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Skin permeation model of phenylalkylcarboxylic homologous acids and their enhancer effect on percutaneous penetration of 5-Fluorouracil

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Abstract

This study was conducted primarily to establish the rat skin penetration model of acidic homologous series with a wide range of lipophilicity (from phenylpropionic acid to phenylcaprylic acid), and to compare it with other homologous series (alkylanilines) by means of adequate permeability-partition correlations, in order to find out if there could be a common model of penetration for all the compounds. The influence of pH on acid penetration through the skin was also analyzed. Standard in vitro skin permeation methods using rat skin were used to determine the permeability coefficients of phenylalkylcarboxylic acids in the conditions established. Membrane/water partition coefficients were also assessed, and the correlations between permeability and partition values were established. A linear relationship between the logarithms of permeabilities of penetrants and the corresponding membrane/water partition coefficients was found. The apparent discordance between this type of correlation and the probabilistic (i.e. parabolic) model previously established with other homologous series (alkylanilines) is attributed to a self-enhancing effect on penetration in the case of the more lipophilic compounds of the series. In fact, when 5-FU was used as a polar model permeant, pretreatment of the membrane with phenyloenanthic acid gave it an enhancer ratio of 4.5, thus confirming its enhancer effect.

Keywords: Percutaneous absorption; Phenylalkylcarboxylic acids; Enhancer effect; 5-FU; Influence of pH; In vitro models

1. Introduction

Penetration through the skin is known to depend essentially on the lipophilicity of the substances tested. The use of homologous series of

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compounds has made it possible to establish behaviour models that, in theory, allow us to predict the percutaneous absorption capacity of chemically related substances.

Much of the theoretical background information on percutaneous absorption has been developed from studies of nonelectrolytic permeating species (Yalkowsky and Flynn, 1973; Flynn et al., 1974), and only a little is available on the effect of pH and pKa on permeation of ionizable drugs through the skin. The vehicle's pH may have a profound influence upon the percutaneous delivery from topical products (Irwin et al., 1990). Depending on the pKa of the compound and the pH of the vehicle, an equilibrium mixture of ionized and un-ionized species would be present in the immediate vicinity of the skin (Swarbrick et al., 1984). To properly control the rate at which such electrolytes permeate human skin, it is necesary to determine the permeability of both forms of the drug. According to the pH-partition simple hypothesis, only the non-ionized forms of drugs are able to pass through lipoidal membranes in significant amounts. However, the permeation of ionized drugs through skin is possible and therefore must be taken into account (Banerjee and Ritschel, 1989), although ionization reduces topical availability (Flynn, 1989). In order to widen our knowledge of the effect that pH has on skin penetration of ionizable compounds with different lipohilicities and pka, we studied six compounds belonging to the homologous series of phenylalkylcarboxylic acids (from two to seven CH_2 groups in the straight alkyl chain). First, the permeability of phenylpropionic, phenylvaleric and phenylcaproic acids through full-thickness Wistar rat skin were studied in vitro to establish whether both ionized and un-ionized forms over a range of pH values from 3 to 7 are able to permeate rat skin. After that, the permeability coefficients of the six compounds of the series through full-thickness and epidermal membranes from Wistar rat, at pH 4 were determined in order to amplify the experimental data on the influence that lipophilicity has on the permeability of these compounds. A pH value of 4 was chosen because of reports that alterations of the membrane can occur as a result of an excessively acid or alkaline pH (Irwin et al., 1990). Membrane/water partition coefficients of these compounds were also determined at the same pH value. Permeation-lipophilicity relationships were established and attempts were made, through comparative evaluations, to determine the existence of possible differences in behaviour with respect to alkylaniline homologous series, attributable to the different characteristics of the permeants (Díez-Sales et al., 1993). On the other hand, in order to ascertain whether the acids tested can alter membrane permeability, as do several linear-chain fatty acids (Green et al., 1988; Ogiso and Shintani, 1990; Lee et al., 1993), three of the compounds used in this study were applied to the epidermis of Wistar rat skin to evaluate their possible enhancer effect on the penetration of 5-FU, which was used for a similar purpose in other experiments (Díez-Sales et al., 1996).

