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Research Article

The Stability and Solubility of Progabide and Its Related Metabolic Derivatives

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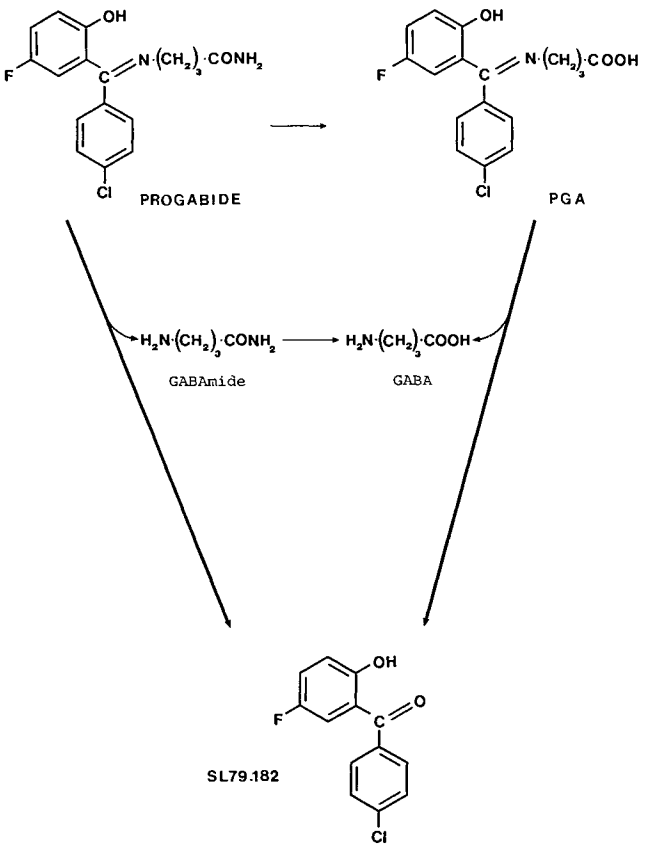
The stability–pH profile of the γ-aminobutyric acid prodrug, Progabide, was found to be bell shaped, with maximum stability occurring at pH 6 to 7 with a $t_{1/2}$ of 126 min. Of its metabolic derivatives, the deamidated product PGA degraded in a similar fashion to Progabide, whereas the hydrolytic degradation product SL79.182 was, as expected, a stable compound. Progabide behaved as a typical weak base, with its solubility increasing with a decrease in pH. SL79.182 behaved as a typical phenolic weak acid, with its solubility increasing with an increase in pH. Both compounds displayed low intrinsic solubilities of 14.5×10^{-5} *M* for Progabide and 33.4×10^{-6} *M* for SL79.182. An increase in temperature resulted in an increase in the solubility but a decrease in the stability of Progabide. The data obtained indicate that the gastric pH and gastric emptying rate will have a profound effect on the oral bioavailability of Progabide.

KEY WORDS: Progabide; metabolic derivatives; stability; solubility.

INTRODUCTION

Progabide, which belongs to the category of compounds known as analogue-prodrug hybrids (1), can be transformed, by deamidation (2), to yield an acid metabolite (PGA) or it can be cleaved at the imine link to yield the carrier moiety, in the form of SL79.182, and GABAmide (Scheme I). The latter can be further metabolized to γ-aminobutyric acid (GABA). PGA biotransformation also yields SL79.182 and GABA. All these compounds, except the carrier moiety, enjoy intrinsic anticonvulsant activity (2,3). The chemical names and structures of these compounds have been given previously (4). In this paper, we report on aspects of the solubility and stability of these compounds which are pertinent to the oral bioavailability of Progabide.



Scheme I. Main biotransformation routes of Progabide.

EXPERIMENTAL

Materials

Progabide, SL79.182, and PGA were used as received from LERS, Paris, France. Trizma base, of reagent grade, and chloroacetic acid (99%) were obtained from the Sigma–Aldrich Group. Formic acid (90%), glacial acetic acid (SLR), and hydrochloric acid (35–38%, w/w) were purchased from Fisons. All other buffer constituents and reagents were of AnalaR quality. Nuclepore 25-mm 0.4-μm polycarbonate membrane filters were used with Millipore Swinnex-25 holders, and where required, Whatman No. 54 filter disks were used as prefilters.

