group may be a potentially useful prodrug approach.

KEY WORDS: desmopressin (dDAVP); prodrug; peptide; enzymatic hydrolysis; lipophilicity; cell culture; transport.

INTRODUCTION

Desmopressin [1-(3-mercaptopropanoic acid)-8-D-arginine vasopressin; dDAVP] (Fig. 1) is a synthetic analogue of the antidiuretic hormone vasopressin used in the treatment of central diabetes insipidus and nocturnal enuresis and as a haemostatic agent (1). The drug is usually administered orally or intranasally. However, the bioavailability of the peptide is only 2–10% for the nasal route (2–4) and 1% for the oral route (3). The poor bioavailability is due largely to its low lipophilicity (5,6), but degradation of the peptide by enzymes in the gut lumen and intestinal and nasal mucosal tissues may also play a role (7–9).

Bioreversible derivatization of desmopressin to give more lipophilic prodrug forms may be a useful approach to enhance its bioavailability. We have recently shown that esterification of the tyrosine phenolic group in α-*N*-acylated tyrosine amide model compounds improved the stability of the tyrosine amide bond toward hydrolysis by the pancreatic enzyme α-chymotrypsin (10). The esters are readily hydrolyzed by plasma and liver esterases with formation of the parent peptide. These findings inspired us to apply this prodrug approach to desmopressin.

In this work, we prepared a number of aliphatic carboxylic acid esters and a carbonate ester of the tyrosine phenolic group in desmopressin (I–VI) (Scheme I) and determined their lipophilicity, reactivity toward α-chymotrypsin, and esterase-catalyzed hydrolysis and chemical stability in aqueous solution. Further, the transport of the compounds was examined across confluent monolayers of Caco-2 cells, as an *in vitro* model the intestinal epithelium for the study of drug transport (11–13).

MATERIALS AND METHODS

Chemicals

Desmopressin acetate was obtained from Carlbiotech A/S, Copenhagen, Denmark. α-Chymotrypsin (type II, from bovine pancreas; 56 U/mg) was purchased from Sigma Chemical Co. (St. Louis, MO). Chemicals and solvents used in the synthesis and the kinetic studies were of reagent grade.

Preparation of Desmopressin Esters (I–VI)

The esters were prepared by reacting desmopressin with an excess of the appropriate acid anhydride or chloroformate in alkaline aqueous solution, in most cases with the addition of acetonitrile to enhance solubility, purified by preparatory HPLC, and isolated as acetate salts formed with the arginine group. Some chloride ions may, however, also be present as counter ions. Under these mild conditions, only the phenolic group at the tyrosine residue reacted with the anhydrides or chloroformates as expected. HPLC analysis of the reaction mixtures revealed only one new peak formed with simultaneous disappearance of the peak due to desmopressin. The esters isolated contained less than 1% of desmopressin and were characterized by their extent of conversion to the parent desmopressin upon incubation in a 0.02 *M* borate buffer of pH 10.0 for 24 hr at 37°C as determined by HPLC (see below).

O-*Propionyl Desmopressin (I).* Propionic anhydride (0.10 ml, 0.72 mmol) was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 20 ml of a 4% aqueous solution of sodium bicarbonate, with the pH being adjusted to 9.5. The mixture was stirred at room temperature (20–25°C) for 1 hr, whereupon the pH of the reaction solution was brought to 5 by the addition of 2 *M* hydrochloric acid. The solution was applied on a preparative HPLC column (Prep Nova-Pak HR C18; 25 × 100 mm; particle size, 6 μm) equipped with a Guard Pak column (Nova-Pak HR C18; 25 × 10 mm; particle size, 6 μm). The column was eluted gradiently with an aqueous 0.05 *M* acetic acid containing increasing amounts (0–60%) of ethanol using a Waters HPLC pump 510. The flow rate was 7 ml min^{-1} and the eluant was monitored at 228 nm using an LKB Broma 2238 Uvicord SII detector. The eluant fractions containing the title compound were collected, concentrated by evaporation *in vacuo,* and lyophilized to yield *O*-propionyl desmopressin as the acetate sale in a yield of 80%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 100.

