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Drug permeation through human skin. III. Effect of pH on the partitioning behavior of a chromone-2-carboxylic acid

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Summary

As part of a continuing study on the permeation properties of drugs through excised human skin, we have investigated the partitioning behavior of proxicromil, a chromone-2-carboxylic acid. One objective of this work was to identify, if possible, a two-phase system that would mimic the behavior of these compounds when partitioning into skin. A second objective was to quantitate the partition coefficients of both the ionized (K′) and unionized (K) forms of the chromone acid. We thus determined the distribution coefficients (D) at 37°C for proxicromil between aqueous buffers ranging in pH from 1 to 9 and butanol, hexanol, octanol, dodecanol and Miglyol (a fractionated, esterified coconut oil). Values for D were also determined for proxicromil between the aqueous buffers and excised human stratum corneum with attached epidermis (SCE). The Miglyol/water system exhibited partitioning behavior in agreement with theory up to pH 8; all other systems, including the SCE/water system, showed significant positive deviations from theory at pHs that were 2 or more units above the pK_a (1.93) of proxicromil. This behavior was shown to be consistent with significant ion-pair formation as the pH was raised. In contrast to K, the values of K′ in the several systems studied were directly related to the hydrophilicity of the organic phase. The data suggested that SCE has a significant hydrophilic component and, in this, more closely resembles butanol than octanol. Butanol/water may therefore be more appropriate than octanol/water when selecting an in vitro system to mimic the partitioning behavior of proxicromil and other compounds into skin.

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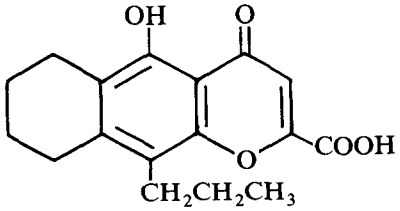
Introduction

In an earlier study (Swarbrick et al., 1984), the permeation of several lipophilic chromone-2-carboxylic acids through excised human skin was found to occur by passage of both the unionized and ionized species. The permeability coefficients for the unionized species were all approximately 10^4 times greater than those for the ionized species. As the pH was reduced, the proportion of the total flux due to the unionized species increased while that due to the ionized species decreased. It was predicted that flux would increase approximately 10^4-fold if the pH was reduced from a value of $(pK_a + 5)$ to a value of $(pK_a - 1)$. Since permeation of both unionized and ionized species took place, the implication is that both species can undergo partitioning from aqueous solutions into skin. If diffusion of these two species through skin occurs by the same route then the large permeability coefficients of the unionized chromone-2-carboxylic acids may be due to the possession of a higher partition coefficient between vehicle and skin than the ionized forms. The ability of ionized species to partition into lipid phases as ion pairs has been studied by several investigators. Of particular relevance is the work of Unger and Feuerman (1979) who, using equations derived by Horvath et al. (1977), studied the partitioning behavior of several lipophilic acids and their anions, including a xanthone-2-carboxylic acid, over a wide pH range. Van der Geisen and Janssen (1982) investigated the pH-dependent distribution of several 4-hydroxycoumarins between octanol and water. Depending on pH, the behavior of these weak acids was consistent with proposed partition mechanisms for unionized molecules, ion pairs, and ion pairs that dissociated in the organic phase. Other recent work has examined the partitioning characteristics of the lipophilic chromone-2-carboxylic acid, proxicromil, from aqueous buffers into octanol and gut tissues as well as its absorption in a perfused rat intestine system (Davis et al., 1984). It was concluded in this study that ion pair formation with simple cations plays a significant role in the gastrointestinal absorption of this compound. Sodium ion-pair formation has also been shown to have an influence on the transport of warfarin through octanol-impregnated membranes (Cools and Janssen, 1983). It is interesting that while many of these studies used octanol to mimic a biologic lipid phase, Beezer et al. (1983) have recently questioned, on thermodynamic grounds, the appropriateness of this solvent.

The present work was undertaken to study the partitioning behavior of the unionized and ionized forms of proxicromil, a lipophilic chromone-2-carboxylic



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acid (I), from aqueous buffer systems into four alkanols, a triglyceride oil, and excised human skin. We hoped thereby to identify an oil/water partitioning system that would be useful in predicting the permeation of this class of compounds through excised human skin.