2. Materials and methods

2.1. Permeants

Six phenylalkylcarboxylic acids belonging to a true homologous series were used in the diffusion experiments. They were supplied as reagent grade products (Merck, A.G., Janssen, Pfaltz and Bauer). They are weak acids, with pKa values from 4.37 to 4.69 (Smith and Martell, 1989). The buffer solutions were used to dissolve these compounds at the following concentrations: phenylpropionic and phenylbutyric acids, 1 mg/ml; phenylvaleric acid, 0.5 mg/ml; phenylcaproic acid, 0.2 mg/ml; phenyloenanthic acid, 0.1 mg/ml, and phenylcaprylic acid, 0.025 mg/ml. Aqueous buffer solutions were prepared over a pH range of 3.0–7.0 using McIlvaine's citrate-phosphate system (Elving et al., 1956). 5-Fluorouracil (Sigma®, lot 54H3417) was prepared in a saturated buffer solution at pH 6.2. Purity for all permeants was checked by HPLC.

2.2. Membranes and their preparation

Diffusion studies were performed with full-thickness and epidermal membranes of Wistar rats (females weighing 50–60 g). Full-thickness

was taken from the abdominal surface and epidermal membranes were prepared as previously reported (Díez-Sales et al., 1993). In experiments carried out with 5-FU as a permeant, epidermal membranes were pretreated with either 400 μl of the ethanolic solutions of phenylpropionic, phenylcaproic and phenyloenanthic acids, respectively (5%, w/w) or the equivalent amount of ethanol overnight.

2.3. Partition coefficients

Full-thickness skin and epidermis of Wistar rat were used to determine the membrane-water partition coefficients of the phenylalkylcarboxylic acids. The values obtained were used to establish permeability-partition correlations. As previously reported (Díez-Sales et al., 1991), partition coefficients were obtained by equilibrating phenylalkylcarboxylic acid solutions, buffered to pH 4, with a known mass (100–300 mg) of the corresponding membrane. The samples were equilibrated in shaker bath at 37 ($\pm$ 0.5)°C for 48 h. Distribution of the phenylalkylcarboxylic acids between the aqueous phase and the membranes was estimated as previously reported (Díez-Sales et al., 1993). Samples of aqueous phase were removed using a glass syringe, centrifuged twice to remove any residual organic phase and then suitably diluted for analysis by HPLC.

2.4. In vitro diffusion procedure

Diffusion experiments with phenylalkylcarboxylic acids were performed in a 6-cell battery system (Durrheim et al., 1980). The membranes were placed in the diffusion cells in vertical position. The actual surface was 4.52 cm^2. The volume of the cell compartments was 22 ml and the temperature was maintained at 37 ($\pm$ 0.5)°C by immersion of the cell in a thermostatic bath. Following this, a volume (22 ml) of the phenylalkylcarboxylic acid test solution was introduced into the stirred donor compartment, while an equal volume of phosphate-buffered saline (pH 7.4) was introduced into the stirred receptor compartment. Samples (1 ml) were taken from the receptor compartment every 30 min. The volumes withdrawn were always replaced with equal volumes of fresh receptor solution. In order to obtain steady-state permeation, the donor cell content was entirely replaced by fresh test solution every 30 min for all of the compounds tested.

Drug penetration of 5-FU through non pretreated skin (control) was determined in order to examine the effect of the pretreatment of skin with ethanol and the ethanolic solutions of the phenylalkylcarboxylic acids. In these experiments a 22 ml aliquot of the saturated solution of the 5-FU at pH 6.2 was applied at zero time to each donor cell. Samples (1 ml) were taken from the receptor compartment every 60 min over a period of 32 h. The volumes withdrawn were always replaced with an equal volume of fresh receptor solution. The linear steady-state expression (Eq. (1)) was used to fit experimental data (Scheuplein, 1967).

$$Q(t) = A \cdot K \cdot h \cdot C\left[D \cdot \frac{t}{h^2} - \frac{1}{6}\right] \quad (1)$$

where $Q(t)$ is the quantity which passes through the membrane and reaches the receptor solution at a given time (t); A represents the actual diffusional surface area (4.52 cm^2 in our particular case); K the partition coefficient of the permeant between membrane and donor vehicle; h the membrane thickness; D the diffusion coefficient of the permeant across the membrane, and C is the concentration of the compound in the donor solution. The terms in Eq. (1) $K \cdot h$ and D/h^2 were replaced by P_1 and P_2, respectively, and obtained by fitting the theoretical equation to the in vitro permeation data using a computerized nonlinear least-squares method (Multi) (Yamaoka et al., 1981). Then the permeability coefficients, $K_p (= P_1 \cdot P_2)$, and fluxes, $J = (K_p \cdot C)$, were calculated. The enhancement ratio (ER) was calculated from the following equation (Williams and Barry, 1991):

$$\mathrm{ER} = \frac{K_p \text{ with pretreatment}}{K_p \text{ without pretreatment}} \quad (2)$$