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Instrumental

(i) Spectral Studies. A Kontron Uvikon 810 double-beam scanning spectrophotometer linked to a Kontron Uvikon 21 linear recorder with an overlay facility was used.

(ii) Stability vs. pH Studies. A 50-ml jacketed beaker was maintained at the required temperature by circulating water from a Grant FH15 water thermocirculator. Evaporation from the beaker was prevented by a Perspex lid with two ports, one for sampling and the other for the temperature probe, which was linked to a Fluke 2180A digital thermometer with a two-decimal display of the temperature. The incubated solution was stirred by the use of a PTFE-coated spin bar (Technilab Instruments) and a stirrer motor unit (Rank Brothers). A Corning 113 pH meter with a standard glass combination electrode was used for pH measurement.

(iii) Stability vs. Temperature Studies. A Grant SS30 shaker water bath with a Grant LC10 cooler–heater thermocirculator was used. The sample analysis was performed on a Cecil CE292 spectrophotometer fitted with a jacketed cell holder.

(iv) Solubility vs. pH Studies. The apparatus used here was similar to that in ii but with the addition of the filter probe (5) for sampling purposes. A Dawe 7532B soniprobe was used in the suspension preparation procedure.

Methods

Unless otherwise stated, all experiements were performed in triplicate.

(i) Standard Stock Solutions. Standard stock solutions of Progabide, SL79.182, and PGA were prepared in methanol and stored at 4°C until required. The solutions were prepared freshly every 3 days. Aliquots were always measured at room temperature.

(ii) Analysis of Samples. The high-performance liquid chromatographic (HPLC) assay procedure which allows the simultaneous analysis of Progabide and its derivatives was described previously (4). It is also possible to utilize visible spectrophotometry for the specific analysis of Progabide in the presence of its degradation product, SL79.182.

Aqueous solutions of Progabide and SL79.182 were prepared at 16.8 and 4.2 μg/ml, respectively, by spiking a series of buffers (6) which covered the required pH range (I = 0.1) with the stock solutions, ensuring that the proportion of methanol in the final solution did not exceed 0.5%. Next, the UV/visible spectra of the solutions were scanned and the necessary conditions for a suitable analytical wavelength for Progabide were determined. Calibration curves for Progabide and SL79.182 were constructed in pH 7.0 buffer and in 0.1 M hydrochloric acid, respectively, to cover the aqueous concentration ranges 0–7.5 × 10^{-5} and 0–1.7 × 10^{-5} M in the same order.

(iii) Rate–pH Profile of Progabide. Buffers of 0.01 and 0.1 M ionic strength were used (6). The ionic strength of the buffer did not affect the rate of reaction in the pH range examined; nevertheless, whenever possible, buffers of I = 0.01 were used. Buffered aqueous solutions of Progabide were prepared at an initial concentration of 30 μg/ml (8.96 × 10^{-3} M) by spiking them with the standard stock solution. The solution was then incubated at 37°C and at suitable intervals 20-μl samples were analyzed by HPLC (4). For the studies above pH 8, a 0.5-ml sample was removed and diluted with 0.5 ml of McIlvaine buffer (6) of pH 5.6 prior to analysis. This procedure avoided column deterioration by the stripping action of the alkaline solution on the stationary phase. The degradation was always followed for a minimum of one half-life, during which time the solution pH remained constant.

(iv) Temperature Effect on the Stability of Progabide. This was determined in pH 7.0 phosphate buffer (I = 0.01) from 15 to 60°C. Because of the solubility limit of Progabide, the initial concentration used was reduced to 15 μg/ml. The solutions prepared were incubated in the shaker water bath, and periodically samples were removed and their absorbance was measured.

