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Physicochemical properties and transport behaviour of piribedil: Considerations on its membrane-crossing potential

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Summary

The lipophilicity (expressed by log P_{oct}) and H-bond donor acidity (expressed by Δ log $P_{oct\text{-}hep}$) of the dopaminergic agonist piribedil in different ionization states were investigated in order to assess its capacity for crossing membranes. The present study showed that piribedil has a relatively high lipophilicity (log $P_{oct} = 2.84$) and is a non- or very weak H-bond donor (Δ log $P_{oct\text{-}hep} = 0.75$), implying optimal properties for transmembranal transport. Based on its microscopic ionization behaviour as studied by ^{13}C-NMR spectroscopy and (log P – log P^+) value (5.04) (a measure of the stability of the ionic vs neutral species in a lipidic phase), protonation proved to be very unfavourable in water-saturated octanol due to the hindrance of solvation by the two bulky groups adjacent to the piperazinyl amino group. In addition, transport of piribedil across a lipophilic membrane was studied according to first-order kinetics in a three-compartment model. The effects of lipophilic counterions on the partitioning behaviour and transfer kinetics were examined and shown to be non-existent at equimolar concentrations.

Introduction

In a recent theoretical study, we investigated the influence of physicochemical and structural properties of penetrants on their percutaneous absorption (El Tayar et al., 1991a). Our results showed that no correlations exist with molecular weight and solvent-accessible surface areas. In most cases, skin permeation was inversely correlated with the parameter Δ log $P_{oct\text{-}hep}$, i.e. log $P_{octanol}$ minus log $P_{heptane}$. This parameter is mainly a measure of the H-bond donor acidity of the solutes, but it is also slightly influenced by their H-bond acceptor basicity and polarity (El Tayar et al., 1991b). Lipophilicity itself, as expressed by log $P_{octanol}$, contributes positively to skin permeation in some cases. The results of this quantitative structure-permeability relationship study thus indicated that compounds with low H-bond donor acidity and/or high lipophilicity could readily permeate across human skin in which the lipophilic stratum corneum has been considered to be the principal barrier (Scheuplein, 1965; Flynn et al., 1981).

It appears that abundant H-bond acceptor groups (ester linkages, phosphate groups, etc.) in the lipidic phase of the stratum corneum may show a tendency to form stable H-bonds with strong H-bond donating solutes in media of low polarity, hence hindering their diffusion. An anal-

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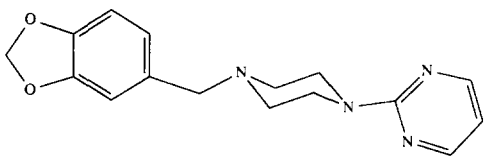


Fig. 1. Molecular structure of piribedil.

ogous relationship can be found in the penetration of drugs through lipid-rich tissues of the blood-brain barrier (Young et al., 1988). In view of the similarities between various biomembranes, it is thus highly likely that the structure-permeability relationship derived from percutaneous absorption can, at least qualitatively, be applied to membrane permeation in general.

While the relationships between lipophilicity (log P) and molecular structure have been abundantly explored (Van de Waterbeemd and Testa, 1987), the hydrogen-bond donor acidity is not always easy to predict quantitatively. This is particularly true for tertiary amines, whose H-bond donor acidity is a priori nil in the neutral state, and poorly understood in the protonated state. Within a broader project, we wished to investigate the relationship between log P_{oct}, Δ log P and pK_a for a dibasic tertiary amine of therapeutic interest, and for this purpose chose the dopamine agonist piribedil (1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidinyl)piperazine, Fig. 1) (Corrodi et al., 1972; Jenner et al., 1973; Caccia et al., 1985).

The initial goal of our study has thus been to achieve a better understanding of the lipophilicity and H-bond donor acidity of piribedil in the neutral state. Although 1-octanol/water has often been used as a reference partition system to simulate partition coefficients into biological membranes (Smith et al., 1975), some discrepancies have been found when comparing the partition coefficients of charged drugs in octanol/water and membranes/water (Mason et al., 1991). Thus, we also assessed the partitioning of monoprotonated piribedil into a lipidic phase and its relative stability therein. In addition, we intended to form ion pairs between piribedil and counterions since these have been proposed to facilitate the permeation of ionic drugs across lipidic biological membrane (e.g. Neubert and Fischer, 1991). While the model of ion-pair absorption has suffered ciriticism (Kakemi et al., 1969; Suzuki et al., 1972), this approach is nevertheless worth investigating. We thus used lipophilic counterions in the form of fatty acids. In this context, we investigated the microscopic ionization behaviour of piribedil, its permeability across an artificial lipophilic membrane in a three-compartment model, and the effect of ion-pair absorption.

Experimental

Materials

Piribedil (free base) was provided by I.R.I.S., Courbevoie, France, and used without further purification. Isopropyl myristate (purity ~ 90–95%), 1-octanol (purity ~ 98%), *n*-heptane (purity > 99%), and all carboxylic acids (purity > 99%) were purchased from Fluka Chemie (Buchs, Switzerland). Deuterated methanol (CD_3OD) of isotopic purity greater than 99.5 atom% was obtained from Dr Glaser AG (Basel, Switzerland), and tetramethylsilane (TMS) (purity > 99.8%) from Ciba-Geigy (Basel, Switzerland). All reagents were of analytical grade. Distilled water was used throughout. Isopropyl myristate was pretreated with very dilute aqueous solutions of sodium bicarbonate to remove residual myristic acid, and then dried using a molecular sieve (0.4 nm; Merck, Darmstadt, Germany).

