International Journal of Pharmaceutics, 46 (1988) 231–239
Elsevier

IJP 01569

Dissociation and partitioning of Progabide and its degradation product

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(Received 4 January 1988)
(Modified version received 17 March 1988)
(Accepted 22 March 1988)

Key words: Progabide; SL79.182; Dissociation; pK_a; Partition; Filter-probe extraction

Summary

The dissociation and partitioning behaviour of Progabide and one of its degradation products, SL79.182, were investigated. Progabide was found to be amphoteric with pK_a values of 3.41 and 12.95. SL79.182 behaved as a typical weak acid with a pK_a value of 8.94. The apparent partition coefficient–pH profile of Progabide for the water/*n*-octanol system was assessed by the filter-probe extractor method whereas that of SL79.182 was determined by the shake-flask method. Log (*P*) values of 2.97 for Progabide and 4.04 for SL79.182 were derived.

Introduction

A number of methods were examined (Albert and Serjeant, 1971) for the determination of the ionisation constant. Only two methods, namely potentiometry and spectrometry, were recommended for use because of their outstanding advantage in terms of accuracy, reproducibility and convenience. Generally, potentiometry can only be applied to drugs with appreciable water solubility. Fleuren et al. (1979) described a differential potentiometric titration procedure suitable for determining the pK_a's of very slightly water-soluble drugs. However, their procedure could not be successfully applied to Progabide or SL79.182. Other authors (Levy and Rowland, 1971; Kaufman et al., 1975; Li Wan Po and Irwin, 1980) adapted the traditional potentiometric technique by simultaneous measurement of another property e.g. precipitation, partition or solubility but these methods involve cumulative errors which render them inaccurate. From a consideration of both the solubility and stability of Progabide and SL79.182 and because the ionised and molecular forms of these compounds absorb differently in the u.v./visible regions of the spectrum (Farraj et al., 1988a), spectrometry was chosen for the determination of pK_a values.

The partition coefficient is another useful parameter for predicting the absorption of drugs from the gastrointestinal tract. For this, the *n*-octanol/water system has become the standard reference system for quoting experimental partition coefficient data. For Progabide, traditional meth-

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ods of partition coefficient determination that involve long incubation periods such as the shake-flask method (Fujita et al., 1964) cannot be used. The low aqueous stability of this compound imposes the requirement that a very rapid partitioning system must be used if reliable data are to be obtained. Of the automated rapid partitioning/analysis systems cited (Davis et al., 1976; Kinkel and Tomlinson, 1980; Tomlinson, 1982) the filter-probe extractor method (Tomlinson, 1982) was chosen for use because of its simplicity, flexibility, and short equilibrium times, typically 0.5–15 min. The validity of the method was confirmed by determining the partition–pH profile of a basic drug with a high partition coefficient, namely, propranolol.

Theory

Spectrophotometry can be used to determine the pK_a of a drug by determining the ratio of unionised species (A_u) to ionised species (A_i) in a series of non-absorbing buffers of known pH values. Albert and Serjeant (1971) derived equations to relate the observed absorbance (A) of the species involved at the chosen analytical wavelength to the pK_a and pH of the solutions examined. For an acidic functional group, Eqn. 1 applies if $A_i > A_u$ and Eqn. 2 if the reverse is the case:

$$pK_a = pH + \log[(A_i - A)/(A - A_u)] \quad (1)$$

$$pK_a = pH + \log[(A - A_i)/(A_u - A)] \quad (2)$$

For a basic functional group, Eqn. 3 is used if $A_i > A_u$, and Eqn. 4 if the reverse is true:

$$pK_a = pH + \log[(A - A_u)/(A_i - A)] \quad (3)$$

$$pK_a = pH + \log[(A_u - A)/(A - A_i)] \quad (4)$$

The experimental values of A_u, A_i, and A are substituted into the appropriate equation above to yield a set of pK_a values from which a mean pK_a can be determined. An alternative method of determining the pK_a is by graphical presentation of the obtained data; Eqn. 1 can be rearranged as:

$$A = A_u + (K_a/[H^+]) \cdot (A_i - A) \quad (5)$$

and a plot of A against $(A_i - A)/[H^+]$ will yield a straight line of slope K_a and intercept A_u. Similarly, Eqn. 2 can be rearranged so that a plot of A against $(A_u - A) \cdot [H^+]$ will give a straight line of slope $1/K_a$ and intercept A_i.

