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Effect of ion-pairing on 1-octanol-water partitioning of peptide drugs. I: The nonapeptide leuprolide acetate

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Summary

Leuprolide acetate is a nonapeptide with virtually no lipid solubility. It has multiple ionizable sites and exists in an ionized form across a wide pH range of physiologic interest. Studies were conducted to determine the effect of counterions on the distribution of leuprolide into octanol. Counterions from the following acids were investigated: alkylsulfonic acids (C = 1, 4, 6, 8, 10), salicylic acid, and dehydrocholic acid. The distribution behavior was studied as a function of pH and counterion concentration. Results showed that methane- and butanesulfonate do not help partitioning of leuprolide into the octanol phase although there is a slight improvement in lipophilicity of the drug with increasing pH. For the C6–C10 alkylsulfonates the partitioning increases significantly in the following order: hexane- < octane- < decanesulfonate. A mathematical model was developed to describe the complex partitioning behavior of this peptide. Data for salicylate indicated marginal effect on partitioning of leuprolide. Results obtained for dehydrocholate showed no improvement in lipophilicity of the drug suggesting that the acid is too weak (high pK_a) and may be sterically hindered from forming an effective ion pair. It was observed that increase in lipophilicity of leuprolide ion pairs may be proportional to the extent of ionization of the imidazolyl nitrogen of histidine, the type of counterion, and number of lipophilic counterions per molecule. Also, the lipophilicity of the ion pairs may be proportional to pK_a of the acid from which the anion is derived, i.e., sulfonic acid (pK_a less than 2.0) > salicylic acid ($pK_a \simeq 3.0$) > dehydrocholic acid (pK_a greater than 6.0). For the alkylsulfonate series a plot of log K (where K represents ion pair equilibrium constant) versus number of carbon atoms in the alkyl chain yielded a straight line with a slope of 0.5 per methylene group. This value is in good agreement with literature values of the Hansch π constant for a methylene group. Implications of these findings relative to dosage form development of leuprolide are discussed.

Introduction

It is generally accepted that drugs in their unionized form have better transport properties through biologic membranes compared to their ionized forms (Cools and Jansen, 1983). From delivery standpoint, this becomes a major problem for peptides (Hirai et al., 1981; Huang, 1983) which have multiple sites for ionization at physiological pH. One way to overcome the adverse effect of ionization is to use a lipophilic counterion to form an ion pair and facilitate partitioning into the biologic membrane (Okada et al., 1983).

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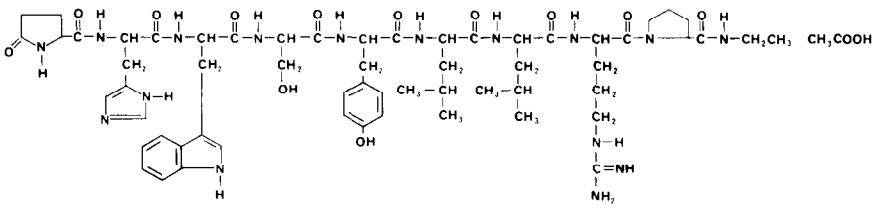


We have used this approach for leuprolide, a leuteinizing hormone-releasing hormone (LH-RH) analog. Leuprolide acetate is a nonapeptide and is designated by its functional amino acid components, namely: 5-oxo-L-prolyl-L-histidyl-L-tryptophanyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-L-prolylethylamide acetate. This LH-RH analog is structurally defined as shown above.

Leuprolide has three ionization sites, namely, the imidazolyl nitrogen of histidine ($pK_a \simeq 6.0$), the phenolic hydroxyl of tyrosine ($pK_a \simeq 10.0$), and the guanidine nitrogen of arginine ($pK_a \simeq 13.0$). The tryptophan moiety does not ionize in water and therefore does not participate in salt formation at typical formulation pH. Since the guanidine nitrogen is extremely basic, this peptide as synthesized exists in the protonated form and is generally associated with at least one mole of acetic acid. The compound therefore exists as an acetate salt, which, although an ion pair ($-NH_3^+ \cdot {}^-OOCCH_3$), is highly hydrophilic as demonstrated by its very low partition coefficient (Adjei et al., 1989). Leuprolide acetate is not orally active (Okada et al., 1982). The drug is therefore commercially available for daily s.c. administration (1 mg) or monthly single intramuscular (3.75 or 7.5 mg) depot injection. The studies reported here were designed in an attempt to elucidate basic formulation factors that might aid in development of non-parenteral dosage forms of leuprolide.