2.5. Analytical procedure

Quantification of the test compounds in the samples was done by HPLC using a Perkin-Elmer liquid chromatograph which included a Binary LC Pump 250, a Rheodyne P/N 7125-047 model injector, a Perkin-Elmer, LC 90 UV detector set at 258 nm and an LCI-100 integrator. An analytical Novapak C-18 column (150/39 mm) with a 5 mm Guardpack precolumn was employed. Mixtures of methanol and aqueous 0.1 M acetic acid (pH 3.0) in variable proportions, depending on the tested solutes were used as eluents at a flow rate of 1 ml/min, at room temperature.

Calibration curves covering the entire range of concentrations assayed for every compound were prepared in triplicate. The accuracy and precision of the method were validated. Accuracy was evaluated by calculating the relative error, which was always less than 5%. Precision was evaluated by calculating the variation coefficient, which was in no case higher than 9.9%. These results were considered completely suitable (Karnes and March, 1993).

3. Results and discussion

3.1. Partition coefficients

The data for the partitioning of the phenylalkylcarboxylic acids in full-thickness and epidermis are included in Table 1. In all cases, the relationships obtained between the partition coefficients, P, and alkyl chain lengths, n, are linear and have excellent correlation coefficients.

$$\text{Epidermis } \log P_n = n \cdot 0.41(\pm 0.02) - 0.53(\pm 0.11) \quad (r > 0.993) \tag{3}$$

$$\text{Full-thickness skin } \log P_n = n \cdot 0.32(\pm 0.02) - 0.07(\pm 0.09) \quad (r > 0.992) \tag{4}$$

Statistical comparison using Student's t-test shows that the slopes of regression lines for partition coefficients obtained for both membranes are significantly different ($p < 0.001$). This can be attributed to the presence of the hydrophilic dermal layer in full-thickness skin sheets, which produces a larger retention and a greater partition coefficient than those obtained in epidermal membrane for the more hydrophilic compounds of the series.

Table 1
Partition coefficient (P, $cm^3 \cdot g^{-1}$), in the rat skin (full-thickness and epidermis) for tested acids at pH 4

Tested acids	Partition coefficient (P, $cm^3 \cdot g^{-1}$)	
	Full-thickness skin	Epidermal membrane
Phenylpropionic	3.72 (0.48)	2.64 (0.22)
Phenylbutyric	8.94 (1.15)	4.01 (0.71)
Phenylvaleric	13.34 (1.76)	13.02 (1.43)
Phenylcaproic	45.60 (4.79)	36.00 (3.55)
Phenyloenanthic	71.33 (9.88)	71.00 (10.67)
Phenylcaprylic	148.57 (32.63)	240.09 (42.44)

The standard deviations are given in brackets ($n = 6$).

3.2. In vitro diffusion tests

In order to analyze the effect of pH on the acids' penetration through full-thickness skin of Wistar rat, diffusion tests were carried out with the phenylpropionic acid at five different pH values (ranging from 3 to 7). With a phenylpropionic acid pKa value of 4.37, at the lowest pH used in the experiments, the acid was largely (95.9%) in its non-ionized form but at the highest pH the acid was practically in the ionized form (99.7%).

In Fig. 1, accumulative amounts (Q, mg) of this acid at different pH values are plotted as a function of time. As can be observed this compound shows greater penetration at a lower pH. Assuming that both the ionized (A^-) and non-ionized (HA) species contribute to the steady-state flux of permeating electrolytes (monoprotic acid), then the total observed flux (J_{obs}) is dependent upon the flux of the ionized fraction (J_i, α) and the flux of the non-ionized fraction (J_u, $1 - \alpha$) and can be expressed by the following equation (Irwin et al., 1990).