Theory

The distribution of carboxylic acid HA between organic (o) and aqueous (w) liquid phases is influenced by the dissociation constant, K_a, of the acid and the pH of the aqueous layer. Thus:

$$K_a = \frac{[H^+]_w[A^-]_w}{[HA]_w} \tag{1}$$

Eqn. 2 defines the o/w partition coefficient K of the undissociated compound:

$$K = \frac{[HA]_0}{[HA]_w} \tag{2}$$

while Eqn. 3 defines the distribution coefficient D as:

$$D = \frac{[HA]_o}{[HA]_w + [A^-]_w} \tag{3}$$

Substitution of Eqns. 1 and 2 into Eqn. 3 results in:

$$\log(K/D - 1) = pH - pK_a \tag{4}$$

The relationship expressed by Eqn. 4 is shown in Fig. 1 for a monoprotic acid having a pK_a of 1.93 and a log K equal to 5.00.

Recently, this relationship has been extended to cover the case where the ionized species A^- enters the organic phase in the form of an ion-pair. This situation becomes significant when the ratio of the aqueous phase concentration of the anion A^- to the undissociated acid, HA, is similar to the ratio of the partition coefficients of the undissociated species (K_{HA}) to the anionic species (K_{A^-}). Eqn. 3 is accordingly modified such that:

$$D = \frac{[HA]_o + [A^-]_o}{[HA]_w + [A^-]_w} \tag{5}$$

Based on these equations, Horvath et al. (1977) derived the expression:

$$D = \frac{K + K' \cdot \frac{K_a}{[H^+]}}{1 + \frac{K_a}{[H^+]}} \tag{6}$$

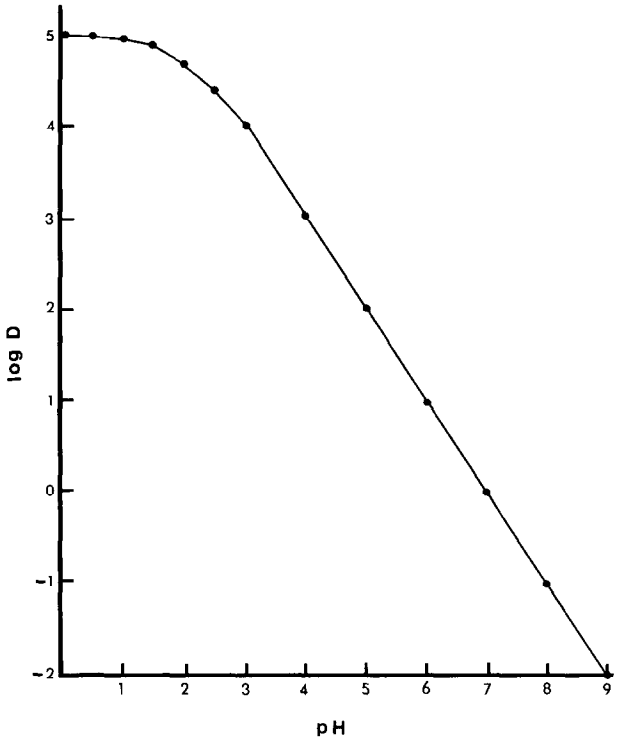

Fig. 1. Theoretical relationship between log D and pH for a mono-carboxylic acid, where $pK_a = 1.93$ and log P = 5.0.

where K and K′ are the partition coefficients for the unionized acid and the anion, respectively. While Eqn. 6 was derived to describe the effect of solute ionization on the retention of weak acids on octadecyl silica columns in HPLC analysis, it has also been applied to the determination of octanol/water partition and distribution coefficients of lipophilic acids (Unger and Feuerman, 1979).

Materials and Methods

Skin samples

Stratum corneum plus attached epidermis (SCE) was prepared from excised human skin taken from the mid-line of the chest at autopsy. The methods of separation, drying and rehydration of samples have been described previously (Swarbrick et al., 1982).