On the assumption that only the unionised species can partition into the organic phase, the apparent partition coefficient, P_{app}, can be related (Kubinyi, 1979) to the true partition coefficient, P, by the following relationships:

$$\text{For acids: } P_{app} = P/(1 + \text{antilog}(pH - pK_a)) \quad (6)$$

$$\text{For bases: } P_{app} = P/(1 + \text{antilog}(pK_a - pH)) \quad (7)$$

However, if the ionised species can partition into the organic phase then the above expression for a base is modified to (Irwin and Li Wan Po, 1979):

$$P_{app} \cdot (1 + \text{antilog}(pH - pK_a)) = P_i + P_u \cdot \text{antilog}(pH - pK_a) \quad (8)$$

where P_i and P_u indicate the partition coefficient of the ionised and unionised species, respectively. An analogous type of expression can be derived for an acid.

Materials and Methods

Materials

Progabide and SL79.182 (Fig. 1) were used as received from L.E.R.S., Paris, France. Propranolol hydrochloride was kindly provided by I.C.I. Pharmaceutical Division, Macclesfield, U.K. *n*-Octanol (SLR) was purchased from B.D.H. and used without further purification. For the

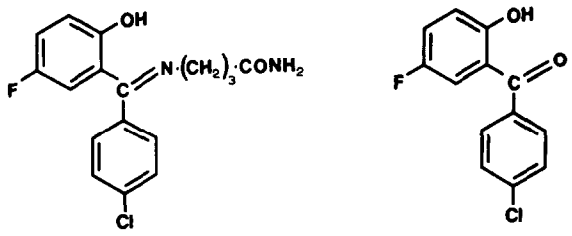

Fig. 1. Structural formulae of Progabide (1) and SL79.182 (2).

filter-probe, Whatman no. 50 filter paper, Acropor hydrophilic membranes of 0.8 μm pore size (Gelman Sciences, Northampton, U.K.), and Whatman 1 PS hydrophobic silicone-treated filters were used. The buffers were prepared using analytical reagents as given by Dawson et al. (1986).

Instrumentation

A Kontron Uvikon 810 double-beam spectrophotometer and a Uvikon 21 linear recorder were used for spectral scans. Fixed wavelength measurements were performed on a Cecil CE292 spectrophotometer fitted with a jacketed cell holder, which was connected to a Grant FH15 thermocirculator. Temperature measurements were performed using a Fluke 2180A digital thermometer. pH measurements were performed using a standard glass combination electrode with a Corning 113 pH meter.

The filter-probe (Fig. 2) was machined from stainless steel by the Engineering Faculty at Nottingham University following a design kindly provided by Professor E. Tomlinson (Ciba-Geigy Pharmaceuticals, U.K.). The filter material was cut to 25 mm discs, two were placed onto the bottom-surface of the steel block and the perimeter of the discs was gripped by the PTFE sleeve which was forced into position by hand pressure. The complete assembly for partition coefficient determination consisted of a 300 ml jacketed glass beaker and a perspex lid with 3 ports, for the pH electrode, temperature probe and filter-probe. A Rodwell monotherm stirrer motor unit with a PTFE-coated magnetic spin bar (Technilab Instruments) provided the necessary mixing. For analysis, the aqueous phase was continually sampled via the filter-probe, and circulated by the pumping action of an LKB 2115 peristaltic pump through a 75 μl Cecil stream analysis cell and back into the beaker. The connecting tubing (0.74 mm i.d.) was made of PTFE except inside the pump where LKB silicone tubing (1.3 mm i.d.) was used. The pumping rate was set to 1.5 ml/min and at any time only 1.2 ml of the incubated solution was present outside the beaker.