$$J_{obs} = \alpha J_i + (1 - \alpha) J_u \tag{5}$$

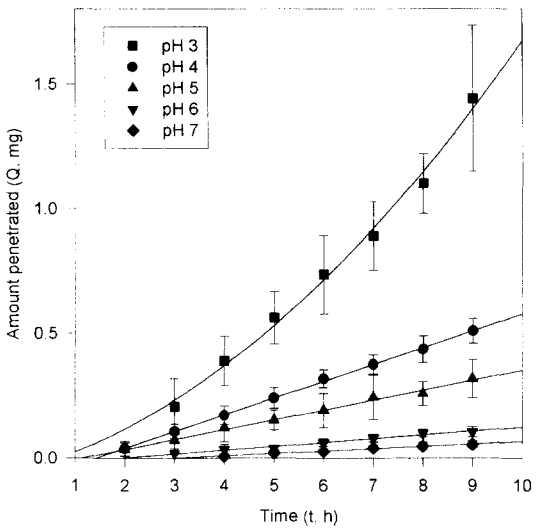

Fig. 1. Amounts of phenylpropionic acid penetrated (Q, mg) at different pH (from 3 to 7) as a function of time (t, h). Each data point was the mean of six experiments with standard deviation.

or its linear form $J_{obs}/\alpha = J_i + (1-\alpha)J_u/\alpha$. Thus, a plot of J_{obs}/α against $(1-\alpha)/\alpha$ makes it possible to estimate the flux of the ionized and non-ionized species. In the case of phenylpropionic acid, the expression obtained is:

$$J_{obs}/\alpha = 39.57 \cdot 10^{-3}(1-\alpha)/\alpha - 7.71 \cdot 10^{-3} \quad (r > 0.999) \qquad (6)$$

There is a good agreement between the theoretical ($39.57 \cdot 10^{-3}$ mg/cm^2·h) and the experimental ($37.76 \cdot 10^{-3}$ mg/cm^2·h) J_u values, but this is not the case for the J_i values because the negative intercept has no practical significance. In fact, at pH 7 phenylpropionic acid is completely ionized and its experimental flux value cannot be considered negligible ($2.54 \cdot 10^{-3}$ mg/cm^2·h). The fact that the experiments were performed with solutions of the acids assayed could explain the discordance between experimental and theoretical J_i values, for the degree of saturation of the solution in contact with the stratum corneum decreases as the pH increases (Irwin et al., 1990).

The permeability coefficients calculated from the steady-state flux at different pH values for the compounds and membranes assayed are shown in Table 2. As could be expected, the results on phenylvaleric acid (pKa 4.68) and phenylcaproic acid (pKa 4.69) confirm the behaviour of phenylpropionic acid. At pH 3 the permeation rates observed through rat skin are as high as expected for non-ionized species. However, the permeation of ionized forms (pH 7), although lower, is not negligible.

3.3. Permeability/structure correlations

Correlations between permeability coefficients and partition constants, solubilities, molecular weight or other structural parameters for homologous series of xenobiotics are a suitable source of information on the passive penetration mechanisms. However it is essential to reach a knowledge as exact as possible of the nature of these relationships, in order to establish a very useful tool in conducting percutaneous absorption design studies for new drugs. In Fig. 2, permeability coefficients, K_p, through full-thickness rat skin for the tested acids are plotted as a function of the number of carbons in the alkyl chain (n). Linear relationships with excellent correlation coefficients ($r > 0.99$) were found at all the pH values studied. This is consistent with the view that the diffusion process is controlled by the transfer across the stratum corneum, which is lipophilic in nature.

On the other hand, the data in Table 2 show that the absence of the dermis produces a small increase in the K_p values for all the compounds as a consequence of the reduction in membrane thickness. Correlations between permeability values (full-thickness and epidermis of Wistar rat) and the number n of methylene groups in the chain were linear.

$$\text{Epidermis } \log K_p = n \cdot 0.25(\pm 0.01) - 2.02(\pm 0.03) \quad (r > 0.998) \qquad (7)$$

$$\text{Full-thickness skin } \log K_p = n \cdot 0.28(\pm 0.01) - 2.30(\pm 0.07) \quad (r > 0.993) \qquad (8)$$

Since the membrane/water partition coefficients show a high correlation with the number n of CH_2

Table 2
Permeability coefficients ($K_p \cdot 10^3$, cm·h^{-1}) through rat skin (full-thickness and epidermis) for tested acids at different pH

Tested acids	Permeability coefficient ($K_p \cdot 10^3$, cm·h^{-1})					
	Full-thickness skin					Epidermis
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 4
Phenylpropionic	37.76 (6.39)	15.81 (1.86)	8.73 (1.46)	2.80 (0.80)	2.54 (0.78)	29.21 (3.20)
Phenylbutyric	–	42.40 (4.16)	– –	– –	– –	53.83 (6.71)
Phenylvaleric	105.83 (14.32)	63.30 (5.31)	–	–	22.33 (2.18)	89.53 (4.43)
Phenylcaproic	217.70 (42.16)	124.47 (6.89)	–	–	35.13 (4.65)	163.74 (7.55)
Phenyloenanthic	–	267.14 (23.50)	–	–	–	324.05 (20.67)
Phenylcaprylic	–	396.22 (30.27)	–	–	–	483.68 (92.44)