Chemicals

Proxicromil (FPL 57,787; 6,7,8,9-tetrahydro-5-hydroxy-4-oxo-10-propyl-4H-

naphtho(2,3,-b)-pyran-2-carboxylic acid) [1], 1-butanol [2], 1-hexanol [3], 1-octanol [4], 1-dodecanol [4] (all reagent grade) and Miglyol 812 [5] (a triglyceride of fractionated coconut oil esterified with caprylic and capric fatty acids) were all used as received. Buffer capsules [6] were used to prepare buffer solutions in the range pH 5.0–9.0. Buffer solutions within the range pH 1.0–4.0 were prepared according to Gomori (1955).

Determination of distribution coefficients

For each determination, a known weight of proxicromil was allowed to partition between equal volumes of an oil and the appropriate buffer solution for 48 h at 37°C ± 0.5°C. The flasks containing the two phases were gently shaken at frequent intervals. Under these conditions, mutual saturation of the two phases and equilibration of proxicromil between these phases was achieved. The concentrations of compound in the equilibrated butanol, hexanol, octanol and buffer phases were measured using the HPLC method described previously (Swarbrick et al., 1982). Ultraviolet spectrophotometry was used to assay for proxicromil present in the dodecanol and Miglyol 812 phases since these two oils were immiscible with the HPLC solvents used. Absorbance was measured at 249 nm with $E^{1\%}$ equal to 601 and 433 for dodecanol and Miglyol 812, respectively. The densities of the various mutually-saturated oil and buffer phases were determined gravimetrically and used to express the distribution coefficients of proxicromil on a w/w basis. Accordingly:

$$D = \frac{\mu g \text{ FPL } 57{,}787 \text{ per g oil phase}}{\mu \text{ FPL } 57{,}787 \text{ per g buffer phase}} \tag{7}$$

This approach permitted comparison with the distribution coefficients for the chromone acid determined between SCE and buffers, where weighed SCE samples were equilibrated in 25 ml volumes of the buffer solutions containing a known concentration of the compound. After equilibration, the distribution coefficients were calculated as:

$$D = \frac{\mu \text{ FPL } 57{,}787 \text{ per g of dry SCE}}{\mu g \text{ FPL } 57{,}787 \text{ per g of buffer phase}} \tag{8}$$

Results and Discussion

Plots of log D versus pH are shown in Fig. 2 for proxicromil distributed between aqueous buffer solutions ranging in pH from 1.0 to 9.0 and SCE, Miglyol, and the

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[5] Kay-Fries, Montvale, NJ 07645, U.S.A.
[6] pHydrion, Micro Essential Laboratories, Brooklyn, NY 11210, U.S.A.

four alkanols studied. The data on which these plots are based are presented in Table 1. Where standard deviations, based on log D, are shown, these were obtained using 3–5 replicates.

The theoretical relationship between D and pH expressed by Eqn. 4, and shown in Fig. 1, is based on the presumption that only unionized solute molecules partition into the organic phase; ionized solute is held to be absent from this phase. In Fig. 2 it is clear that the curves for proxicromil between the various water/organic systems follow this relationship at low pH. However, departure from this relationship takes place at pHs above 4, with the 1-butanol system being the first to give a distribution coefficient which is independent of pH. The expected distribution pattern followed at low pH is separable from the more complex partition behaviour observed at higher pHs, as shown in Fig. 3 where the values of D at each pH studied have been substituted into Eqn. 4 to calculate apparent values of K. The curves in Fig. 3 exhibit a break (designated pH_c) that denotes the pH at which Eqn. 4 is no longer followed. The consisted pattern of behaviour of the alkanols in this regard is