Methods

Determination of Progabide pK_a values

Stock solutions of Progabide were prepared in methanol. The buffer solutions were allowed to equilibrate at 37 °C and then one at a time, these were spiked with 100 μl of a stock solution to produce a 9×10^{-5} M solution (for pK_{a_1}) or a 6.9×10^{-5} M solution (for pK_{a_2}) with a final methanol concentration of 0.4%. The absorbance of the resulting solution was immediately assessed at the predetermined analytical wavelength (Farraj

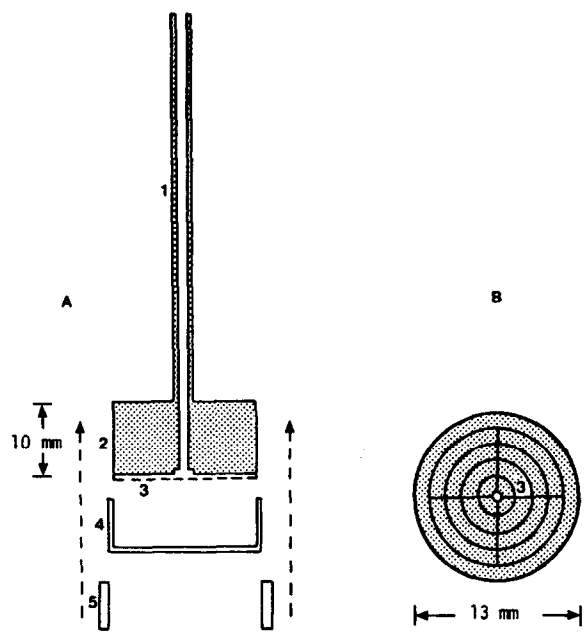

Fig. 2. Section (A) and bottom-view (B) of the filter-probe extractor; 1, 1.0 mm bore stainless-steel tube; 2, cylindrical stainless steel block; 3, radial and concentric 0.2 mm deep channels, 4, 25 mm filter discs and 5, PTFE sleeve.

et al., 1988a) of 410 nm at 37°C. All the determinations were performed in triplicate. At 410 nm, $A_u > A_i$ for both functional groups. Eqn. 4 was therefore used to calculate the experimental pK_{a_1} value of the basic functional group. For the acidic functional group, a value of A_i could not be determined because of Progabide instability at very high pH. Hence, the graphical treatment of Eqn. 2 was used to arrive at the value of pK_{a_2}.

Determination of SL79.182 pK_a

The extremely low solubility of this compound in water (Farraj et al., 1988a) meant that the absorbance measurements for equimolar concentrations at pH values above and below the pK_a were low and thus susceptible to large errors. The obvious answer was to use higher concentrations of the compound. Although these were feasible at pH values above the pK_a, they could not be maintained at lower pH values because the compound precipitated out of solution. Thus, no value for the absorbance of the unionised species, A_u, could be obtained. However, the use of the graphical treatment of Eqn. 5 allowed the value of the pK_a to be determined without the knowledge of A_u. The analytical wavelength for SL79.182 was chosen as 395 nm. A 4.2 mg/ml solution of the compound in methanol was prepared. As with Progabide, aqueous solutions of SL79.182 were prepared at 6.7×10^{-5}M in the buffer solutions at 37°C with a final methanol concentration of 0.4%, and their absorbance was measured.

Partition measurements

Separation of mixed phases. Two filter-probes can be used, in conjunction with the correct choice of hydrophilic or hydrophobic filters, to sample either the aqueous or organic phase under mild agitation conditions. As a general rule, under vigorous agitation, it is only possible to monitor the continuous phase and not the dispersed phase. In addition, the functionality of the probe depends on the rate of phase-flow through the sampling circuit. For the aqueous phase, any hydrophilic membrane or filter paper can be used on condition that it is initially wetted with the aqueous phase. Acropor hydrophilic membranes were compared with Whatman no. 50 filter paper and when no difference in their performance was observed, the latter were chosen for routine use. A suitable maximum pumping rate of 1.5 ml/min was determined and used thereafter. For the *n*-octanol phase, Whatman 1PS hydrophobic filters were used successfully under mild agitation conditions. However, the problems encountered in measuring octanol spectrophotometrically, and the difficulty found in probing the organic phase under the vigorous agitation conditions used ruled out the analysis of the octanol phase.

Propranolol partition–pH profile. This compound was conveniently chosen because its molar absorptivity in water is known to be independent of pH (Davis et al., 1976). The octanol and water phases were mutually saturated at 22.5°C. The pH of the aqueous phase was varied by using sodium hydroxide and hydrochloric acid so that the values obtained from the partition experiments could be compared with literature values. All the experiments were performed, in triplicate, and in the dark, as propranolol is a light-sensitive material.