Standard deviations are given in brackets ($n = 6$).

groups in the aliphatic chain of homologous series, they were used to obtain the plots of data (Fig. 3) found for the phenylalkylcarboxylic acids assayed in the present work and for alkylanilines through hairless mouse skin assayed previously (Díez-Sales et al., 1993), according to the best fitting model equation.

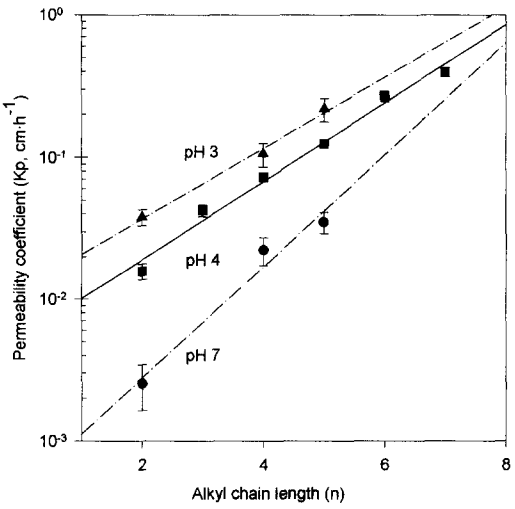

Fig. 2. Permeability coefficients ($K_p \cdot 10^3$, cm·h^{-1}) determined at different pH values through full-thickness rat skin, for the phenylalkylcarboxylic acids as a function of the number of carbons in the alkyl chain (n). Each data point was the mean of six experiments with standard deviation.

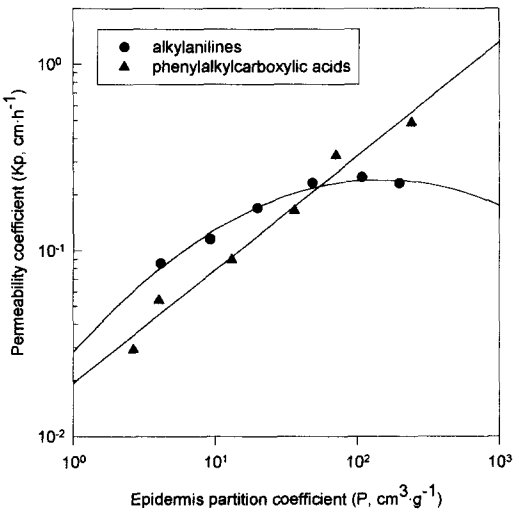

Fig. 3. Comparative plots of permeability coefficients (K_p, cm·h^{-1}) found for phenylalkylcarboxylic acids and alkylanilines through rat skin as a function of the respective epidermis partition coefficient (P, cm^3·g^{-1}), according to the best fitting model equation.

Clearly the linear model established for the phenylalkylcarboxylic acids assayed is not similar to those obtained with the alkylaniline homologous series (Díez-Sales et al., 1993) which constitutes a probabilistic (i.e. parabolic) model. A parabolic correlation implies the existence of an optimum of lipophilicity for permeation and the compounds which have a lipophilicity higher or lower than this optimum value will probably diffuse with more difficulty through the membrane. In our opinion, the cause of these differences is probably the different nature of the compounds assayed. As can be deduced from the data given in the literature, it seems that fatty acids (oleic and lauric acids) increase the penetration of other compounds by disorganising the skin structure (Green et al., 1988; Ogiso and Shintani, 1990; Koyama et al., 1994). For the same reason, in our case the phenylalkylcarboxylic acids of the homologous series assayed, in particular the most lipophilic compounds, could produce an enhancing action by fluidizing the intercellular skin lipids, which as a consequence would promote their penetration.

In order to check this hypothesis, we did a new series of experiments using 5-FU as a hydrophilic penetrant model and determined whether its intrinsic permeability values suffer some modification when membranes were pretreated with ethanolic solution of phenylalkylcarboxylic acid prepared at a concentration of 5% w/w similar to that used in various studies to evaluate the enhancing effect of acidic compounds (Pershing et al., 1993; Lee et al., 1993).