The artificial membrane (VSWP 02500) was obtained from Millipore (Molsheim, France).

Methods

pK_a measurements The pK_a value of piribedil was determined by potentiometry and UV spectrophotometry at 25.0 ± 0.2°C and an ionic strength of 0.1. The initial concentration of piribedil was 3.2 mM. The method and equipment used for the potentiometric pK_a determination have been described previously (El Tayar et al., 1985). The spectrophotometric pK_a determination was performed using a Philips model PU8700 UV spectrophotometer. Piribedil was dissolved in 10 different buffered solutions of pH values ranging from 6.0 to 7.8, and the ab-

sorbance measured at 240 nm. Non-linear calculations based on the Henderson-Hasselbach equation yielded the pK_a value.

^{13}C chemical shift measurements ^{13}C-NMR spectra of piribedil were recorded at 50 MHz on a Varian VXR-200 spectrometer. The assignment of chemical shifts of piribedil in deuterated methanol was carried out by means of a DEPT experiment (distortionless enhancement by polarization transfer, which is based on polarization transfer in a heteronuclear *J*-coupled spin system and generates subspectra identifying carbon multiplicity) (Doddrell et al., 1982), and with the help of the spectra of three reference compounds, pyrimidine, piperazine, and 1,2-methylenedioxybenzene (Pretsch et al., 1986).

Distribution coefficient measurements Distribution coefficients of piribedil in octanol/buffer and heptane/buffer systems were measured by horizontal flow-through centrifugal partition chromatography (CPC) using a coil planet type centrifuge. The design of the instrument has been described (Ito, 1980; Ito and Oka, 1988). The apparatus from Pharma-Tech Research Corp. (Baltimore, MD, U.S.A.) used three columns, each of which was helically wound with five layers of PTFE tubing (3.00 mm i.d., 3.94 mm o.d.). The three columns made a total capacity of 350 ml. A Kontron model 432 UV/Vis detector coupled with a Hewlett-Packard 3392A integrator was used to detect the solutes. A Phase Separations flow meter allowed precise measurement of flow rates.

For log $D < 0$, measurements began by filling the columns with 1-octanol presaturated with 0.1 M phosphate buffer depending upon the pH used. While the columns were revolving at a speed of 1000 rpm along the central axis at that moment, they were also rotating along their own axis in a mode of planetary motion. The mobile phase (aqueous phase) was then pumped into the columns in a 'head-to-tail' mode during the rotation. As for log $D > 0$, the columns were first filled with aqueous solutions; this was then followed by pumping the mobile phase (organic phase) into the columns in a 'tail-to-head' mode during the rotation. Depending upon the expected distribution coefficients, the flow rate of the mobile phase was adjusted from 0.5 to 6.0 ml/min (flow rates of 0.5 ml/min for log $D <$ -2.3 or > 2.3; 1 ml/min for log D between -2.3 and -1.3 or between 1.3 and 2.3; and 6 ml/min for log D between -1.3 and 0 or between 0 and 1.3). A Merck injector was used to inject samples of 200 μl (in mobile phase solution, 1–50 mM). The solute was detected at a wavelength of 254 nm and all measurements were performed at 25 ± 0.1° C and in triplicate. Concentration effects were negligible, the difference in calculated log D values being smaller than 0.05 units.

At flow rates of 0.5, 1, and 6 ml/min, approx. 310, 305, and 270 ml of the stationary phase were retained, respectively. The retention time of the solvent front (t_0) was determined using non-retained solutes (potassium dichromate, when using aqueous solution as mobile phase; anthracene, when using octanol as mobile phase). It follows that the distribution coefficients can be calculated from Eqn 1:

$$\log D = \log \frac{(t_R - t_0) \cdot U}{V_t - U \cdot t_0}$$

(mobile phase: aqueous solution)

$$\log D = \log \frac{V_t - U \cdot t_0}{(t_R - t_0) \cdot U}$$

(mobile phase: octanol) (1)

where t_R is the retention time of the solute, U denotes the flow rate of the mobile phase, and V_t is the total capacity of the three columns (El Tayar et al., 1991c).

Taking as negligible the partitioning of the ionic species into the organic phase, the partition coefficient of a dibase such as piribedil can be calculated from the distribution coefficient using Eqn 2 (Van de Waterbeemd and Testa, 1987):

$$\log P = \log D + \log(1 + 10^{pK_{a1} - pH} + 10^{pK_{a1} + pK_{a2} - 2pH}) \quad (2)$$

(for $pK_{a1} > pK_{a2}$)

The distribution coefficients for piribedil in an isopropyl myristate/buffer system were measured by the traditional shake-flask method. For all measurements aqueous solutions of 0.1 M phosphate buffers were prepared. The volume ratios of the two phases were chosen to leave 30–60% of the solute in the aqueous phase. Both phases were submitted to gentle mechanical shaking for 3 h, a time amply sufficient to ensure attainment of equilibrium. After centrifugation for 20 min at 4000 rpm the concentration of solute in the aqueous phase was determined at 240 nm using a Perkin Elmer model 557 UV spectrophotometer. A detailed description of the experimental determination of partition coefficients can be found elsewhere (Leo et al., 1971; Mayer et al., 1982).

Assessment of H-bond donor acidity In a theoretical study on partitioning in different solvent systems and the contribution of H-bonding capacity, $\Delta \log P_{\text{oct-hep}}$ was shown to express mainly the capacity of solutes to donate hydrogen bonds (El Tayar et al., 1991a). Hence, the H-bond donor acidity of piribedil was expressed by the difference ($\log P_{\text{oct}} - \log P_{\text{hep}}$).