(v) Stability of PGA. Aqueous buffered (pH 7.0, I = 0.1) solutions of PGA at 30 μg/ml were incubated at 37°C. At suitable intervals, aliquots were removed and analyzed by HPLC.

(vi) Solubility Determination of Progabide and SL79.182. The preliminary investigations showed that a 30-times excess Progabide powder over the expected solubility limit was required to achieve a very rapid approach to equilibrium and maintain the ratio of the dissolution rate to the degradation rate sufficiently large for enough time to allow a meaningful solubility determination to be performed. The excess powder was first wetted, by sonication at 20 kHz for 1 min, with a 3-ml quantity of the buffer. The crude suspension was then added to the remainder of the buffer solution and incubated at 37°C, and periodically samples of the solution were removed via the filter probe. The filter probe was fitted with a single Whatman No. 54 filter paper to act as a prefilter and a Nuclepore 0.4-μm polycarbonate membrane. The PTFE sleeve of the probe ensured that only the filtrate was sampled. The analysis was performed by diluting the collected midfiltrate in pH 7.0 buffer, followed by spectrophotometric analysis against an appropriate blank. Progabide solubility was determined in the pH range 1.5 to 10. The solubility of SL79.182 in the same pH range was determined by traditional "shake-flask" method. Excess powdered SL79.182 (50 times the solubility limit) was first wetted with the buffer as outlined above and later incubated at 37°C for 1 week, by which time the equilibrium solubility was attained. Samples of the saturated solution were withdrawn via the filter probe, then diluted in 0.1 M hydrochloric acid, and their absorbance was measured against an appropriate blank.

(vii) Temperature Effect on the Solubility of Progabide. The solubility of Progabide from 18 to 45°C was determined in pH 7.0 buffer (I = 0.1) by the same procedure given above.

RESULTS AND DISCUSSION

The effect of pH on the spectra of Progabide and SL79.182 is summarized in Figs. 1 and 2, respectively. The variations observed are caused by the ionization of the two compounds in aqueous solutions where the ionized and non-ionized species display different light-absorption characteristics. For Progabide, the secondary visible-light absorption maximum at 410 nm shifts with a decrease in pH to 363 nm. In comparison, the primary absorption maximum shows

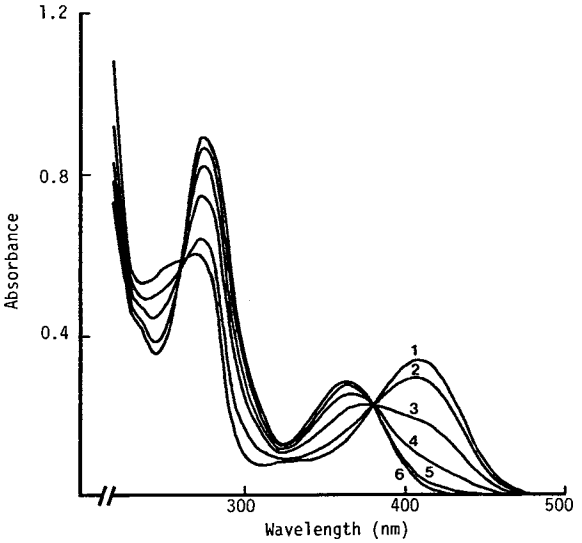

Fig. 1. Effect of pH on the UV/visible absorption spectrum of Progabide, present at 16.8 μg/ml in aqueous buffered solutions at 23°C: 1, pH 7.2; 2, pH 4.2; 3, pH 3.5; 4, pH 3.0; 5, pH 2.3; and 6, pH 1.5.

little shift, from 270 to 275 nm, for the same pH change. The comparable shifts for SL79.182 are from 393 to 340 and from 260 to 265 nm. The important observation to be made from Figs. 1 and 2 is that below pH 8 the absorbance of the SL79.182 solution at 410 nm becomes insignificant. In fact, even for a saturated solution at pH 7.0, the absorbance recorded at 410 nm was just 0.004. Thus, $\lambda_{410\ nm}$ can be used to measure Progabide quantitatively regardless of the concomitant presence of SL79.182 as long as the pH is maintained near 7.0. For SL79.182, the absorption maximum at 265 nm was used to construct its calibration curve in 0.1 *M* hydrochloric acid. Progabide and SL79.182 calibration curves obeyed the two linear equations