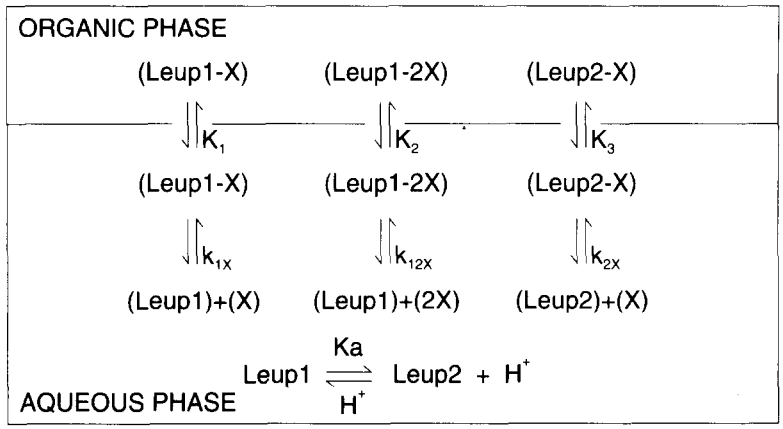
Theoretical Considerations

The partition of bioactive compounds from water to water-immiscible organic solvents has been regarded as a simple model system for drug transport across biomembranes (Nernst, 1981). Solvent systems which are almost completely immiscible (i.e., alkanes and water) are fairly well behaved and lend themselves to more rigorous thermodynamic treatment of partitioning data than solvent systems which are partially soluble in each other (Cratin, 1968; Aveyard et al., 1969; Leo et al., 1971; Hansch et al., 1973). Although immiscible liquids do deviate from ideality, these departures from ideality, especially by polar solvent systems, are not so great as to render mathematical treatment valueless (Leo et al., 1971). In fact in such systems, and at concentrations where Henry's Law is applicable, the thermodynamic partition coefficient is equal to the mole fraction ratio X_o/X_w. There are sufficient literature data to show that the octanol/water system for determining drug partitioning characteristics is sensitive to solute changes in the partitioning medium. Therefore, the octanol/water system was chosen as the partitioning medium for this study. This manuscript discusses detailed results of partitioning experiments conducted with leuprolide and several counterions of varying lipophilicity, size and shape. The partitioning behavior of the respective salts was also evaluated under various pH conditions. Insights from these studies relative to rational design of various drug delivery systems are presented.

Experimental

Chemicals and reagents

The chemicals used in this study were: leuprolide acetate (Abbott-43818); 1-octanol, glacial acetic acid, salicylic acid sodium salt, and 1-decanesulfonic acid sodium salt (Aldrich Chemical



$$Ka = \frac{(Leup2)(H^+)}{(Leup1)}$$

Ion Pairing:

$$k_{1X} = \frac{(Leup1\text{-}X)}{(Leup1)\,(X)}$$

$$k_{12X} = \frac{(Leup1\text{-}2X)}{(Leup1)\,(X)^2}$$

$$k_{2X} = \frac{(Leup2\text{-}X)}{(Leup2)\,(X)}$$

Partitioning:

$$K_1 = \frac{(Leup1\text{-}X)_{ORG}}{(Leup1\text{-}X)_{AQ}}$$

$$K_2 = \frac{(Leup1\text{-}2X)_{ORG}}{(Leup1\text{-}2X)_{AQ}}$$

$$K_3 = \frac{(Leup2\text{-}X)_{ORG}}{(Leup2\text{-}X)_{AQ}}$$

$$[Leup]_{T\,AQ} = [Leup1]_{AQ} + [Leup2]_{AQ} + [Leup1\text{-}X]_{AQ} + [Leup1\text{-}2X]_{AQ} + [Leup2\text{-}X]_{AQ} \quad \textbf{Eq. 1}$$