Equilibrium constant of the protonated species in the octanol phase The relative stability of the ionic vs neutral species in a lipidic phase can be assessed from their pK_a difference (ΔpK_a) in aqueous and water-saturated octanol solutions (Scherrer and Crooks, 1989). A linear free-energy relationship based on the partitioning and ionization of a base, HB, in octanol/water systems (Fig. 2) yields Eqn 3:

$$\log P - \log P^+ = pK_a^w - pK_a^o \qquad (3)$$

where P^+ is the partition coefficient of the protonated species, pK_a^w represents the dissociation constant in the water phase, and pK_a^o is the dissociation constant in the octanol phase. Thus, ΔpK_a can be obtained indirectly from the partition coefficient difference ($\log P - \log P^+$); in other words, ($\log P - \log P^+$) provides a measure of the relative stability of the protonated vs neutral species in lipid-like media.

Transport kinetics of piribedil across an artificial lipidic membrane The membrane permeation of drugs has been described according to a three-compartment model (Houk and Guy, 1988). In this model, a drug penetrates by passive diffusion from an aqueous donor compartment, across a membrane (neutral or synthetic), into an aqueous acceptor compartment. Since the process of transport by passive diffusion usually follows first-order kinetics, the observed transmembranal rate constant (k^{obs}) can be readily deduced from the derived kinetic equation (Kroon and Janssen, 1982):

$$\log(1 - C_2/C_0) = -(k^{\text{obs}}/2.303) \cdot t \qquad (4)$$

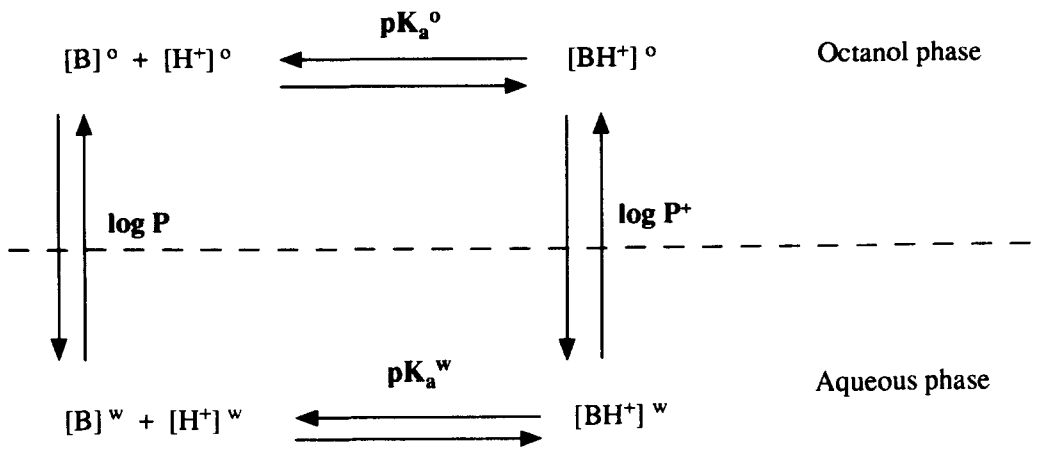


Fig. 2. Thermodynamic cycle of the ionization and partitioning of a base, B, in octanol/water systems.

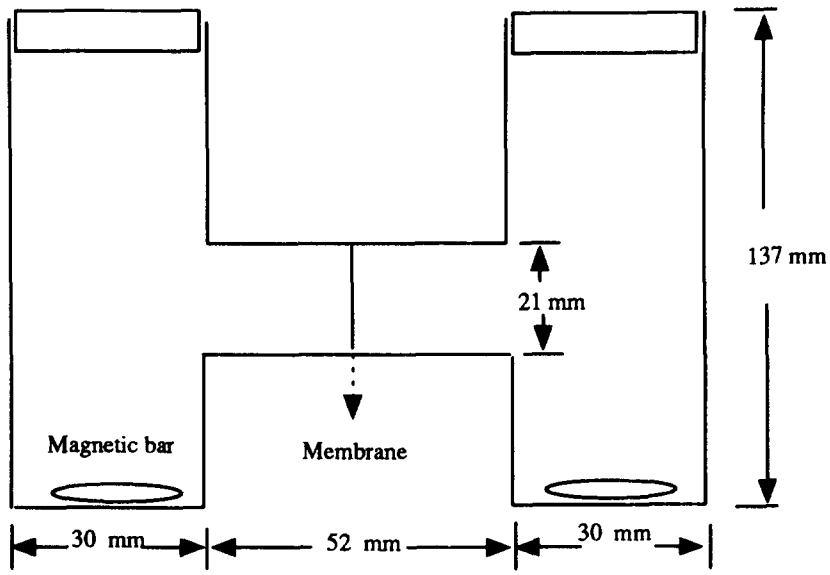


Fig. 3. Diffusion cell apparatus used for the determination of transfer rate constants.

where C_0 is the initial concentration of the drug applied in the donor compartment, C_2 being the concentration of the drug measured at time t. The observed rate constant can then be taken as a measure of the permeability of drugs across the membrane.