O-*Pivaloyl Desmopressin (II).* A solution of pivalic an-

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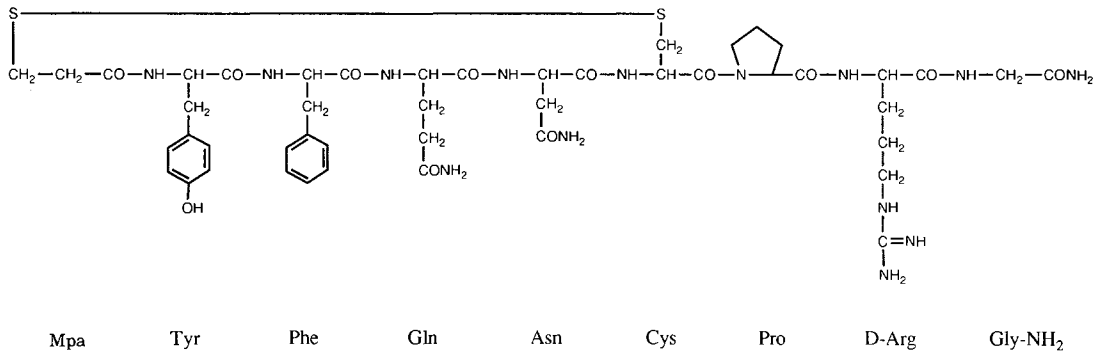
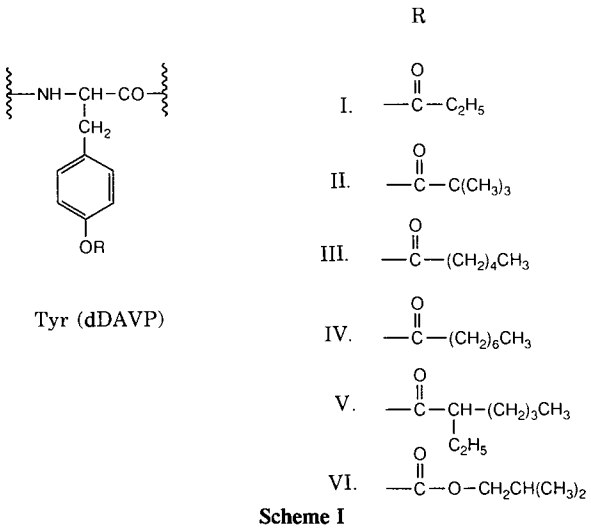


Fig. 1. Structural formula of desmopressin.

hydride (0.15 ml, 0.72 mmol) in 8 ml of acetonitrile was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 16 ml of a 4% aqueous solution of sodium bicarbonate, with the pH being adjusted to 9.5. The mixture was stirred at room temperature for 20 hr and then treated as described for compound I. The acetate salt of the title compound was isolated as a white solid in a yield of 70%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 100.

O-*Hexanoyl Desmopressin (III).* A solution of hexanoic anhydride (0.17 ml, 0.72 mmol) in 16 ml of acetonitrile was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 30 ml of a 4% aqueous solution of sodium bicarbonate (pH 9.5). The mixture was stirred at room temperature for 2 hr and then treated as described for compound I. The acetate salt of the title compound was isolated as a white solid in a yield of 75%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 101.

O-*Octanoyl Desmopressin (IV).* A solution of octanoic anhydride (0.22 ml, 0.72 mmol) in 24 ml of acetonitrile was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 30 ml of a 4.5% aqueous solution of sodium bicarbonate (pH 9.5). The mixture was stirred at room temperature for 2 hr and then treated as described for compound I except that the purification was done with a Kromasil Eka C8 column (15 × 150 mm; particle size, 10 μm). The acetate salt of the title compound was isolated as a white solid in a yield of 75%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 100.