$$[Leup]_{T\,ORG} = [Leup1\text{-}X]_{ORG} + [Leup1\text{-}2X]_{ORG} + [Leup2\text{-}X]_{ORG} \quad \textbf{Eq. 2}$$

$$K_{obs} = \frac{[Leup]_{T\,ORG}}{[Leup]_{T\,AQ}} = \frac{(Leup1\text{-}X)_{ORG} + (Leup1\text{-}2X)_{ORG} + (Leup2\text{-}X)_{ORG}}{(Leup1 + Leup2 + Leup1\text{-}X + Leup1\text{-}2X + Leup2\text{-}X)_{AQ}} \quad \textbf{Eq. 3}$$

$$K_{obs} = \frac{K_1\,(Leup1\text{-}X)_{AQ} + K_2\,(Leup1\text{-}2X)_{AQ} + K_3\,(Leup2\text{-}X)_{AQ}}{(Leup1)_{AQ} + (Leup2)_{AQ} + (Leup1\text{-}X)_{AQ} + (Leup1\text{-}2X)_{AQ} + (Leup2\text{-}X)_{AQ}} \quad \textbf{Eq. 4}$$

$$K_{obs} = \frac{(K_1 \cdot k_{1X} \cdot X \cdot H^+) + (K_2 \cdot k_{12X} \cdot X^2 \cdot H^+) + (K_3 \cdot k_{2X} \cdot Ka \cdot X)}{H^+ + Ka + k_{1x} \cdot X \cdot H^+ + k_{12x} \cdot X^2 \cdot H^+ + k_{2x} \cdot Ka \cdot X} \quad \textbf{Eq. 5}$$

$$K_{obs} = \frac{(K'_1 \cdot X \cdot H^+) + (K'_2 \cdot X^2 \cdot H^+) + (K'_3 \cdot Ka \cdot X)}{H^+ + Ka + (k_{1x} \cdot X \cdot H^+) + (k_{12x} \cdot X^2 \cdot H^+) + (k_{2x} \cdot Ka \cdot X)} \quad \textbf{Eq. 6}$$

For the equilibrium, k_{1X}, k_{12X}, and k_{2X} are negligible.

$$K_{obs} = \frac{[X]_{AQ}}{H^+ + Ka}\ \{K'_1[H^+] + K'_2[H^+]\,[X]_{AQ} + K'_3\,Ka\} \quad \textbf{Eq. 7}$$

Scheme 1. Schematic representation of ion-pair partition model.

canesulfonic acid sodium salt (Aldrich Chemical Co.); 1-octane and 1-butanesulfonic acid sodium salts, and methane sulfonic acid (Eastman Kodak Co.); 1-hexanesulfonic acid sodium salt (Fisher Scientific Products); dehydrocholic acid sodium salt (United States Biochemical); sodium hydroxide (J.T. Baker Co.); and double distilled deionized water. All chemicals were used as received.

Methods

Equipment, methods and supplies

The equipment and supplies used in the study were as follows: Constant temperature water bath (Blue M Instruments); pH meter (Beckman Instruments); glass scintillation vials (Wheaton Glass); centrifuge (International Instruments); culture tubes (Kimble). The analytical systems used included the following:

n-Alkylsulfonates: Ion chromatography was used to determine *n*-alkylsulfonate concentrations in the aqueous phase. Anion concentrator/precolumn, separator, and suppressor column, respectively, Dionex Part nos 03096, 030985 and 030828, with a conductivity detector were used. A solution of 0.005 M sodium carbonate with 2–5% methanol was used as mobile phase for the separation of decanesulfonate, octanesulfonate, and hexanesulfonate. A solution of 0.015 M sodium bicarbonate was used for the separation of methanesulfonate and butanesulfonate. Mobile phase flow rate was 69 ml/h.

Leuprolide acetate: The test method, reverse-phase HPLC, was previously described by Sutherland and Menon (1987). Concentrations of leuprolide in the organic phase were calculated by mass difference based on initial and final drug concentrations in the aqueous phase. Preliminary studies produced quantitative results which were used to validate recovery of the drug.