The transport of piribedil in a three-compartment model was studied in the diffusion cell apparatus (modified according to Tanaka et al., 1978) shown in Fig. 3. The synthetic Millipore membrane was impregnated with the solvent isopropyl myristate (IPM) which has been shown to be a valuable substitute for skin tissues (Albery and Hadgraft, 1979). Thus, the membrane was immersed in IPM overnight before being mounted in the diffusion cell; 40 ml samples of piribedil solutions at concentrations ranging from 0.03 to 0.16 mM and pH levels ranging from 4.8 to 8.3 were added carefully to the donor compartment, while equal volumes of phosphate buffers at pH 7.4 were added to the acceptor compartment. The all-glass apparatus was then partly immersed in a thermostatically controlled water bath at 37°C.

Measuring the concentration of piribedil in the acceptor compartment allowed calculation of the observed rate constants according to Eqn 4. In all experiments, the acceptor compartment was sampled using a Knauer HPLC pump (flow rate 1.3 ml/min) and analyzed spectrophotometrically at 240 nm using a Kontron model 7305 UV spectrophotometer. Fig. 4 depicts a diagram of the experimental set-up. The measurements were automated by coupling to a Knauer recorder.

Results and Discussion

Microscopic ionization behaviour of piribedil

Two pK_a values ($pK_{a1} = 6.91$, $pK_{a2} = 1.3$) have been reported in the literature for piribedil (Caccia et al., 1985). The correct assignment and the precise pK_a value for the first protonation site are of importance in order to gain a better understanding of the possible complexation of counter-

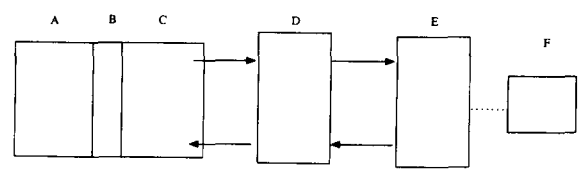


Fig. 4. Experimental set-up for the automated determination of transfer rate constants. (A) Stirred aqueous donor compartment; (B) artificial membrane impregnated with isopropyl myristate; (C) stirred aqueous acceptor compartment; (D) pump; (E) UV spectrophotometer; (F) recorder.

ions. The results of the ionization constant measurements were as follows: by potentiometry, $pK_a = 6.94 \pm 0.01$ ($n = 3$); by UV spectrophotometry, $pK_a = 6.92 \pm 0.22$ ($n = 3$). Since the pK_a value determined from the potentiometric titration was the most precise, it was used in the remainder of the study.

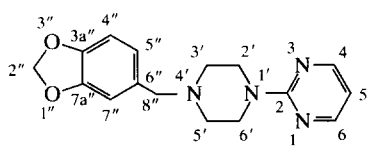
In the determination of the first protonation site, a DEPT experiment initially identified the primary, secondary, and tertiary carbon atoms (Doddrell et al., 1982). The ^{13}C chemical shifts of pyrimidine, piperazine, and 1,2-methylenedioxybenzene (Pretsch et al., 1986), all of which are moieties of piribedil, further confirmed the assignments. The ^{13}C-NMR spectra under various conditions of acidity or alkalinity were recorded so that changes in the chemical shifts could be rationalized in terms of changes in atom charge densities due to nitrogen protonation (Kuroda et al., 1980; Cossette and Vocelle, 1987). The ^{13}C chemical shifts of piribedil under various conditions are compiled in Table 1. A diagrammatic comparison of the chemical shift changes of each carbon atom reveals that the resonances of C(8″), C(3′) and C(5′) were significantly shifted upfield for a change from neutrality to slight acidity, implying that the nitrogen atom attached to the methylenedioxybenzyl group is the first protonation site (Kuroda et al., 1980). In addition, the resonance of C(2) was dramatically shifted upfield on change from slight to strong acidity, implying that the second protonation site is the guanidinyl moiety. The lower sensitivity of chemical shifts to pH changes from alkalinity to neutrality is presumably due to the effect of methanol on ionization.

Lipophilicity and H-bond donor acidity of piribedil

The distribution coefficients of piribedil as measured by CPC for various ionization states in 1-octanol/water and *n*-heptane/water systems are shown in Fig. 5. The log D_{oct} value at pH 7.4 (2.71) is similar to the literature value (2.72) (Caccia et al., 1985). The lipophilicity of piribedil

TABLE 1

^{13}C chemical shifts of piribedil under various conditions [a]



C atom [b]	Alkalinity (CD_3OD/NaOH)	Neutrality (CD_3OD)	Slight acidity (CD_3OD/CH_3COOH)	Strong acidity (CD_3OD/HCl)
C(5″)	132.073	132.230	126.633	123.008
C(6″)	111.235 [c]	111.178 [e]	112.140 [g]	112.356 [i]
C(7″)	110.790 [c]	110.733 [e]	111.580 [g]	112.192 [i]
C(4″)	108.827 [c]	108.809 [e]	109.345 [g]	109.752 [i]
C(7a″)	148.390 [d]	148.403 [f]	149.594 [h]	149.840 [j]
C(3a″)	149.164 [d]	149.206 [f]	149.833 [h]	150.793 [j]
C(2″)	102.301	102.292	102.828	103.193
C(8″)	63.691	63.729	62.109	61.408
C(3′),C(5′)	44.594	44.620	42.883	43.228
C(2′),C(6′)	53.704	53.767	52.627	51.239
C(2)	162.802	162.862	162.503	154.724
C(4),C(6)	159.046	159.008	159.191	158.244
C(5)	124.052	123.940	125.744	127.125

[a] Chemical shifts (δ in ppm) relative to TMS.

[b] Non-systematic numbering in order to encompass all the heavy atoms.

[c–j] May be interchanged.