Scheme I

O-*2-Ethylhexanoyl Desmopressin (V).* A solution of (±)-2-ethylhexanoic anhydride (0.19 g, 0.72 mmol) in 32 ml of acetonitrile was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 40 ml of a 5% aqueous solution of sodium bicarbonate (pH 9.5). The mixture was stirred at room temperature for 48 hr and then treated as described for *O*-octanoyl desmopressin. The acetate salt of the title compound was isolated as a white solid in a yield of 50%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 100.

O-*Isobutyloxycarbonyl Desmopressin (VI).* A solution of isobutyl chloroformate (46 μl, 0.35 mmol) in 14 ml of acetonitrile was added to a stirred solution of desmopressin acetate (200 mg, 0.18 mmol) in 8 ml of a 4% aqueous solution of sodium bicarbonate (pH 9.5). The mixture was stirred at room temperature for 30 min and then treated as described for *O*-propionyl desmopressin. The acetate salt of the title compound was isolated as a white solid in a yield of 60%. The yield (mol%) of desmopressin formed upon hydrolysis at pH 10 was 96.

HPLC Assays

Desmopressin and its ester derivatives were determined by isocratic reversed-phase HPLC procedures using a Shimadzu system consisting of an LC-6A pump, an SPD-6A variable-wavelength UV detector operated at 215 nm, and a Rheodyne 7125 injection valve with a 20-μl loop. For the analysis of desmopressin a Spherisorb ODS-2 column (100 × 3 mm; 5-μm particles) was eluted at ambient temperature with a mobile phase consisting of 20% (v/v) acetonitrile in 0.1% (v/v) phosphoric acid, with triethylamine added at a concentration of 10^{-3} *M* to improve the peak shape. A deactivated Supelcosil LC-8-DB column (33 × 4.6 mm; 3-μm particles) in conjunction with a Supelguard precolumn was eluted with mixtures of 30–50% (v/v) acetonitrile and 0.1% (v/v) phosphoric acid containing 10^{-3} *M* triethylamine for analysis of the prodrug derivatives. The flow rate was 1.0 ml min^{-1} and quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

In the cell transport studies analysis of desmopressin was performed with a system consisting of a Merck Hitachi pump (Model L-6200), a variable UV detector (Merck Hitachi Model 4200), and an autosampler Merck Hitachi (Model

AS-4000). Data acquisition and processing were performed using a Merck Hitachi HPLC manager (Model D-6000). The analytical column was a reversed-phase Supelcosil LC-18-DB column (33 × 4.6 mm; 3-μm particles) protected with a Supelguard precolumn. The mobile phase consisted of 14% (v/v) acetonitrile in 0.1% (v/v) phosphoric acid adjusted to pH 2.8 and with triethylamine added at a concentration of 5×10^{-3} *M*. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 200 nm. Under these conditions desmopressin showed a retention time of 7.3 min.

Kinetic Measurements

The stability of the compounds was studied in aqueous buffer solutions at 37 ± 0.2°C. Temperature-accelerated stability studies of the pivalate ester (II) were also performed at 60–80°C in order to predict the stability at room and lower temperatures. The buffers used were hydrochloric acid, acetate, phosphate, borate, and carbonate buffers at a total concentration of 0.02 *M*. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The reactions were initiated by adding 100 μl of a stock solution of the compounds in water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being 10^{-5} *M*. The solutions were kept in a water bath at a constant temperature, and at appropriate intervals samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the hydrolysis of the derivatives were determined from the slopes of linear plots of the logarithm of residual derivative against time.

The stability of the compounds in the presence of α-chymotrypsin was examined at 37°C in a 0.1 *M* phosphate buffer solution of pH 7.40 containing the enzyme at a concentration of 0.50 mg ml^{-1}. The initial concentration of the compounds was 2×10^{-5} *M*. At various intervals samples of 250 μl of the reaction solutions were withdrawn and added to 250 μl of a 5% (v/v) aqueous solution of perchloric acid in order to deproteinize the samples and stop the reaction. After mixing and centrifugation for 3 min at 13,000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Hydrolysis of the compounds was also studied in human plasma diluted to 80% with 0.05 *M* phosphate buffer, pH 7.40, and, in some cases, in 20% rabbit liver homogenate at 37°C. The latter was prepared by homogenizing the liver in 5 parts of 0.05 *M* phosphate buffer (pH 7.40) at 4°C. It was not centrifuged but used as such. The initial concentration of the compounds was 2×10^{-5} *M*. Samples of 250 μl were withdrawn and deproteinized by the addition of 500 μl of a 2% solution of zinc sulfate in methanol–water (1:1, v/v). The supernatant obtained following centrifugation was analyzed by HPLC as described above.