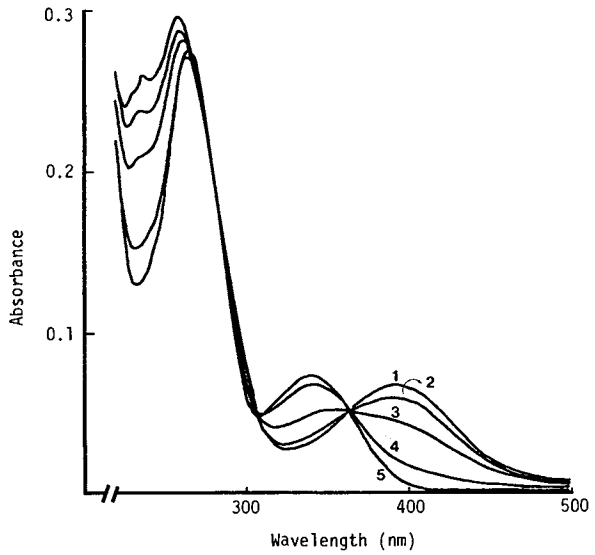

Fig. 2. Effect of pH on the UV/visible absorption spectrum of SL79.182, present at 4.2 μg/ml in aqueous buffered solutions, at 23°C: 1, pH 10.0; 2, pH 9.5; 3, pH 9.0; 4, pH 8.0; and 5, pH 1.4.

$$\text{absorbance} = 6076.2 \times \text{concentration} + 5.5 \times 10^{-3}$$

and

$$\text{absorbance} = 22719.7 \times \text{concentration} - 0.9 \times 10^{-3}$$

respectively. The imine functional group in the side chain of Progabide is particulary sensitive to hydrolytic degradation by both acids and bases. Progabide was found to degrade in aqueous solutions to give SL79.182 at all pH values examined and the HPLC results further confirmed that PGA was not involved in the degradation. The process obeyed apparent first-order kinetics (Fig. 3). Linear regression analysis of the data allowed the rate constants to be computed and these were then used to obtain the rate–pH profile shown in Fig. 4. The effect of pH on the rate of hydrolysis can be related to the ionization state of Progabide. This drug is an amphoteric compound for which two pK_a values were observed (5): 3.41 for the transition immonium cation (H_2A^+)-neutral form and 12.95 for the transition phenolate anion (A^-)-neutral form. However, the system is complicated by the existence of the tautomeric equilibrium phenol–imine/orthoquinone (7) for the neutral form, which can be represented by HA $\rightleftharpoons$ HB. Maupas and others (7) showed that in water–methanol mixtures, the percentage of the HA form of Progabide decreased as the percentage of water increased. By extrapolation, it can be estimated that in pure water the HA form of Progabide will represent only 15% of the total. Thus, depending on the pH, Progabide can exist in the cationic H_2A^+ form, and/or in the neutral form