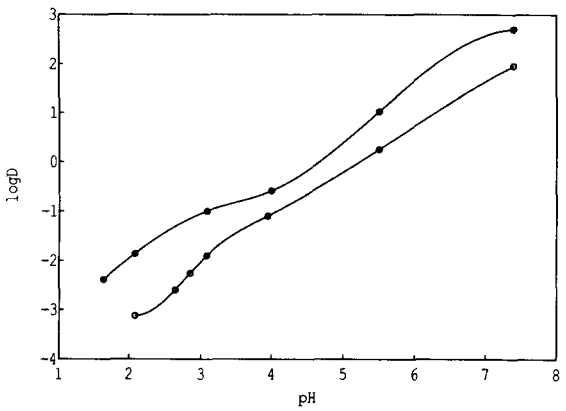


Fig. 5. Distribution coefficients of piribedil in various ionization states in octanol/buffer (●) and heptane/water (○) systems. The average standard deviation is 0.02.

in the neutral form (log $P_{oct} = 2.84$) suggests a relatively high affinity for cutaneous tissues, since the partition coefficients in the 1-octanol/water system were shown to be closely correlated with those in a skin/water system (El Tayar et al., 1991a). Its log P_{hep} value (2.09) can be calculated from the log D_{hep} value at pH 7.4 (1.96) in Fig. 5.

In the theoretical study of El Tayar et al. (1991b) on the correlation between H-bond donor acidity and Δ log $P_{oct\text{-}hep}$, 75 compounds with zero, one or two H-bond donor groups (e.g. $-OH$, $-NH_2$, etc.) were shown to have Δ log $P_{oct\text{-}hep}$ values ranging from -0.79 (*n*-pentane) to 4.65 (sulfathiazole). In comparison, the Δ log $P_{oct\text{-}hep}$ value of piribedil in the neutral form (0.75) im-

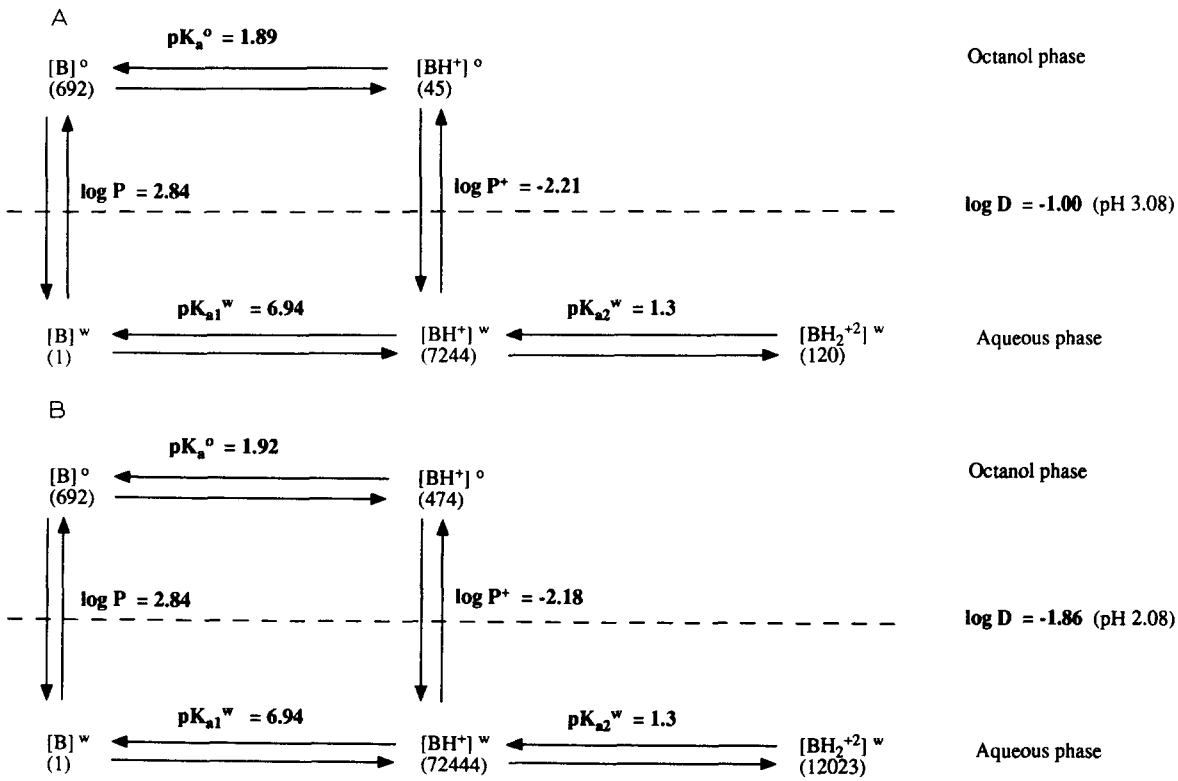


Fig. 6. Equilibria of piribedil in octanol/water systems. $[B]^w$, $[BH^+]^w$, and $[BH_w^{+2}]^w$ represent the concentrations of neutral, mono-protonated, and di-protonated species in the aqueous phase, respectively, $[B]^o$, $[BH^+]^o$, and $[BH_2^{+2}]^o$ being those in the octanol phase. The number in parentheses indicates the population ratio of the species. While log *P*, pK_{a1}^w, pK_{a2}^w, and log *D* are measured values, the pK_a^o and log P^+ values can be calculated from the mass action law. (A) Equilibria at pH 3.08; (B) equilibria at pH 2.08.

plies that it is a non- or very weak H-bond donor. It follows that piribedil in the neutral form can be predicted to be a good skin penetrant on the basis of its high lipophilicity and low H-bond donor acidity according to the quantitative structure-permeability relationship study of El Tayar et al. (1991a), assuming that no specific interactions occur between the drug and endogenous molecules (e.g., proteins, enzymes, etc.).