Determination of Partition Coefficients

The apparent partition coefficients (*P*) of desmopressin and the ester derivatives were determined in an octanol–buffer system at 21°C. The aqueous phase was a 0.05 *M* phosphate buffer of pH 7.4. The buffer solution and octanol were mutually saturated before use. The compounds were dissolved in the aqueous buffer at a concentration of 10^{-5} *M* and the octanol–buffer mixtures were shaken for about an hour. HPLC analysis of the aqueous phase was used to assure that distribution equilibrium was reached after this period and that no measureable degradation of the compounds occurred. The concentration of the compounds in the aqueous phase before and after partitioning was determined by HPLC analysis, and the partition coefficients were calculated from the following equation:

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_o}\right) \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous buffer phase before and after distribution, respectively, and V_w represents the volume of the aqueous phase and V_o the volume of the octanol phase. The octanol/buffer volume ratio ranged from 1000:1 to 1:2.

Transport Studies

Confluent monolayers of Caco-2 cells (14,15) were used in the transport studies. The cells were obtained from the American Tissue Culture Collection (Rockville, MD) and maintained in tissue culture flasks as previously described (16). Approximately 2×10^6 cells in 2 ml of growth medium were transferred to uncoated polycarbonate filter inserts with an internal diameter of 24.5 mm, a thickness of 10 μm, and a pore size of 0.4 μm (Transwell, MA). The cells were fed every second day and used 23 ± 3 days after seeding. Cells of passage numbers 49–63 were used throughout this study. The cells were mycoplasma negative as determined by the Mycoplasma Laboratory, Statens Seruminstitut, Copenhagen. The integrity of the monolayers was checked by measurements of transmembrane resistance before and after the experiments and by determination of the permeability of the hydrophilic marker [^{14}C]polyethylene glycol (MW 4000) (16). The transmembrane resistance of 23 ± 3-day-old cell monolayers was 432 ± 42 (n = 25) Ω cm^{-2} and the permeability to [^{14}C]polyethylene glycol was less than 0.1%/hr of the dose applied.

The transport studies were performed at 37°C as described previously (16). Briefly 2.50 ml of the transport medium (Hank's balanced salt solution) containing desmopressin or prodrug at a concentration of $2–5 \times 10^{-5}$ *M* was added to the apical compartment and 3.00 ml of transport medium was then added to the basolateral compartment. Agitation was performed by a plate shaker, positioned at an angle of 2.5° relative to horizontal, at 300 rpm. At various intervals 50-μl samples were taken from both compartments. In the case of the prodrugs the samples were added to 40 μl of a 0.1 *M* borate buffer, pH 10.5, and the mixtures allowed to stand at room temperature for complete hydrolysis to desmopressin, which was then determined by HPLC as described above.

The apparent permeability coefficients (P_{app}) were calculated according to

$$P_{app} = \frac{dQ}{dtAC_o} \quad (2)$$

where dQ/dt is the flux across the cell monolayer, A is the surface area of the membrane (4.7 cm^2), and C_o is the initial drug concentration in the donor compartment. In all runs,

the permeability data were obtained under sink conditions, i.e., less than 10% of the dose applied had disappeared.

RESULTS AND DISCUSSION

Chemical Stability of Desmopressin Esters

The kinetics of hydrolysis of the derivatives I–VI was studied in 0.02 *M* phosphate buffer solution, pH 7.40, at 37°C. The stability of the pivalate ester (II) was further investigated over a wide range of pH values and at different temperatures. At constant pH and temperature the hydrolysis of the esters followed strict first-order kinetics over several half-lives, and for all compounds the parent desmopressin was formed in stoichiometric amounts. The half-lives observed for hydrolysis at pH 7.40 and 37°C are listed in Table I. Esters II and V are seen to be appreciably more stable than the other compounds, which may be ascribed to their sterically hindered alkyl side chains. The rates of hydrolysis of the desmopressin esters are comparable to those of similar esters of *N*-acetyl-L-tyrosine amide (10), indicating no noticeable influence of the rest of the desmopressin molecule on the tyrosine ester stability.