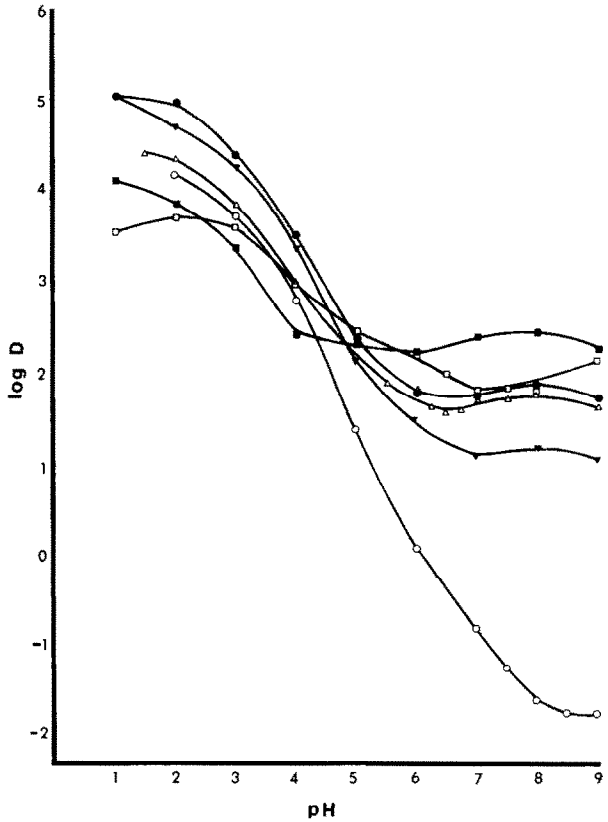

Fig. 2. Effect of pH on the partitioning of proxicromil between alkanols, Miglyol or SCE and aqueous buffers. Key: ■, 1-butanol; ●, 1-hexanol; △, 1-octanol; ▼, 1-dodecanol; ○, Miglyol; □, SCE.

TABLE 1

LOG D VALUES FOR PROXICROMIL IN VARIOUS LIPOPHILE/WATER SYSTEMS AT 37°C

pH	Components	Ionic strength (M)	log D in lipophile/water system (S.D.)					
			Butanol	Hexanol	Octanol	Dodecanol	Miglyol	SCE
1.0	HCL; KCl	0.134	4.12	5.04	–	5.06	–	3.57
1.5	HCl; KCl	0.091	–	–	4.40	–	–	–
2.0	HCl; KCl	0.063	3.90	4.98	4.36	4.72	4.22	3.74
3.0	Citric acid; Na_2HPO_4	0.300	3.37	4.39	3.87	4.29	3.76	3.63
4.0	Citric acid; Na_2HPO_4	0.250	2.42	3.56	3.04 (0.14)	3.39	2.80	3.04
5.0	KH_2PO_4; Na_2HPO_4	0.074	2.32	2.44	2.39 (0.01)	2.15	1.41	2.45 (0.30)
5.5	Citric acid; Na_2HPO_4	0.238	–	–	1.91	–	–	–
6.0	KH_2PO_4; Na_2HPO_4	0.097	2.25	1.78	1.82	1.53	0.09	2.23
6.25	KH_2PO_4; NaOH	0.061	–	–	1.63 (0.02)	–	–	–
6.5	KH_2PO_4; NaOH	0.061	–	–	1.59 (0.01)	–	–	2.00
6.75	KH_2PO_4; NaOH	0.072	–	–	1.63 (0.01)	–	–	–
7.0	KH_2PO_4; Na_2HPO_4	0.077	2.40	1.79	1.72 (0.04)	1.12	−0.81	1.80
7.5	KH_2PO_4; NaOH	0.092	–	–	1.63 (0.01)	–	−1.23	1.82
8.0	KH_2PO_4; Na_2HPO_4	0.083	2.48	1.91	1.82 (0.03)	1.22	−1.60	1.92
8.5	H_3BO_3; NaOH; KCl	0.337	–	–	–	–	−1.73	–
9.0	KH_2PO_4; $Na_2B_4O_7$	0.078	2.29	1.73	1.67 (0.05)	1.09	−1.74	2.18