Relative stability of the protonated vs neutral form of piribedil in water-saturated octanol

Assuming that the di-protonated species does not partition into the octanol phase, the partition coefficient of the mono-protonated species (log P^+) and its dissociation constant in the octanol phase (pK_a^o) can be calculated using Eqn 3, based on the measured distribution coefficient (log D), ionization constant in aqueous solution (pK_a^w), and partition coefficient of the neutral species (log P). In Fig. 6A and B, the calculated results are shown: log $P^+ = -2.21$ and -2.18; $pK_a^o = 1.89$ and 1.92. It appears that ΔpK_a is equal to (log P − log P^+) (5.04) as indicated by Eqn 2. Testa and Murset-Rossetti (1978) previously determined the (log P − log P^+) values for diaryl antihistamines and 1-*n*-alkylamines, the average being 3.53 ± 0.41 ($n = 19$) and 2.26 ± 0.10 ($n = 7$), respectively. The relatively high (log P − log P^+) value for piribedil (5.04) is suggestive of a remarkably unstable protonated species in the octanol phase. Thus, the partitioning of protonated piribedil is highly unfavourable.

Scherrer and Crooks (1989) used ΔpK_a ($pK_a^w - pK_a^o$) as a measure of the stability of protonated basic agents in a water-saturated octanol phase and found that protonated primary amines are more stable than tertiary amines, implying that bulky substituents on the amino group are unfavourable to the solvation of the charge. Indeed, the two adjacent aryl groups surrounding the protonated tertiary amino group of piribedil must be energetically unfavourable to its solvation in the hydrophilic core of the water-centred complex with four octanol molecules hydrogen-bonded to a water molecule in a tetrahedral orientation (Fig. 7) which is believed to be the predominant structure in the water-saturated 1-octanol phase (Smith et al., 1975).

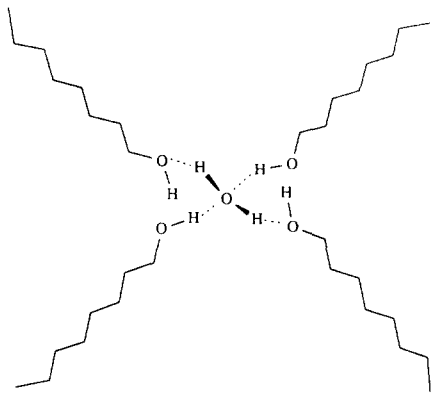


Fig. 7. Tetrahedral orientation of hydrogen-bonded 1-octanol around the oxygen atom of water; this complex is believed to be the most abundant species in water-saturated octanol phase (Smith et al., 1975).

Permeation of piribedil across an artificial lipidic membrane and the effect of ion-pair absorption

The transport kinetics of piribedil, from a donor compartment at pH 5.0, across the IPM-saturated membrane, into an acceptor compartment at pH 7.4, is demonstrated in Fig. 8. The calculated transfer rate constants of piribedil and its corresponding distribution coefficients at vari-

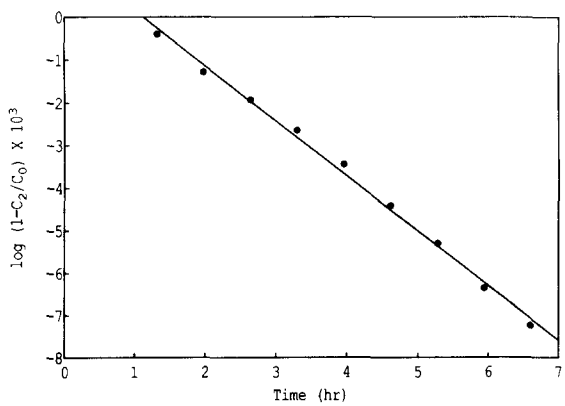


Fig. 8. Permeation kinetics of piribedil in a three-compartment model. C_0 denotes the initial concentration of piribedil in the donor compartment at pH 5.0 (0.16 mM); C_2 is its concentration in the acceptor compartment (pH fixed at 7.4) measured at time t.

TABLE 2

Observed rate constants of piribedil at different ionization states and its corresponding distribution coefficients in an IPM / water system

pH of donor compartment [a]	Percent protonation in donor compartment [b]	Log k^{obs} (s^{-1}) [c] (±S.D.)	Log D_{IPM} [d] (±S.D.)
5.0	98.9	−5.56±0.02	0.77±0.01
5.5	96.5	−5.32±0.05	1.25±0.02
6.0	87.2	−5.00±0.04	1.72±0.01
6.5	73.4	−4.62±0.04	2.14±0.04
7.0	46.6	−4.43±0.10	2.44±0.02
7.4	25.7	−4.29±0.05	2.58±0.04
8.0	9.9	−4.29±0.03	2.66±0.05
8.3	4.1	−4.30±0.02	2.69±0.04

[a] pH of the acceptor compartment: 7.4.
[b] Calculated based on $pK_{a1} = 6.94$, $pK_{a2} = 1.3$.
[c] Values calculated based on Eqn 4.
[d] Distribution coefficients measured by the shake-flask method in isopropyl myristate/water systems.

ous pH values in IPM/buffer systems are listed in Table 2. The relationship between these two properties is linear (Eqn 5),

$$\log k^{obs} = 0.70(\pm 0.02) \log D_{IPM} - 6.15(\pm 0.05) \quad (5)$$
$$n = 8;\ r = 0.996$$

TABLE 3

Effect of lipophilic carboxylate counterions on the distribution coefficient of protonated piribedil in an IPM / water system at pH 5.5

Carboxylate	Log D^{a}_{IPM} (±S.D.)
Control (0.1 M phosphate)	1.29±0.05
Propanoate	1.25±0.03
Butanoate	1.34±0.04
Pentanoate	1.31±0.03
Hexanoate	1.36±0.04

[a] Measured by the shake-flask method.
The molar ratio of piribedil to carboxylate used was 1/1.