150 ml of the octanol-saturated water was placed in the jacketed beaker and the sampling circuit was operated to achieve thermal equilibrium. The pH was adjusted to about 4 by addition of 0.2 M hydrochloric acid and the absorbance at 290 nm was adjusted to zero. Then, 10 ml of the water-saturated octanol was added to the beaker while vigorous mixing was continued and the spectrophotometer reading checked for any change. On no occasion did this vary by more than 0.005 units. Then, 1.6 ml of a 5 mg/ml solution of propranolol hydrochloride was added and the steady absorbance resulting at 290 nm, A_t, noted. Lowering the pH further had no effect on the absorbance reading indicating that all the drug was in the aqueous phase. Using a microsyringe, volumes of 0.25 M sodium hydroxide were added at intervals and following each addition, the new steady absorbance reading, A, was noted. The time required after each pH step for the system to re-equilibrate was about 5 min with the total volume of acid and alkali added to the system amounting to only 0.3 ml.

In accordance with the theory, the apparent and true partition coefficients for each set of pH

and absorbance measurements were calculated by:

$$P_{app} = ((A_t - A)/A) \cdot (V_w/V_o)$$

and $$P = P_{app} \cdot (1 + \text{antilog}(pK_a - pH))$$

where V_w and V_o were the volumes of the aqueous and organic phases, respectively. The pK_a value of propranolol was taken as 9.45 (Newton and Kluza, 1978).

Progabide partition–pH profile. The above procedure could not be applied to Progabide because of its low solubility and its poor stability in acidic media (Farraj et al., 1988a). An alternative modified procedure utilising buffered solutions was used. The aqueous and organic phases were mutually saturated at 37 °C prior to use. The aqueous and half the organic phase were mixed vigorously in the jacketed beaker at 37 °C and the absorbance reading of the aqueous phase, at 273 nm, was adjusted to zero. The other half of the octanol phase was then added to the system containing the required amount of Progabide, D_o, in solution. The equilibrium absorbance reading achieved in about 3–5 min was noted. From the calibration curve, the amount of drug in the aqueous phase, D, was calculated. The apparent partition coefficient was then computed using:

$$P_{app} = ((D_o - D)/D) \cdot (V_w/V_o) \qquad (9)$$

Triplicate runs were performed for each pH in the range 2.5–7.3. As the molar absorptivity of Progabide was pH-dependent, calibration curves for Progabide were prepared for each of the octanol-saturated buffer solutions used.

SL79.182 partition coefficient. The stability of this compound permitted the use of the conventional shake-flask method for partition coefficient determination. The buffers were mutually saturated with octanol at 37 °C. A 40 mg/g solution of SL79.182 was prepared in octanol and 5.5 ml of this solution was shaken with 150 ml of each of the buffer solutions for one week. A sample of the aqueous phase was then withdrawn, via the filter-probe, into a glass syringe, diluted appropriately and assayed spectrophotometrically at 265 nm. Using the law of mass balance, the partition coefficients were calculated by application of the general form of Eqn. 9.