Salicylic acid: Salicylate concentrations in the aqueous phase were determined using HPLC. Separation of the anion was achieved on a Bondapak C18 column (Waters Associates) at 313 nm with a 0.1% acetic acid (30:70% v/v) mobile phase at a flow rate of 1.5 ml/min.

Dehydrocholic acid: Dehydrocholic acid concentrations were determined by HPLC at 205 nm on a Nucleosil C18 column (Alltech Associates). The mobile phase consisted of 0.014 M monobasic sodium phosphate monohydrate, acetonitrile, methanol, triethylamine, and 85% phosphoric acid in a ratio of 50:35:15:0.02:0.2% v/v, and a flow rate of 1.0 ml/min.

Partitioning studies

These studies were conducted in 20 ml glass vials provided with polyethylene capliner. All counterions were prepared in octanol-saturated water at the respective concentrations. Leuprolide acetate was added to these solutions to a final concentration of 4×10^{-4} M. 10 ml portions of the resulting solutions were adjusted to various pH levels (pH 3, 5, or 7) with 2% acetic acid or 1% sodium hydroxide and transferred into glass vials. 10 ml of water-saturated octanol were added to each vial. The vials were tightly capped and placed in a 30°C water bath and shaken at 120 cycles per min for at least 1 h. Studies showed a 1 h equilibration period under the above shaking conditions was adequate to achieve equilibrium. After equilibration, samples were transferred into culture tubes and centrifuged at 2000 rpm for 10 min. The octanol phase was aspirated off, and aliquots of the aqueous phase analyzed for leuprolide and free counterion content. All experiments were conducted in triplicate.

Results and Discussion

Theoretical considerations

Scheme 1 above is a schematic representation of the ion pair partition model used in the investigation. In this model [Leup1] represents the concentration of leuprolide species possessing two positive charges (guanidine and histidine). [Leup2] corresponds to the concentration of leuprolide species with one positive charge (guanidine). Species designated as Leup1 may interact with two counterions to form Leup1–2X where X represents the lipophilic counterion. Leup1 species may also have only one lipophilic counterion in which case the other counterion may be any other anion, e.g., acetate. This latter species (i.e., one with only one lipophilic counterion) is repre-

TABLE 1

Effect of pH and concentration of alkyl sulfonic acids on apparent partition coefficient of leuprolide at 30°C

Ratio [a]/pH	Decane	Octane	Hexane	Butane	Methane
1:2 (3)	4.01	0.86	0.03	0.02	0.01
1:2 (5)	18.67	1.87	0.19	0.02	0.03
1:2 (7)	38.36	4.14	0.55	0.11	0.15
1:1 (3)	0.72	0.30	0.02	0.02	0.02
1:1 (5)	2.25	0.55	0.09	0.02	0.03
1:1 (7)	6.49	1.57	0.32	0.16	0.15
2:1 (3)	0.27	0.10	0	0.01	0.02
2:1 (5)	0.66	0.33	0.05	0.03	0.02
2:1 (7)	1.11	0.60	0.17	0.15	0.15

[a] Molar ratio of leuprolide to counterion concentration before partitioning. K_{obs} for leuprolide acetate at pH 3, 5, and 7: $2.0E-06$, $3.5E-05$, and $2.0E-02$, respectively.

sented by Leup1-X in the model. The equilibrium between Leup1 and Leup2 is governed by the dissociation constant, K_a. The model assumes that the pairing of leuprolide and lipophilic counterions occurs in the aqueous phase prior to partitioning into the organic phase and that k_{1x}, k_{12x}, and k_{2x} are smaller than K_1, K_2, and K_3. This is supported by independent partitioning data (not reported) for the respective counterions and leuprolide acetate under the same experimental conditions.

Eqn 7 describes partition coefficients of leuprolide as a function of $[H^+]$ and counterion concentration $[X^-]_{aq}$ at equilibrium. K_1', K_2', and K_3' are equal to $(k_{1x} \cdot K_1)$, $(k_{12x} \cdot K_2)$, and $(k_{2x} \cdot K_3)$, respectively. In this investigation, best fits for the observed partition coefficient were obtained using Eqn 7. Values for $[H^+]$, calculated from pH, and K_a were known and therefore fixed as constants in the experimental protocol. Values for $[X^-]_{aq}$ were determined analytically using the chromatographic procedures described earlier. Estimates of K_1, K_2 and K_3 were mathematically computed using nonlinear regression fits (Statgraphics*) of the variables expressed in Eqn 7.