TABLE 4

Observed rate constants (k^{obs}) of piribedil in a three-compartment model in the presence of carboxylate

Carboxylate	Log k^{obs} (s^{-1}) (±S.D.)
Control (0.1 M phosphate)	−5.00±0.04
Butanoate	−5.02±0.03
Pentanoate	−4.89±0.04
Hexanoate	−4.95±0.05
Heptanoate	−5.03±0.05
Octanoate	−4.93±0.05
Nonanoate	−5.05±0.08

The donor compartment was maintained at pH 6.0 while the acceptor compartment was kept at pH 7.4. The initial concentration of piribedil in the donor compartment was about 0.13 mM, together with 1 mM carboxylate.

clearly corroborating the 'pH-partition theory' in this three-compartment model (Kroon and Janssen, 1982).

The influence of lipophilic counterions on the distribution coefficient of protonated piribedil in a biphasic system is shown in Table 3, demonstrating that no ion-pair extraction occurred when 1/1 molar ratios of piribedil and carboxylate were used. In a further inspection of the concept, the observed rate constants of piribedil (at pH 6.0) in the presence of carboxylic acids in a three-compartment model were determined and found to be indistinguishable from controls, as seen in Table 4, again suggesting that no ion-pair absorption existed in this model.

Conclusions

In the present study, piribedil has been shown to have a high lipophilicity (log $P_{oct} = 2.84$) and a non-existent or very weak H-bond donor acidity (Δ log $P_{oct\text{-}hep} = 0.75$), the two properties being known to influence skin penetration (El Tayar et al., 1991b).

Assessment of the relative stability of the mono-protonated vs neutral species in a water-saturated octanol phase by its (log P − log P^{+}) value (5.04) implies a high energy cost for its solvation, probably due to steric effects. It should

be noted that steric factors play equally important roles in the transport of protonated molecules into biological membranes. X-ray crystallography, small angle X-ray scattering studies and structural comparison of calcium channel blockers led Mason et al. (1989) to conclude that a hydrophobic interaction with the phospholipid acyl chains and an ionic interaction between the anionic oxygen of the phosphate head group and the protonated primary amino group of amlodipine account for its relatively long pharmacokinetic half-lives. In contrast, the presence of a phenyl group adjacent to the charged tertiary amine of nicardipine renders the electrostatic interactions energetically unfavourable and could explain the pharmacokinetic behaviour of this drug. In the light of the microscopic protonation site of piribedil and the stability of the protonated species in water-saturated octanol, it seems unlikely that this species can contribute to membrane permeation if indeed octanol mimics lipidic membranes. Neutral piribedil must therefore be the sole species permeating across membranes.

As for ion-pair absorption effects using lipophilic counterions in a 1/1 molar ratio, they were shown to be non-existent in organic phase extraction ($\log D_{IPM}$) as well as in permeation across a lipophilic membrane ($\log k^{obs}$).

In summary, the neutral form of piribedil shows promising potential in penetrating across lipid-rich tissues; therein its protonated species containing charged tertiary amine is relatively unstable and should not contribute to partitioning into membranes. Studies are currently in progress in our laboratory aimed at the elucidation of the conformational behaviour of the protonated species by quantum chemical calculations.

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References

Albery, W.J. and Hadgraft, J., Percutaneous absorption: in vivo experiments. *J. Pharm. Pharmacol.*, 31 (1979) 65–68.

Caccia, S., Fong, M.H. and Guiso, G., Disposition of the psychotropic drugs buspirone, MJ-13805 and piribedil, and of their common active metabolite 1-(2-pyrimidinyl)-piperazine in the rat. *Xenobiotica*, 15 (1985) 835–844.

Corrodi, H., Farnebo, L.-O., Fuxe, K., Hamberger, B. and Ungerstedt, U., ET495 and brain catechol amine mechanisms. Evidence for stimulation of dopamine receptors. *Eur. J. Pharmacol.*, 20 (1972) 195–204.

Cossette, D. and Vocelle, D., Protonation of a conjugated Schiff base by weak to strong acids: carbon-13 and nitrogen-15 nuclear magnetic resonance study. *Can. J. Chem.*, 65 (1987) 661–665.

Doddrell, D.M., Pegg, D.T. and Bendall, M.R., Distortionless enhancement of NMR signals by polarization transfer. *J. Magn. Reson.*, 48 (1982) 323–327.

El Tayar, N., Van de Waterbeemd, H. and Testa, B., Lipophilicity measurements of protonated basic compounds by reversed-phase high-performance liquid chromatography. II. Procedure for the determination of a lipophilic index measured by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, 320 (1985) 305–312.

El Tayar, N., Tsai, R.-S., Tests, B., Carrupt, P.-A., Hansch, C. and Leo, A., Percutaneous penetration of drugs: a quantitative structure-permeability relationship study. *J. Pharm. Sci.*, 80 (1991a) 744–749.