Results and Discussion

Progabide is an amphoteric compound for which two pK_a values were previously observed in a solvent system of 80% water and 20% methanol (Fleury et al., 1985). The values reported were 3.35 for the transition immonium cation-neutral form (pK_{a_1}) and 12.8 for the transition neutral form-phenolate anion (pK_{a_2}). The system is complicated further by the existence of the tautomeric equilibrium phenol-imine/orthoquinone (Maupas et al., 1984) as shown in Fig. 3. In the present work, the percentage of methanol in the solvent system was reduced to 0.4%. At this concentration, it is expected to have a much smaller effect, if any, on the determined pK_a values. The pK_{a_1} value of Progabide at 37 °C was determined from the data summarised in Table 1 to be 3.41 with a S.D. of 0.02. Linear regression analysis of a plot of A versus $(A_u - A) \cdot [H^+]$ (Table 1) gave an equation of the form $Y = 8.89 \cdot 10^{12} X - 0.035$ with a correlation coefficient of 0.995. The 95% confidence limits of the slope were $8.89 \cdot 10^{12} \pm 1.64 \cdot 10^{12}$. By this method, the pK_{a_2} value was found to be 12.95. Further, although the carbamoyl group ($-CONH_2$) of Progabide is capable of ionisation, it is an extremely weak basic function and its pK_a will lie outside the normal pH range of 1–14. In fact, its value will be close to -0.43 and -0.44 which are the pK_a values of 1-carbamoyl-propane and 1-carbamoyl-3, 3-dimethyl-butane (Kezdy and Bruylants, 1959), respectively. The pK_a of SL79.182 was determined from the data summarised in Table 2. Linear regression analysis of a plot of A versus $(A_i - A)/[H^+]$ gave an equation of the form $Y = 1.16 \cdot 10^{-9} X + 0.08$ with a correlation coefficient of 0.967. The 95% confidence limits of the slope were $1.16 \cdot 10^{-9} \pm 5.58 \cdot 10^{-10}$. By this method, the pK_a was found to be 8.94.

Propranolol was chosen to check the validity of the filter-probe extractor method. The results obtained are shown in Table 3. At low pH, where the drug is ionised, it is contained totally in the aqueous phase but as the pH is increased it is extracted

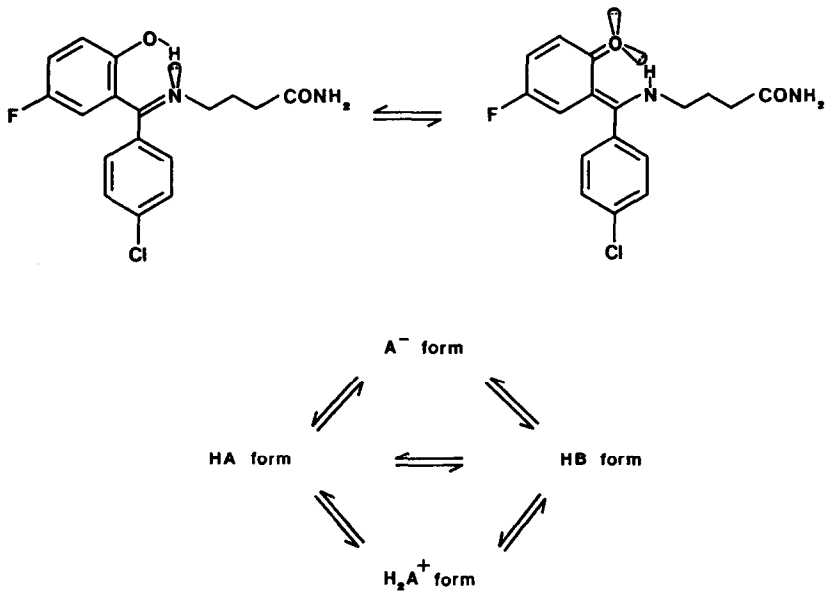

Fig. 3. Acid-base and tautomeric equilibria of Progabide.

into the organic phase. In fact, almost total extraction of the compound occurs, as expected, at pH values well below the pK_a of the free base. In other words, the inflexion point of the apparent partition coefficient–pH profile occurs at a pH lower than the pK_a of the free base. This so called "pH-shift" phenomenon is related to the partition coefficient of the unionised form of the drug. Illum et al. (1983) used the partition–pH relationships to derive an equation which predicted that the greater the partition coefficient of the free base the greater will be the shift of the inflexion point from the pK_a value. For the triplicate runs performed, the mean log (P) value obtained and its S.D. were 3.61 ± 0.05. This compares favourably with literature values of 3.56 by the AKUFVE