The influence of pH on the rate of hydrolysis of the pivalate ester (II) at 60°C is shown in Fig. 2, in which the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. The U-shaped pH–rate profile can be accounted for by the following rate expression:

$$k_{obs} = k_H a_H + k_o + k_{OH} a_{OH} \quad (3)$$

where a_H and a_{OH} are the hydrogen ion and hydroxide ion activity, respectively, k_H and k_{OH} are second-order rate constants for specific acid- and base-catalyzed hydrolysis, respectively, and k_o is a first-order rate constant for spontaneous or water-catalyzed hydrolysis. The following values were found for these rate constants at 60°C:

Table I. Half-Lives of Hydrolysis of Desmopressin and Various Prodrug Derivatives in Buffer Solution, in the Presence of α-Chymotrypsin, and in 80% Human Plasma at 37°C

	Half-life		
Compound	Buffer, pH 7.40[a]	Buffer, pH 7.40,[b] with α-chymotrypsin (0.50 mg ml^{-1})	80% human plasma
Desmopressin	Stable[c]	19 min	>100 hr[d]
I	66 hr	10 min	34 min
II	784 hr[e]	40 min	5.9 hr
III	190 hr	6 sec	19 min
IV	165 hr	<2 sec	7 min
V	4460 hr[e]	2.0/15 min	14.1 hr
VI	115 hr	3 min	8.2 min

[a] Phosphate buffer, 0.02 *M*.
[b] Phosphate buffer, 0.1 *M*.
[c] No degradation of desmopressin was seen after incubation in the buffer for 7 days at 37°C.
[d] About 10% degradation occurred upon incubation for 24 hr.
[e] Half-life calculated from extrapolation of rate data obtained at pH 9.5–11.

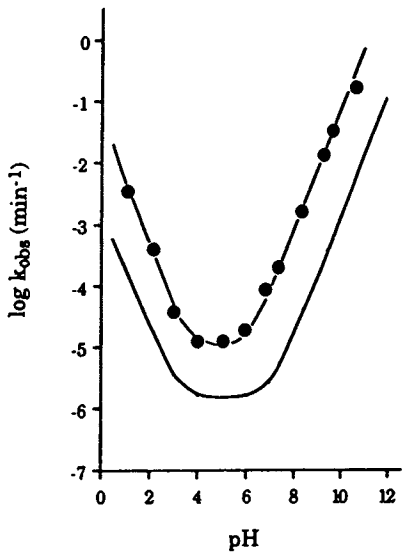


Fig. 2. The pH–rate profiles for the decomposition of the *O*-pivalate ester of desmopressin (II) in aqueous buffer solution (μ = 0.5) at 60°C (●) and at 25°C (——). The latter profile was calculated on the basis of temperature-accelerated studies.

$$k_H = 4.1 \times 10^{-2}\ M^{-1}\ \text{min}^{-1}$$
$$k_o = 9.0 \times 10^{-6}\ \text{min}^{-1}$$
$$k_{OH} = 80\ M^{-1}\ \text{min}^{-1}$$

The effect of temperature (60–80°C) on the rate of hydrolysis of compound II was studied in 0.1 *M* hydrochloric acid (pH 1.1), 0.02 *M* acetate buffer (pH 5.0), and 0.02 *M* borate buffer (pH 10.0). At these pH values either the k_H, the k_o, or the k_{OH} reaction is the predominating degradation pathway. From Arrhenius-type plots values of the activation energy, E_a, and the frequency factor, *A*, were obtained (Table II). On the basis of these data and the Arrhenius equation,

$$\log k = \log A - \frac{E_a}{2.303\ RT} \quad (4)$$

where *R* is the gas constant and *T* is the absolute temperature (K), the pH–rate profile for compound II was constructed at 25°C (Fig. 2). Maximal stability at 25°C occurs at pH 5.0 and the time for 10% degradation at this pH and temperature is estimated to be 49 days. The corresponding $t_{10\%}$ at 4°C is approximately 237 days.