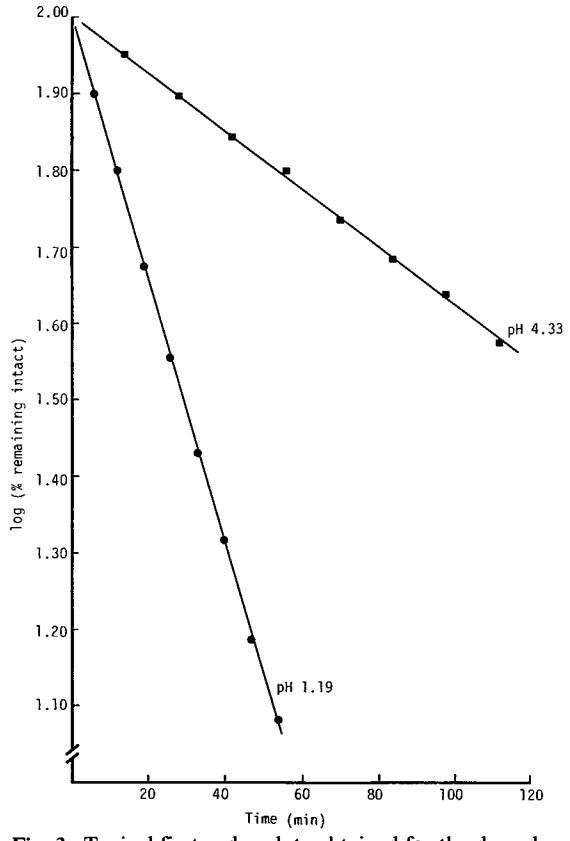

Fig. 3. Typical first-order plots obtained for the degradation of Progabide in aqueous buffered solutions at 37°C.

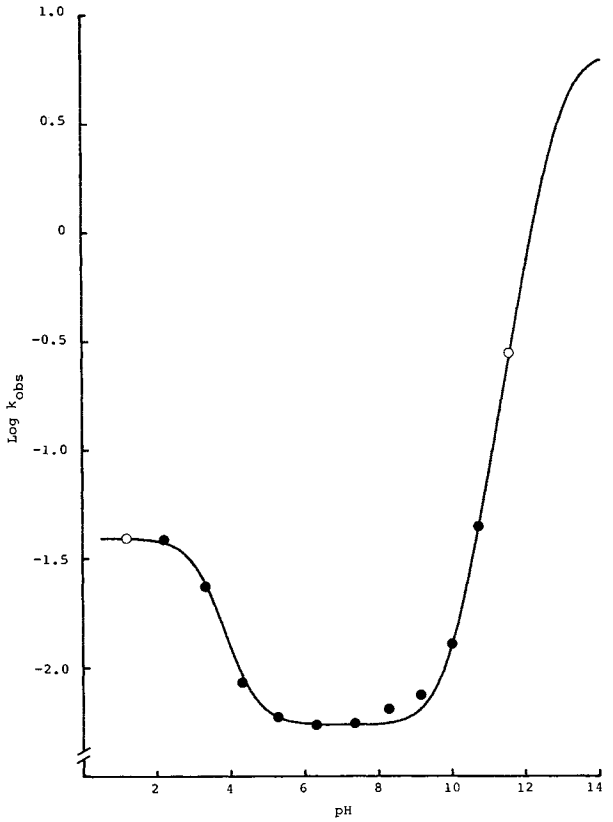

Fig. 4. Rate–pH profile of Progabide at 37°C in aqueous buffered solutions. Filled and open circles indicate 0.01 and 0.1 ionic strength, respectively.

(HB mainly), and/or in the anionic A^- form. The reaction rate (v) can be represented by an equation of the form

$$v = k_2 \cdot [H_2A^+] + k_3 \cdot [HB] + k_4 \cdot [HB] \cdot [OH^-]$$

The dissociation constants of Progabide, K_1 and K_2, can be given by

$$K_1 = [HA + HB][H^+]/[H_2A^+]$$

and

$$K_2 = [A][H^+]/[HA + HB]$$

Thus the fractions of H_2A^+ and HB can be given, respectively, by

$$F_1 = [H^+]/\{[H^+] + K_1 + (K_1K_2/[H^+])\}$$

and

$$F_2 = K_t/\left((1 + K_t) \cdot \{([H^+]/K_1) + (K_2/[H^+]) + 1\}\right)$$

It is normal to express the reaction rate by an equation of the form

$$v = k_{obs} \cdot [S]$$

where k_{obs} is the observed reaction rate constant and S is the substrate total concentration. Since

$$[S] = [H_2A^+] + [HA + HB] + [A^-] \text{ and } [OH^-] = K_w/[H^+]$$

it follows that

$$k_{obs} = (k_2 \cdot F_1) + (k_3 \cdot F_2) + (k_4 \cdot F_2 \cdot K_w/[H^+]) \quad (1)$$

where K_w is the dissociation constant of water at 37°C.