The amounts of 5-FU that accumulated in the receptor compartment as a function of time in the experiments without pretreatment (control) and with pretreatment of the skin with ethanol and ethanolic solutions of phenylalkylcarboxylic acids are shown in Fig. 4. As can be seen, in all cases pretreatment of the skin with acids produces an increase in the amount of 5-FU penetrated (Q, mg).

The permeability coefficients obtained in different experimental conditions (non pretreated and pretreated membranes) are shown in Table 3. In order to compare them a logarithmic transformation of the data was performed to obtain homogeneous variability. Homogeneity was confirmed by Barlett's test. One- and two-way ANOVA were used prior to the Tukey test. The statistical analysis is also shown in Table 3. As can be seen, there are no significant differences between the permeability coefficients obtained through the membrane without pretreatment and with pretreatment with ethanol. However, significant differences between the permeability coefficients obtained through the membrane without pretreatment and with pretreatment with ethanolic solutions of the phenylalkylcarboxylic acids were found.

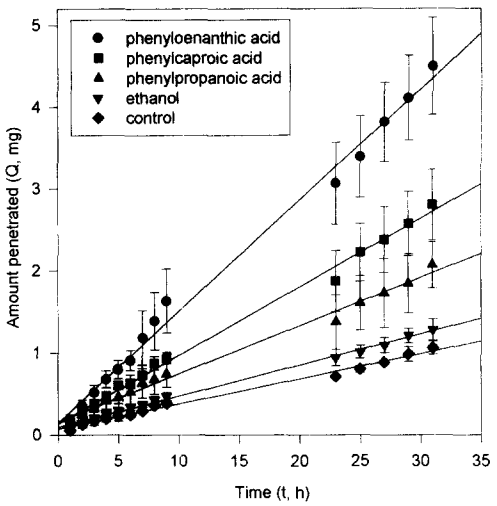

Fig. 4. Graphical plot showing the amount penetrated (Q, mg) for the 5-FU tested under different conditions; control, ethanol, phenylpropionic acid (5%, w/w), phenylcaproic acid (5%, w/w) and phenyloenanthic acid (5%, w/w). Each data point was the mean of six experiments with standard deviation.

Fig. 5 shows the enhancement ratios calculated for all conditions, and it clearly shows the enhancer effect of the phenylalkylcarboxylic acids. As can be seen, the high enhancer effect was observed for the acid with the longest alkyl chain length of all the acids assayed as 5-FU enhancers (phenyloenanthic acid).

In conclusion, phenylalkylcarboxylic acids seem to induce a decrease in the skin's barrier function. This effect could explain the permeability/lipophilicity linear model found for the series.

Table 3
5-FU Permeability coefficients obtained without pretreatment and with different pretreatments of Wistar rat epidermis

Compound of pretreatment	5-FU Permeability coefficient ($K_p \cdot 10^3$, cm·h^{-1})	Statistical differences ($p<0.05$)			
		I	II	III	IV
Without (I)	0.67 (0.06)	–	–	–	–
Ethanol (II)	0.81 (0.04)	NS[a]	–	–	–
Phenylpropionic acid (III)	1.34 (0.22)	S[b]	S	–	–
Phenylcaprylic acid (IV)	2.00 (0.15)	S	S	S	–
Phenyloenanthic acid (V)	3.00 (0.45)	S	S	S	S

Standard deviations are given in brackets ($n = 6$). Statistical analysis of permeability coefficients obtained in the different conditions are also shown.
[a]No significant differences.
[b]Significant differences.

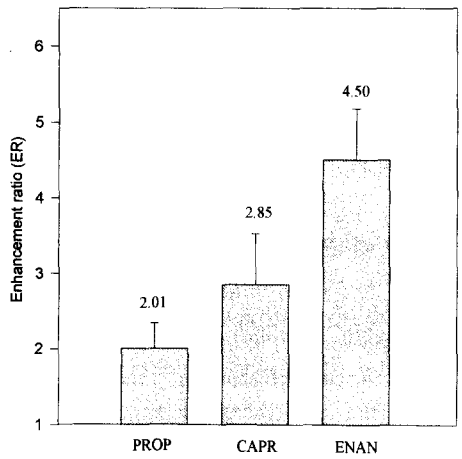

Fig. 5. The penetration enhancing activities of phenylpropionic acid (PROP), phenylcaproic acid (CAPR) and phenyloenanthic acid (ENAN) expressed as 5-FU enhancement ratios (ER).

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