Bioconversion

In order to be useful as prodrugs of desmopressin, the ester derivatives should be readily converted to the parent peptide *in vivo*. Therefore, the stability of the esters was examined in 80% human plasma solutions (pH 7.40) at 37°C. Under the conditions used, all esters were quantitatively hydrolyzed to desmopressin as illustrated in Fig. 3 for propionate ester I. This conversion followed strict first-order kinetics, and the half-lives (Table I) indicate that plasma enzymes efficiently catalyze the ester hydrolysis. As is the case for chemical hydrolysis, the sterically hindered esters II and V are the most stable compounds toward plasma-catalyzed hydrolysis. Hydrolysis is also catalyzed by liver esterases. Thus, the half-lives of hydrolysis of these esters in

Table II. Energies of Activation (E_a) and Frequency Factors (A) for the Hydrolysis of *O*-Pivaloyl Desmopressin (II) in Aqueous Solution (μ = 0.5)

Rate parameter	E_a (kJ mol^{-1})	log$A$ (min^{-1})
k_H	80.0	10.1
k_{OH}	72.1	9.8
k_o	50.7	3.1

a 20% rabbit liver homogenate (pH 7.4) at 37°C were only 0.1 min (ester II) and 1.0 min (ester V). Both esters were quantitatively hydrolyzed to desmopressin, which itself was also degraded in the homogenate more slowly, with a half-life of 26 min.

Stability Toward α-Chymotrypsin

The stability of desmopressin and its various ester derivatives was examined at 37°C in a 0.1 *M* phosphate buffer solution, pH 7.40, containing 0.5 mg ml^{-1} of α-chymotrypsin, corresponding to 2×10^{-5} *M*. The substrate concentration was 2×10^{-5} *M*. Under these conditions all compounds except ester V were found to degrade according to strict first-order kinetics (Fig. 4 and Table I). The degradation of ester V consisted of an initial rapid phase with a half-life of 2.0 min followed by a slower degradation with a half-life of 15 min (Fig. 4). This behavior most likely results from different reactivities of the stereoisomeric forms of the ester with (±)-2-ethylhexanoic acid. Product inhibition can be excluded since similar half-lives were found at different substrate concentrations. Also, the addition of (±)-2-ethylhexanoic acid to the reaction solution did not change the kinetics.

The observed rate of degradation of desmopressin by α-chymotrypsin agrees with previous findings of Matsui *et al.* (17), who reported 54% degradation of desmopressin upon incubation with α-chymotrypsin at a concentration of 0.2 mg ml^{-1} for 30 min at 37°C. On the other hand, other studies have shown that desmopressin remains intact or shows only very little degradation by incubation for 1 hr with pig pancreatic juice (8) or rabbit intestinal juice (18). The concentration of α-chymotrypsin used in the present study

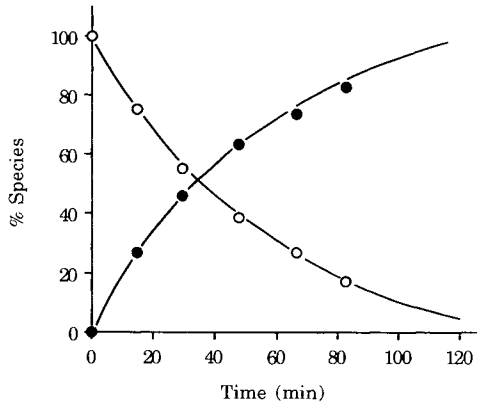


Fig. 3. Plots showing the time courses of the degradation of compound I (○) and formation of desmopressin (●) in 80% human plasma at 37°C.