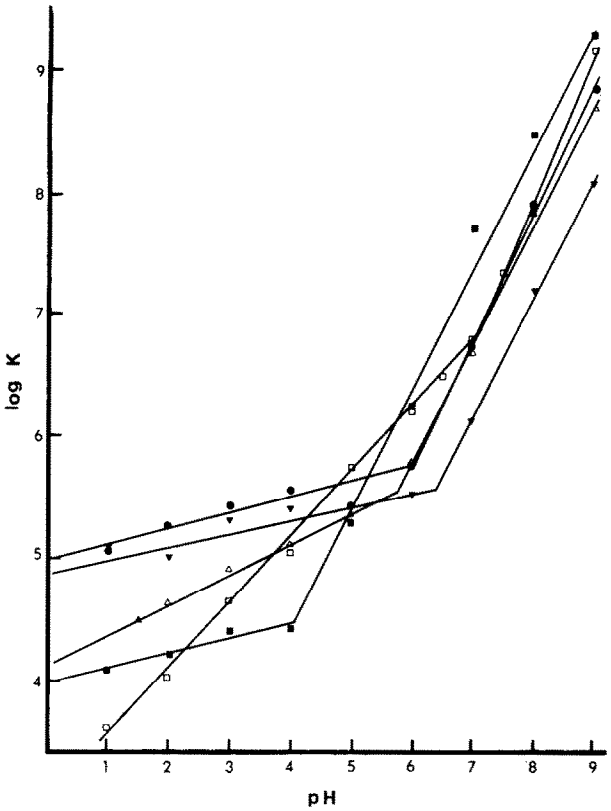

Fig. 3. Variation in apparent values of log K of Proxicromil with pH. Key: as in Fig. 2, with Miglyol data omitted for clarity.

emphasized by the similar slopes over the pH ranges both below and above pH_c (Table 2). In contrast, the SCE/water system differs from the alkanol/water systems by its different slopes both below and above pH_c, and by the higher value of pH_c. Although the data for the Miglyol/water systems (not plotted in Fig. 3) are more

TABLE 2

LOG K VERSUS pH FOR PROXICROMIL

System	Initial slope (r)	Final slope (r)	pH_c
butanol/water	0.11 (0.943)	1.01 (0.996)	4.07
hexanol/water	0.18 (0.994)	1.00 (0.998)	6.16
octanol/water	0.25 (0.999)	1.02 (0.996)	5.94
dodecanol/water	0.11 (0.925)	0.99 (0.998)	6.44
SCE/water	0.54 (0.999)	1.24 (0.999)	7.12
Miglyol/water	0.01 (0.089)	–	–

scattered, Table 2 shows that proxicromil exhibits close to ideal behaviour in this system over the pH range studied, having a slope close to zero and no pH_c.

Unger and Feuerman (1979) have used the logarithmic form of Eqn. 6, namely:

$$\log D = \log\left(K + K' \cdot \frac{K_a}{[H^+]}\right) - \log\left(1 + \frac{K_a}{[H^+]}\right) \quad (9)$$

to model the partitioning behaviour of unionized and ionized lipophilic acids between octanol and water and computed the respective partition coefficients of the two species. Such an analysis of the proxicromil ($pK_a = 1.93$) data collected in Table 1 results in the values for log K and K′ shown in Table 3. In three of the four alkanol/water systems (hexanol being the exception), K′ increases with chain length. In all four alkanol/water systems, as might be expected, K′ decreases as the lipophilicity of the respective organic phases increase with chain length. The close-to-ideal behaviour of the Miglyol/water system apparent from Fig. 1 is confirmed by the value of log K′ (−1.95) which indicates that very little ion-pair extraction occurs into the Miglyol phase. The results for the SCE/water system show that the partitioning behavior of proxicromil into this biophase more closely resembles that which occurs with butanol. This is perhaps best illustrated by the ratio K/K′ (Table 3) where again the value for SCE (141-fold in favor of K) is closest to that for butanol (81-fold in favor of K). In contrast, K for proxicromil in the frequently used octanol/water system is 1480 times that for K′.

The partitioning behavior of proxicromil in all systems, with the exception of Miglyol/water, is consistent with significant ion-pair formation at higher pHs. Two factors control the occurrence of this phenomenon, namely: (i) the relationship between the pK_a of the acid and the pH of the aqueous phases; and (ii) the lipophilicity of the organic or bio-phase. Thus, as the pH increases above the pK_a, the concentration of unionized species falls and hence the concentration of undissociated chromone-2-carboxylic acid in the organic phase. At the same time, the relative concentration of the ionized species increases, and with it the tendency for

TABLE 3

LOG K AND LOG K′ FOR PROXICROMIL

System	log K (SE)	log K′ (SE)	$K/K' \times 10^3$
butanol/water	4.24 (0.068)	2.33 (0.051)	0.081
hexanol/water	5.38 (0.079)	1.78 (0.090)	3.98
octanol/water	4.87 (0.086)	1.70 (0.062)	1.48
dodecanol/water	5.25 (0.063)	1.14 (0.081)	12.9
Miglyol/water	4.49 (0.100)	−1.95 (0.260)	2750
SCE/water	4.24 (0.217)	2.09 (0.146)	0.141

Fitted to Eqn. 7 using the 'FIT FUNCTION' procedure, RS/1 package, BBN Research Systems, Cambridge, MA, U.S.A.

ion-pair formation. However, the formation of ion-pairs in the aqueous phase occurs independent of the nature of the organic phase unless molecules of the organic (in particular the alkanol) phase dissolved in the aqueous phase are closely associated with the ion-pair. This is felt to be unlikely.