TABLE 1

Spectrophotometric pK_a determination of Progabide

pK_{a_1}				pK_{a_2}		
pH	Absorbance at 410 nm ($A \pm$ S.D.)	$\log[(A_u - A)/(A - A_i)]$	pK_{a_1} values	pH	Absorbance at 410 nm ($A \pm$ S.D.)	$(A_u - A) \cdot [H^+] \times 10^{14}$ (M)
7.23	0.623 ± 0.003 [a]	–	–	7.20	0.480 ± 0.005 [a]	–
4.23	0.557 ± 0.004	−0.855	3.38	12.42	0.365 ± 0.005	4.37
3.75	0.457 ± 0.002	−0.352	3.40	12.61	0.314 ± 0.004	4.08
3.52	0.392 ± 0.003	−0.125	3.40	12.78	0.272 ± 0.003	3.45
3.28	0.313 ± 0.002	0.132	3.41	13.07	0.190 ± 0.004	2.47
2.99	0.232 ± 0.001	0.422	3.41	13.24	0.138 ± 0.003	1.97
2.73	0.176 ± 0.001	0.687	3.42			
2.30	0.120 ± 0.001	1.145	3.45			
1.46	0.084 ± 0.002 [b]	–	–			

[a] A_u
[b] A_i

TABLE 2

Spectrophotometric pK_a determination of SL79.182

pH	Absorbance at 295 nm $A \pm$S.D.	$(A_i - A)$	$((A_i - A)/[H^+]) \cdot 10^{-6}$ (M^{-1})
12.23	0.255 ± 0.004	–	–
9.93	0.238 ± 0.002	0.017	144.69
9.62	0.226 ± 0.004	0.029	120.89
9.48	0.218 ± 0.002	0.037	111.74
9.28	0.201 ± 0.001	0.054	102.90
8.87	0.157 ± 0.003	0.098	72.65

method (Davis et al., 1976) and 3.65 by the HPLC method (Mirrlees et al., 1976).

For Progabide, the primary absorption maxima shifted slightly from 274 nm to 270 nm for a pH change of 2.52 to 7.30 (Farraj et al., 1988a). A λ_{max} of 273 nm was thus chosen for use in the partition experiments. At this wavelength, SL79.182 can absorb if present in the aqueous phase but the small duration of the experiment, the low concentration of Progabide in the aqueous phase, and the high partition coefficient of SL79.182 meant that under the experimental conditions employed, SL79.182, if formed, did not interfere with the assay procedure.

Typical recordings of the aqueous phase absorbance with time are shown in Fig. 4. The data obtained for Progabide are summarised in Table 4. It can be seen that, as the pH decreases, the apparent partition coefficient increases which agrees with the theoretical predictions for a weak base. The mean log (P) value of the free base was

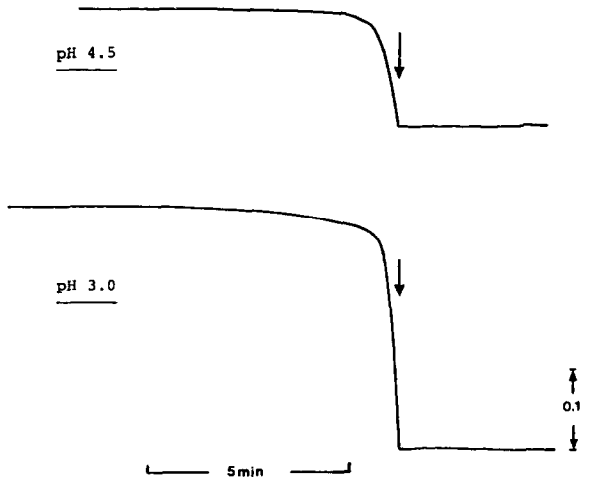

Fig. 4. Typical recordings of the absorbance-time profiles of Progabide in the aqueous phase of the agitated buffer–octanol system following the introduction (arrows above) of the drug into the organic phase. The pH of the aqueous phase is given.

calculated as 2.97 with a S.D. of ±0.09. In accordance with Eqn. 7 for a weak base, a plot of $\log[(P - P_{app})/P_{app}]$ against pH should yield a straight line of slope −1 and intercept = pK_a. This plot for Progabide (Fig. 5) gave a slope of −0.93 and an intercept of 3.44 which compare favourably with values of −1 and 3.41. A plot of $P_{app} \cdot [1 + \text{antilog}(pH - pK_a)]$ vs $\text{antilog}(pH - pK_a)$ should yield a straight line of slope P_u and intercept P_i (Eqn. 8). This plot for Progabide gave a slope of 1151 and an intercept of −761. The negative intercept is caused probably by experimental errors and the effect of buffers on the partition coefficient. However, this demonstrates