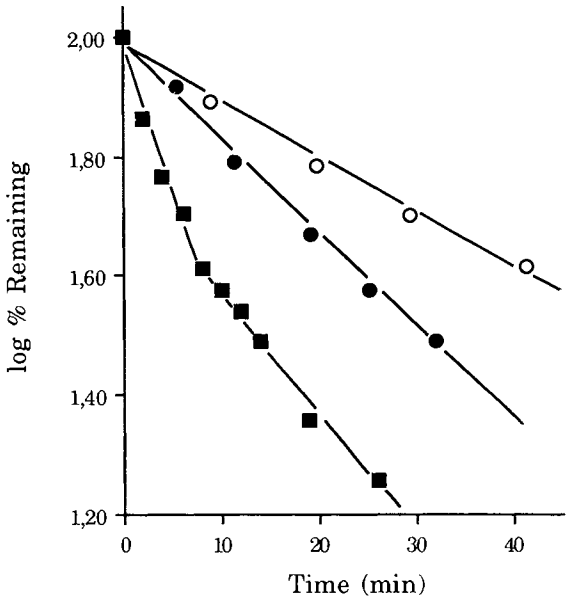


Fig. 4. Time courses of the degradation of desmopressin (●), compound II (○), and compound V (■) in 0.1 *M* phosphate buffer solution, pH 7.4, containing 0.5 mg ml^{-1} of α-chymotrypsin (at 37°C).

(0.5 mg ml^{-1}) corresponds to the normal concentration of the enzyme in the gut including the stool (19,20); however, part of the enzyme may be bound to gut components, thereby having less proteolytic activity. The known substrate specificity of α-chymotrypsin (21,22) suggests cleavage of the Tyr–Phe bond and/or the Phe–Gln bond but this remains to be studied.

Apart from the pivalate ester the desmopressin esters are more readily degraded by α-chymotrypsin than the parent peptide (Table I). The rapid degradation of esters III and IV, however, does not result from increased cleavage of the peptide bond, but of the tyrosine ester bond. Thus, incubation of esters III and IV with α-chymotrypsin resulted in quantitative release of desmopressin, which then degraded more slowly. The rate data obtained for the desmopressin esters qualitatively agree with our previous study (10) on various esters of *N*-α-acylated tyrosine amides, in which aliphatic carboxylic acid and carbonate esters with a short or branched side chain were shown to be the most resistant esters toward hydrolysis by α-chymotrypsin.

Lipophilicity of the Prodrugs

The lipophilicity of the derivatives was assessed by measuring the apparent partition coefficients (P) of the compounds between octanol and 0.05 *M* phosphate buffer, pH 7.4. The log$P$ values obtained are shown in Table III. The results obtained show that it is feasible to obtain ester derivatives possessing a much higher lipophilicity than the parent desmopressin. The partition coefficients of desmopressin and its propionate ester were too low to obtain reliable values even when an octanol/buffer volume ratio of 1000:1 was used. Lundin and Artursson (5) have previously reported a log$P$ value (octanol–water) of −1.95 for tritiated desmopressin, which is in great contrast to the present findings. The purity of the labeled peptide is important for the deter-

Table III. Partition (P) and Permeability (P_{app}) Coefficients for Desmopressin and Various Ester Derivatives

Compound	log$P$[a]	$P_{app} \times 10^7$ (cm sec^{-1})[b]
Desmopressin	< -3.5	5.15 ± 1.67 (5)[c] 5.99 ± 1.88 (15)[d]
I	< -3	9.44 ± 1.54 (4)
II	−2.25	15.1 ± 1.1 (3)
III	−1.25	9.77 ± 1.19 (4)
IV	0.41	8.50 ± 1.60 (3)
V	0.09	ND
VI	−1.77	5.99 ± 0.57 (4)

[a] P is the partition coefficient between octanol and aqueous buffer solution, pH 7.40 (at 21°C).
[b] Values are given as means ± SD (n).
[c] Initial apical concentration, 2.0×10^{-5} M.
[d] Initial apical concentration, 5.0×10^{-5} M.

mination of the partition coefficient and may account for the discrepancy. Both desmopressin and the esters are highly protonated at pH 7.4 due to the arginine group, and the apparent partition coefficients may therefore be dependent on the anion in the aqueous phase. Further, the difference in the log$P$ values determined for the esters do not quantitatively agree with that expected on the basis of the π substituent parameter. Thus, the increase in log$P$ from the hexanoate ester (III) to the octanoate ester (IV) amounts to 1.65, which is higher than the π value (1.04) for an ethylene group (23).