The value of pH_c may be regarded as that pH where undissociated acid partitioning into the organic phase is replaced by partitioning of the ionic species. This occurrence is determined by the ability of the ion pair to now penetrate the organic phase. This is related to the lipophilicity of the organic phase which, in the case of the alkanols, will depend on their chain length, water content at saturation, and dielectric constant. Available literature data (Table 4) confirm the trend observed for pH_c for the alkanols, with the exception of hexanol. Alkanols of chain length C4 to C12 exist as linear and cyclic tetramers, which in the presence of water rearrange to form a water-centered complex (A_4W). This complex is believed to be a tetrahedral orientation of hydrogen-bonded alkanol molecules surrounding the oxygen atom of a water molecule (Diamond and Wright, 1969), and as such retains a high degree of lipophilicity. As the water content of butanol (moles of water to moles of butanol, W/A = 1.050) is far in excess of the stoichiometric amount needed to form the A_4W complex, there is much non-complexed water present. This gives the butanol phase a very low lipophilicity, similar in magnitude to water saturated with butanol (McDonald, 1967) enabling ion-pairs to partition quite readily at a relatively low pH ($pH_c = 4.07$). Octanol and dodecanol have W/A values of 0.28 and 0.25, respectively, suggesting that most of the water molecules present are involved in the A_4W complex (where W/A would equal 0.25). These two alkanols therefore retain their lipophilicity, in spite of the water dissolved within them. Ion-pair extraction occurs at higher pHs ($pH_c = 5.94$ and 6.44, respectively). Hexanol, with a W/A value of 0.34, has a lipophilicity intermediate between butanol and octanol. However, the values for D in this alkanol at pH values below 4.0 are higher than those for the other alkanols (Fig. 2). In addition, as noted earlier, the pH_c for hexanol (6.16) lies between octanol and dodecanol. Taken together, these data indicate that hexanol behaves as a stronger lipophile than its structure suggests.

TABLE 4

ALKANOL/WATER SOLUBILITY DATA

Alkanol	Solubility of alkanol in water at 25°C [a] (molal)	Solubility of water in alkanol at 25°C [b] (molal)	Dielectric constant of pure alkanol at 20°C [c]
butanol	1.006	3.27	17.8
hexanol	6.14×10^{-2}	5.67×10^{-1}	13.3 (25°C)
octanol	4.51×10^{-3}	2.89×10^{-1}	10.3
dodecanol	2.30×10^{-5}	6.76×10^{-2}	–

[a] Amidon et al. (1974).

[b] McDonald (1967).

[c] 'Handbook of Chemistry and Physics', 49th edn., Chemical Rubber Co., page E59, 1968/1969.

The partitioning behavior of proxicromil between SCE and aqueous buffers shows a greater deviation from the theoretical relationship than any of the other systems studied. This is not surprising, given the complexity of the stratum corneum composition (Elias et al., 1981). The value for pH_c is higher than in any of the alkanol/water systems, yet the pre-pH_c slope (Fig. 3; Table 2) indicates that the hydrophilicity of this membrane is quite high. At the same time, the post-pH_c slope differs from all other systems studied. Proxicromil has been shown to be protein bound in the serum of several laboratory animal species to the extent of 99% or more (Smith et al., 1984). It is likely that such behavior also occurs in SCE and this may be responsible, totally or in part, for the differences observed between this system and the four alkanol/water systems. Nevertheless, the results presented do indicate the unique nature of SCE and the factors that need to be taken into account when attempting to devise in vitro models to mimic its properties. Our data suggest that the octanol/water system is not an adequate model and should be avoided. On the basis of current studies it would appear that an organic phase having lipophilic characteristics similar to butanol would be more suitable when attempting to mimic the partitioning behavior of proxicromil and related molecules into SCE, even though no account is taken of protein binding.