Alkylsulfonates

Results of the experimentally determined partition coefficients, K_{obs}, for leuprolide using several alkylsulfonates are reported in Table 1. The decanesulfonate salt (C10) shows the greatest improvement in lipophilicity while methane- (C1) and butane- (C4) sulfonic acids do not appear to have any effect on the apparent partition coefficient of leuprolide. The data for C10, C8 and C6 also demonstrate a consistent increase in K_{obs} as a function of increasing pH and counterion concentration.

Mathematical treatment of the data to provide estimates of partition coefficients for the respective ion pairs was carried out using the model described in Eqn 7 (see Scheme 1). Results of the equilibrium constants, K_1, K_2 and K_3 are tabulated in Table 2. For the given counterion, lipophilicities of different leuprolide species in-

TABLE 2

Equilibrium constants of ion pairs of leuprolide effect of alkyl chain length on lipophilicity of leuprolide

Carbon number	log K_1	log K_2	log K_3
C10	3.84	7.75	4.98
C8	3.33	6.35	3.89
C6	1.76	4.83	2.88
C4	NR	NR	NR
C1	NR	NR	NR
Slope	0.52	0.98	0.52

Note: the slope for the K_2 curve represents a di-anionic salt. Therefore, the effect of carbon number is half this value, i.e., 0.49. NR: data not reported due to the lack of statistial significance.

crease in the following order: Leup1-X < Leup2-X < Leup1-2X (i.e., $K_1 < K_3 < K_2$). Although the Leup1-2X species (which exist under acidic conditions) have the highest partition coefficient, their contribution to the observed partition coefficient is relatively low in the range of counterion concentrations studied. This is due to the fact that mass law is operative under these experimental conditions. Fig. 1 shows the correlation between observed and predicted partition coefficients for the decyl, octyl, and hexyl ion pairs based on the model. The predicted and observed partition coefficients for the butyl salt do not correlate nearly as well as those of the other analogs. This is possibly due to very small changes in partition coefficient and therefore larger errors associated with experimental determinations. A three-dimensional plot of the data which gives a