El Tayar, N., Tsai, R.-S., Testa, B., Carrupt, P.-A. and Leo, A., Partitioning of solutes in different solvent systems: the contribution of hydrogen-bonding capacity and polarity. *J. Pharm. Sci.*, 80 (1991b) 590–598.

El Tayar, N., Tsai, R.-S., Vallat, P., Altomare, C. and Testa, B., Measurements of partition coefficients by various centrifugal partition chromatographic techniques: a comparative evaluation. *J. Chromatogr.*, 556 (1991c) 181–194.

Flynn, G.L., Durrheim, H. and Higuchi, W.I., Permeation of hairless mouse skin. II. Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.*, 70 (1981) 52–56.

Houk, J. and Guy, R.H., Membrane models for skin penetration studies. *Chem. Rev.*, 88 (1988) 455–471.

Ito, Y., New horizontal flow-through coil planet centrifuge for counter-current chromatography. I. Principle of design and analysis of acceleration. *J. Chromatogr.*, 188 (1980) 33–42.

Ito, Y. and Oka, H., Horizontal flow-through coil-planet centrifuge equipped with a set of multilayer coils around the column holder. Counter-current chromatography of proteins with a polymer-phase system. *J. Chromatogr.*, 457 (1988) 393–397.

Jenner, P., Taylor, A.R. and Campbell, D.B., Preliminary investigation of the metabolism of piribedil (ET495); a new central dopaminergic agonist and potential anti-Parkinson agent. *J. Pharm. Pharmacol.*, 25 (1973) 749–750.

Kakemi, K., Sezaki, H., Muranishi, S. and Tsujimura, Absorption and excretion of drugs. XL. Enhancement of the rectal absorption of pharmaceutical amines with lauryl sulfate and saccharinate anions. *Chem. Pharm. Bull.*, 17 (1969) 1641–1650.

Kroon, J.D.L. and Janssen, L.H.M., An in vitro experiment to demonstrate kinetic aspects of pH partition theory. *Am. J. Pharm. Educ.*, 46 (1982) 241–245.

Kuroda, Y., Lee, H. and Kuwae, A., Direct evidence for the site of protonation of 4-aminoazobenzene by nitrogen-15 and carbon-13 nuclear magnetic resonance spectroscopy. *J. Phys. Chem.*, 84 (1980) 3417–3423.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. *Chem. Rev.*, 71 (1971) 525–616.

Mason, R.P., Campbell, S.F., Wang, S.-D. and Herbette, L.G., Comparison of location and binding for the positively charged 1,4-dihydropyridine calcium channel antagonist amlodipine with uncharged drugs of this class in cardiac membranes. *Mol. Pharmacol.*, 36 (1989) 634–640.

Mason, R.P., Rodes, D.G. and Herbette, L.G., Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. *J. Med. Chem.*, 34 (1991) 869–877.

Mayer, J.M., Testa, B., Van de Waterbeemd, H. and Bornand-Crausaz, A. Non-additivity and intramolecular effects in the partition coefficient of ω-functionalized alkylpyridines. *Eur. J. Med. Chem.*, 17 (1982) 443–459.

Neubert, R. and Fischer, S., Influence of lipophilic counter ions on the transport of ionizable hydrophilic drugs. *J. Pharm. Pharmacol.*, 43 (1991) 204–206.

Pretsch, E., Clerc, T., Seibl, J. and Simon, W., *Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden*, Springer, Berlin, 1986.

Scherrer, R.A. and Crooks, S.L., Titrations in water-saturated octanol: a guide to partition coefficients of ion pairs and receptor-site interactions. In Fauchère (J.L. (Ed.), *QSAR: Quantitative Structure-Activity Relationships in Drug Design*, A.R. Liss, New York, 1989, pp. 59–62.

Scheuplein, R.J., Mechanism of percutaneous adsorption. I. Routes of penetration and the influence of solubility. *Invest. Dermatol.*, 45 (1965) 334–346.

Smith, R.N., Hansch, C. and Ames, M.M., Selection of a reference partitioning system for drug design work. *J. Pharm. Sci.*, 64 (1975) 599–606.

Susuki, E. Tsukigi, M., Muranishi, S., Sezaki, H. and Kakemi, K., The absorption from the gut of quinine and chloropheniramine given with various anionic agents. *J. Pharm. Pharmacol.*, 24 (1972) 138–144.

Tanaka, M., Fukuda, H. and Nagai, T., Permeation of drug through a model membrane consisting of Millipore filter with oil. *Chem. Pharm. Bull.*, 26 (1978) 9–13.

Testa, B. and Murset-Rossetti, L., The partition coefficient of protonated antihistamines. Its calculation and interpretation in terms of hydrophobic fragmental constants. *Helv. Chim. Acta*, 61 (1978) 2530–2537.

Van de Waterbeemd, H. and Testa, B., The parametrization of lipophilicity and other structural properties in drug design. In Testa, B. (Ed.), *Advances in Drug Research*, Academic Press, London, 1987, Vol. 16, pp. 85–225.

Young, R.C., Mitchell, R.C., Brown, T.H., Ganellin, C.R., Griffiths, R., Jones, M., Rana, K.K., Saunders, D., Smith, I.R., Nerina, E.S. and Wilks, T.J., Development of a new physicochemical model for brain penetration and its application to the design of centrally acting H_2 receptor histamine antagonists. *J. Med. Chem.*, 31 (1988) 656–671.