It has been suggested (Moser et al., 1975) that measurement of the partition coefficient of an acidic electrolyte will only be correct if made at a pH of less than three units above the pK_a. As we have shown with proxicromil, there is a marked deviation with SCE and the four alkanols due to ion-pair formation and extraction that starts as low as pH 4 — two pH units above the pK_a. Prudence dictates therefore that partition coefficients are best measured at pH values equal to or below the pK_a of the solute. If such measurements are not possible due to the solute's low solubility at these pHs, then the partition coefficient should be measured using a system containing a solvent such as Miglyol. As this solvent has near-ideal behavior, K can be accurately calculated from measurements of D made at higher pHs.

Finally, the fact that proxicromil readily forms ion-pairs with simple counter ions explain the ability of this compound to permeate biological membranes at physiologic pHs that are well removed from its pK_a (Davis et al., 1984).

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References

Amidon, G.L., Yalkowsky, S.H. and Leung, S., Solubility of nonelectrolytes in polar solvents II: Solubility of aliphatic alcohols in water. J. Pharm. Sci., 63 (1974) 1858–1866.

Beezer, A.E., Hunter, W.H. and Storey, D.E., Enthalpies of solution of a series of *m*-alkoxy phenols in water, *n*-octanol and water-*n*-octanol mutually saturated: derivation of the thermodynamic parameters for solute transfer between these solvents. J. Pharm. Pharmacol., 35 (1983) 350–357.

Cools, A.A. and Janssen, L.H.M., Influence of sodium ion-pair formation on transport kinetics of warfarin through octanol-impregnated membranes. J. Pharm. Pharmacol., 35 (1983) 689–691.

Davis, M.G., Manners, C.N., Payling, D.W., Smith, D.A. and Wilson, C.A., Gastro-intestinal absorption of the strongly acidic drug proxicromil. J. Pharm. Sci., 73 (1984) 949–953.

Diamond, J.M. and Wright, E.M., Molecular forces governing non-electrolyte permeation through cell membranes. Proc. Roy. Soc., B172 (1969) 272–316.

Elias, P.M., Cooper, E.R., Korc, A. and Brown, B.E., Percutaneous transport in relation to stratum corneum structure and lipid composition. J. Invest. Dermatol., 76 (1981) 297–301.

Giesen, van der, W.F. and Janssen, L.H.M., Influence of ionization and ion-pair formation on lipophilicity of some 4-hydroxycoumarin derivatives in the octanol–water system. Int. J. Pharm., 12 (1982) 231–249.

Gomori, G., Preparation of buffers for use in enzyme studies. In Colwick, S.P. and Kaplan, N.O. (Eds.), *Methods in Enzymology, Vol. 1*, Academic Press, New York, 1955, pp. 138–146.

Horvath, C., Melander, W. and Molnar, I., Liquid chromatography of ionogenic substances with nonpolar stationary phases. Anal. Chem., 49 (1977) 142–154.

McDonald, M.P., Molecular association in mono- and dihydric alcohol and alcohol–water systems. In Porter, R.S. and Johnson, J.F. (Eds.), *Ordered Fluids and Liquid Crystals* (Advances in Chemistry series, Vol. 63), American Chemical Society, Washington, DC, 1967, pp. 125–140.

Moser, P., Jakel, K., Krupp, P., Menasse, R. and Sallmann, A., Structure–activity relations of analogs of phenylbutazone. Eur. J. Med. Chem., 10 (1975) 613–617.

Smith, D.A., Brown, K. and Neale, M.G., Metabolism of chromone-2-carboxylic acids. Drug Metab. Rev., (1984) in press.

Swarbrick, J., Lee, G. and Brom, J., Drug permeation through human skin: I. Effect of storage conditions of skin. J. Invest. Dermatol., 78 (1982) 63–66.

Swarbrick, J., Lee, G., Brom, J. and Gensmantel, N.P., Drug permeation through human skin II: Permeability of ionizable compounds. J. Pharm. Sci., 73 (1984) 1352–1355.

Unger, S.H. and Feuerman, T.F., Octanol–aqueous partition, distribution and ionization coefficients of lipophilic acids and their anions by reversed-phase high-performance liquid chromatography. J. Chromatogr., 176 (1979) 426–429.