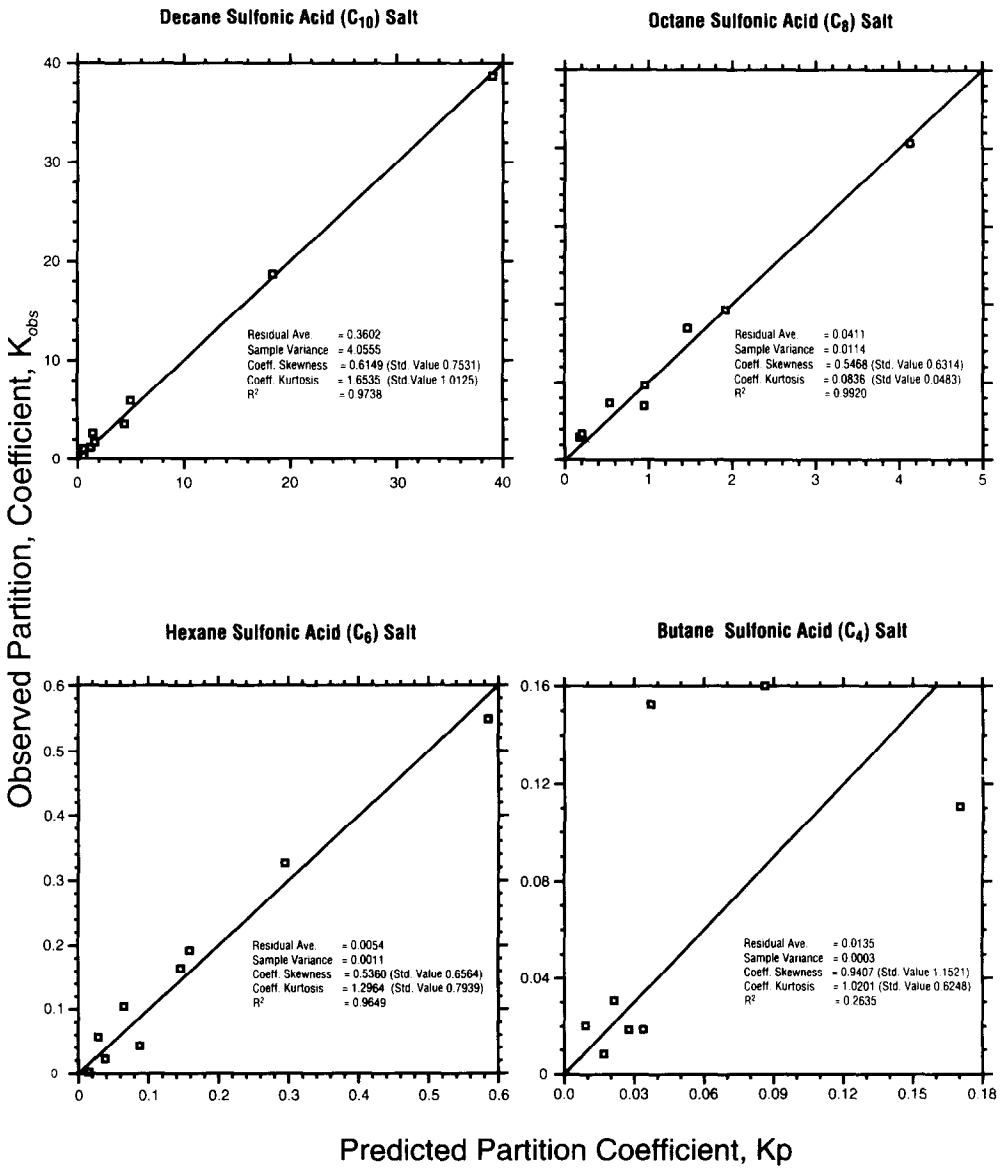


Fig. 1. Correlation of observed and predicted partition coefficients for various ion pairs of leuprolide.

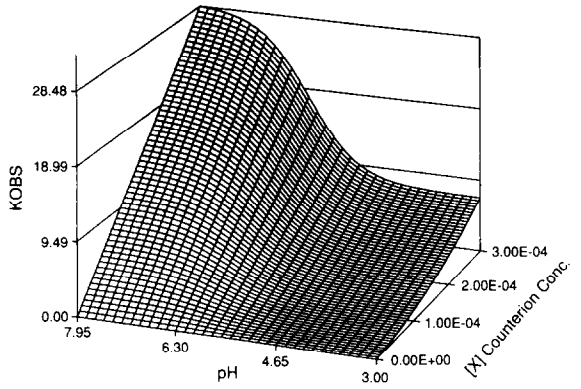


Fig. 2. Effect of pH and decanesulfonic acid concentration on paritioning of leuprolide.

perspective of how K_{obs} changes with pH and counterion concentration for the decyl salt is presented in Fig. 2. Regression (Figs 3–5) of log K vs n (n, carbon number of the alkyl side chain) of these counterions, will have a slope of 0.5 for

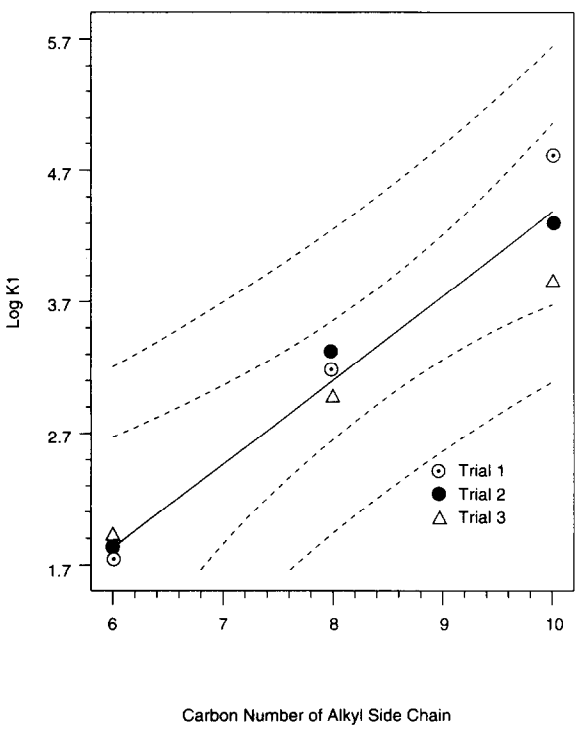


Fig. 3. Regression of equilibrium constant K_1 as a function of carbon number for various leuprolide ion pairs in octanol-water systems.