Transport Across Caco-2 Cells

Representative permeation profiles for the transport of desmopressin and the pivalate ester (II) across the Caco-2 cell monolayer are shown in Fig. 5. The apparent permeability coefficients (P_{app}) were derived from the slopes of such plots and Eq. (2) (Table III). The amount of ester derivative penetrating the cells was measured as desmopressin after alkaline hydrolysis of the basolateral samples and thus represents the sum of prodrug penetrated and any desmopressin being formed by hydrolysis during the transport. In agreement with previous findings (5), desmopressin was found to be stable in the presence of Caco-2 cells. Recoveries of more than 90% of desmopressin and the esters in the apical compartment were found at the end of each run, indicating negligible adsorption of the compounds to the filter inserts or walls and negligible hydrolysis by apical hydrolases on the Caco-2 cells. Ester V, however, showed significant adsorption, and no meaningful transport data were obtained for this compound.

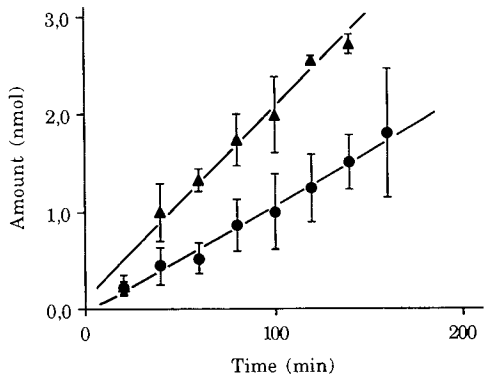


Fig. 5. Transport of desmopressin (●) and the pivalate ester (II) (▲) across confluent Caco-2 cell monolayers. Each point represents the mean standard deviation for at least three determinations.

The observed permeability coefficients P_{app} contain contributions from the permeabilities through the aqueous boundary layer (P_{aq}), across the cell monolayer (P_c), and through the underlying filter (P_f). The agitation intensity used throughout the study leads to an aqueous boundary layer of approximately 250 μm (16). Using previous arguments (16,24), P_{aq} and P_f can be ignored and P_{app} reflects the rate of transport across the cell monolayer.

The P_{app} value found for desmopressin did not vary significantly with the peptide concentration (Table III), in agreement with previous findings (5), indicating that the transport is mediated by passive diffusion. All prodrug esters studied except the carbonate ester VI showed increased permeability relative to the parent peptide (Table III). However, there is no apparent correlation between the permeability and the lipophilicity of these derivatives. The highly hydrophilic desmopressin is largely transported through Caco-2 cells via the paracellular route (5,6). This may also be the case for the ester derivatives but the transcellular route could make some contribution. Increased lipophilicity should increase the transcellular permeability, whereas increased molecular size should decrease the paracellular permeability (12,25). Since the lipophilicity of the desmopressin esters parallels their molecular size, the permeability data observed may be explained in terms of the involvement of both a transcellular and a paracellular transport mechanism. The importance of desolvation energy, as reflected in the number of hydrogen bonding sites, for the diffusion of peptides across Caco-2 cell monolayers as well as the rabbit intestinal mucosa has been demonstrated (13,24). This is, however, not a major factor determining the differences in the permeability of the desmopressin esters studied since they possess the same number of hydrogen binding sites.

In conclusion, this study shows that esterification of desmopressin at its tyrosine phenolic group may be a useful prodrug approach. The pivalate ester is more stable than desmopressin itself toward α-chymotrypsin and shows an increased rate of transport across Caco-2 cell monolayers. Studies are in progress to determine the bioavailability of this and other ester prodrugs in experimental animals following administration by the oral as well as by other routes of administration.

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