TABLE 3

The dependence of the apparent partition coefficient of propranolol on pH for the n-octanol / water system at 22.5 °C

Run 1			Run 2			Run 3		
pH	P_{app}	Log(P)	pH	P_{app}	Log(P)	pH	P_{app}	Log(P)
4.98	0.15	3.66	5.30	0.28	3.60	5.08	0.21	3.69
5.58	0.55	3.61	5.94	1.09	3.54	5.91	1.39	3.68
6.20	2.08	3.56	6.30	2.40	3.53	6.38	3.80	3.65
6.47	4.15	3.59	6.60	5.02	3.55	6.66	7.43	3.66
6.61	5.69	3.59	6.97	11.85	3.55	6.94	14.16	3.66
7.00	14.39	3.60	7.30	26.46	3.57	7.42	43.21	3.66
7.40	38.36	3.63	7.60	53.78	3.58	7.88	121.45	3.66
8.10	182.17	3.62						

TABLE 4

Effect of pH on the apparent partition coefficient of Progabide in the octanol/buffer system

pH	$P_{app} \pm$S.D.	P	log(P)	log[$(P - P_{app})/P_{app}$]
7.30	1151 ± 39	1151	3.06	–
5.04	1117 ± 5	1143	3.06	−1.517
4.50	882 ± 12	954	2.98	−0.516
4.00	698 ± 7	877	2.94	−0.188
3.52	408 ± 4	725	2.86	0.260
3.01	207 ± 3	727	2.86	0.659
2.52	118 ± 1	1034	3.02	0.942

the lack of involvement of the ionised species in the partitioning process. Further evidence was obtained from partition studies performed at pH 1.5 where the rate of disappearance of Progabide from the aqueous phase could be fully accounted for by the degradation process.

For SL79.182, the partition data obtained (Table 5) show that this compound has a very large partition coefficient which is consistent with the very low aqueous solubility found earlier (Farraj et al., 1988a).

TABLE 5

Effect of pH on the apparent partition coefficient of SL79.182 in the octanol/buffer system

pH	log(P_{app})	log(P) ± S.D.
7.30	4.06, 4.07, 4.01	4.05 ± 0.03
4.50	4.01, 4.02, 4.04	4.02 ± 0.02
2.52	4.03, 4.03, 4.06	4.04 ± 0.02

The partition studies thus predict that the absorption of Progabide from the stomach will be small, whereas in the intestine it will be large. In comparison, the absorption of any SL79.182 formed is expected to be high, both in rate and magnitude, along the whole length of the gastrointestinal tract. These predictions are in agreement with the experimental data obtained in animal species and in man (Ferrandes et al., 1985; Farraj et al., 1988b and c).

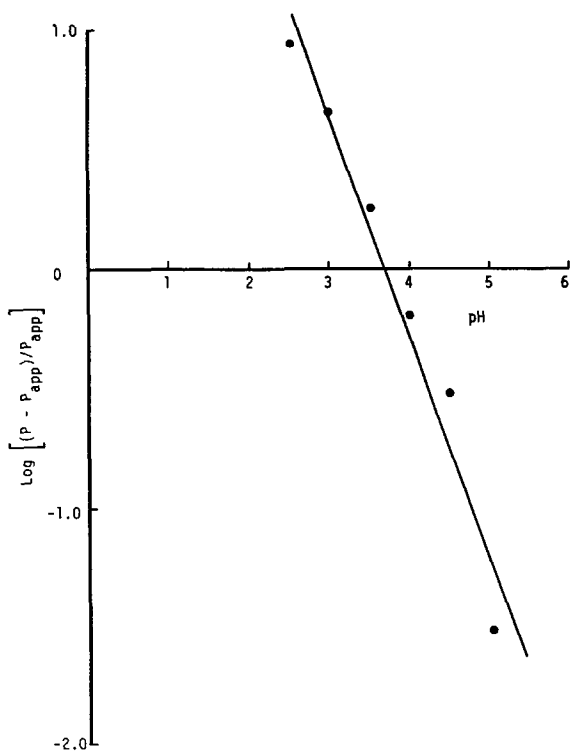

Fig. 5. A plot of log[$(P - P_{app})/P_{app}$] vs pH for Progabide.

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