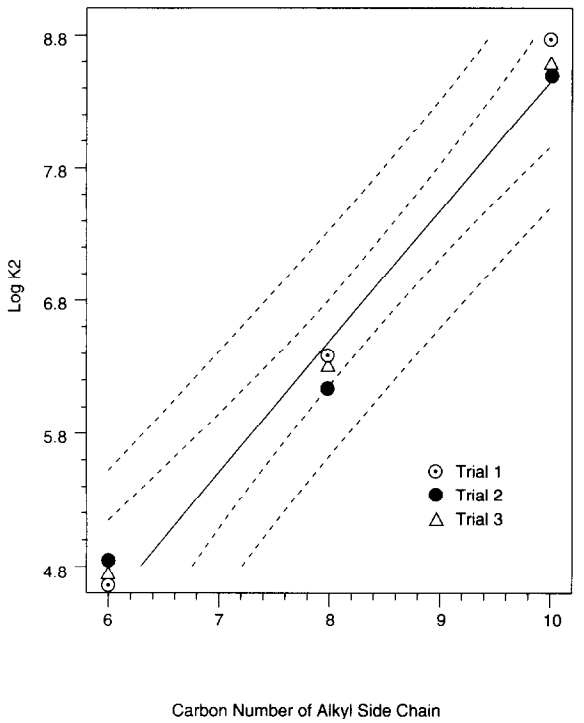


Fig. 4. Regression of equilibrium constant K_2 as a function of carbon number for various leuprolide ion pairs in octanal-water systems.

one methylene. This represents the Hansch π constant (Hansch and Clayton, 1973), and values obtained here were 0.52 for K_1 and K_3 and 0.98 for K_2 (Table 2). The slope of 0.98 for K_2 represents contributions from two counterions (i.e., two methylene groups).

Salicylate

Summarized in Table 3 are K_{obs} data obtained with salicylate counterion for leuprolide. A treatment of the data similar to the alkylsulfonate series did not provide any meaningful correlation indicating that different, and perhaps additional, factors may be involved in ion pair formation of leuprolide with salicylate. As with the alkylsulfonates, there is an increase in K_{obs} with corresponding increase in pH and counterion concentration. The magnitude of the increase in partition coefficient of leuprolide with salicylate is about the same as that of leuprolide with hexanesulfonate.

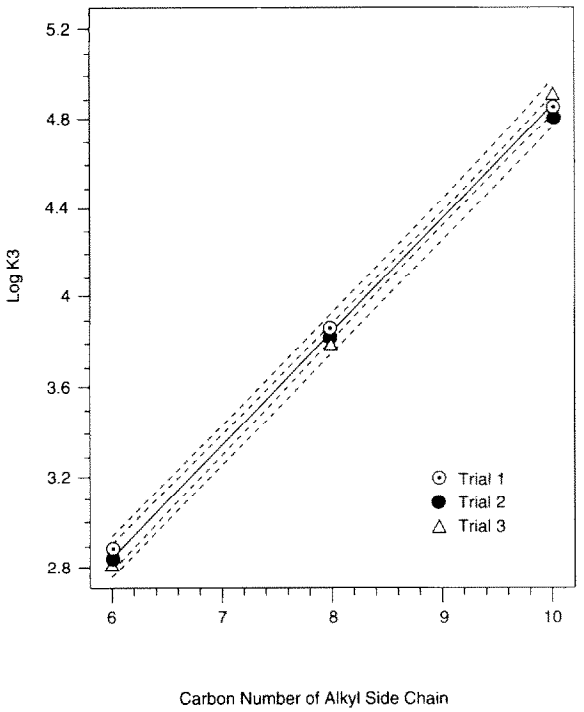


Fig. 5. Regression of equilibrium constant K_3 as a function of carbon number for various leuprolide ion pairs in octanal-water systems.

Dehydrocholic acid

Apparent partition coefficients obtained for leuprolide in the presence of dehydrocholic acid under the same experimental conditions as the alkylsulfonates are summarized in Table 3. Unlike the alkylsulfonates and salicylate, this counterion did not provide any beneficial effect on leuprolide partitioning even at pH 7. The low K_{obs} for leuprolide with cholic acid derivatives at lower pH values would be expected from theoretical considerations. Notably, the pK_a of the counterion, in this study dehydrocholic acid, is greater than 6 and should not be available for ion pairing at pH values lower than pH 7. Steric hindrance from the bulky nature of this molecule may be largely responsible for the low ion pair formation with leuprolide at the pH range (i.e., 3–7) under which the studies were carried out.

TABLE 3

Effect of pH and concentration of salicylate and dehydrocholate on apparent partition coefficient of leuprolide at 30°C

Ratio [a]/pH	Salicylate	Dehydrocholate
1:2 (3)	0	0.01
1:2 (5)	0.16	0.01
1:2 (7)	0.45	0.10
1:1 (3)	0.01	0
1:1 (5)	0.11	0
1:1 (7)	0.39	0.07
2:1 (3)	0	0
2:1 (5)	0.06	0
2:1 (7)	0.25	0.06

[a] Molar ratio of leuprolide to counterion concentration before partitioning.

Conclusion

The work summarized in this paper suggests that there are potentially several counterions capable of enhancing the lipophilicity of leuprolide. A model describing ion pair formation of leuprolide with selected alkylsulfonates was developed. This model accurately predicted the increase in the apparent partition coefficient of leuprolide under increasing pH and counterion concentration (range C1–C10). The theoretical model allowed correlation of leuprolide data with the Hansch model in which the contribution of each methylene group to the equilibrium constant is about 0.5.

The results also showed that methane- and butanesulfonate do not significantly help partitioning of leuprolide into octanol although there is a slight improvement in lipophilicity with increasing pH. Hexyl-, octyl- and decylsulfonates significantly increase K_{obs} of leuprolide with the effect ranking according to the following order: hexane < octane < decanesulfonate. With salicylate, the model did not satisfactorily predict results that were consistent with K_{obs} of leuprolide. The results also showed that dehydrocholic acid does not have any beneficial effect on K_{obs} of leuprolide even at pH 7. The relatively high pK_a of dehydrocholic acid and steric hindrance may have restricted ion pair formation with leuprolide under the conditions of the experiment. These results indicate that the extent of increase in lipophilicity of leuprolide may be proportional to the extent of deprotonation of the imidazolyl

nitrogen of histidine. Also, the effectiveness of ion pair formation (and therefore resulting lipophilicity) is proportional to pK_a of the acid from which the anion is derived, i.e., sulfonic acid ($pK_a < 2.0$) > salicylic acid ($pK_a \simeq 3.0$) > dehydrocholic acid ($pK_a > 6.0$).

The use of formulation adjuvants to increase the bioavailability of poorly absorbed drugs has routinely been employed in drug formula development work. Of particular interest is the finding that salicylates (Nishihata et al., 1980, 1981, 1982), bile acids (Hirai et al., 1981; Okada et al., 1982, 1983), and enamines (Kim et al., 1983) can be used to enhance the bioavailability of biochemically active compounds when administered rectally or nasally. These adjuncts presumably act by increasing permeability of the absorption barrier. Ion pairs of leuprolide with various counterions have been shown to possess greater partitioning into octanol than the acetate form of this drug. The data correlate well with other published studies (Lee et al., 1987) which show that ion-paired drugs attain electrical neutrality and enhanced lipophilic characteristics thereby promoting enhanced permeation through hydrophobic membranes. The data presented in this report suggest that ion pairs of peptides, for example, leuprolide decanesulfonate, might contribute to developing formulations with optimized absorption of these poorly orally absorbed compounds. The use of these agents in development of non-parenteral dosage forms may thus be of benefit in transport of leuprolide across absorption barriers in the body.

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