The continuous line in Fig. 4 shows the theoretical rate–pH profile that can be obtained by using Eq. (1) above with $k_2 = 3.94 \times 10^{-2}$, $k_3 = 6.40 \times 10^{-3}$, and $k_4 = 36.18$ min^{-1}. This theoretical profile compares favorably with the experimental data points. The region of maximum stability occurs at about pH 6–7 with a $t_{1/2}$ of about 126 min.

For Progabide, the variation of temperature did not affect the order of the degradation process. The Arrhenius plot for the degradation conformed to a straight line with a slope of −3516 and an intercept of 9.02. From these values, the activation energy and the frequency factor were calculated to be 67.3 kJ/mol and 10.4×10^8 min^{-1}, respectively. The value of the activation energy is seen to be well within the range of 40–120 kJ/mol indicated by Pope (8) for solvolytic processes.

PGA was found to degrade at pH 7.0 in a similar fashion to Progabide to yield SL79.182. The first-order rate constant was calculated to be 5.49×10^{-3} min^{-1}, with a degradation half-life of 126 min. The striking similarity between the degradation rate of Progabide and that of PGA is not surprising in view of the fact that both compounds possess the same imine functional group that undergoes hydrolytic degradation (4). Thus, although Progabide is only converted to PGA by an enzymatic process (9,10), the latter can be degraded by a nonenzymatic process to SL79.182. Therefore, care has to be exercised when discerning the rates of elimination of the above compounds from body compartments, as will become clear from a later publication (10).

The instability of Progabide imposed restrictions on the

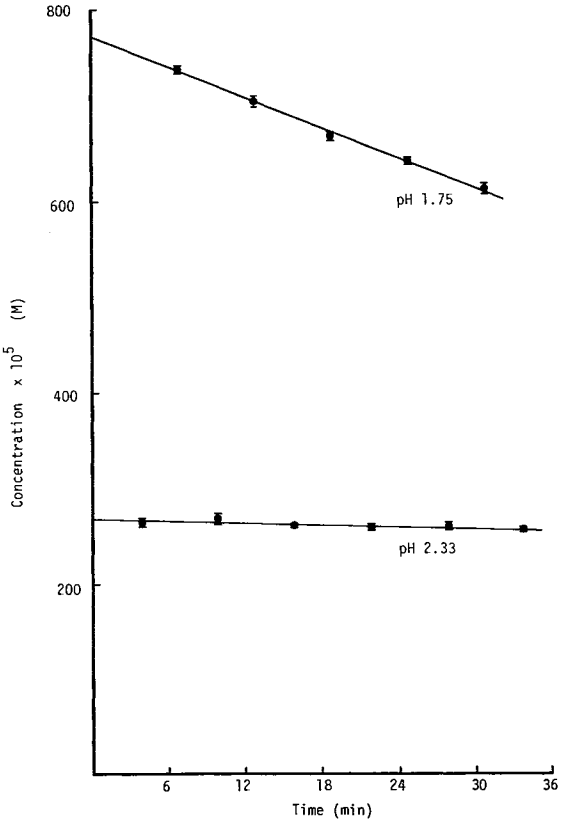

Fig. 5. The aqueous concentration–time profiles of Progabide obtained for the solubility determination at pH 1.8 and 2.3, at 37°C. Each point represents the mean ± SD.

Table I. Variation of the Solubility of Progabide and S179.182 with pH at 37°C

pH	Solubility of Progabide, ($S_t \pm SD$) $\times 10^5$ (M)	pH	Solubility of SL79.182, ($S_t \pm SD$) $\times 10^6$ (M)
1.8	771.9[a] ± 5.1	1.5	32.0 ± 0.84
2.3	267.9[a] ± 1.5	2.5	36.9 ± 0.96
2.8	67.5 ± 0.2	4.1	35.9 ± 0.80
3.5	25.9 ± 0.2	6.9	40.4 ± 0.84
4.1	18.2 ± 0.2	8.0	37.4 ± 0.36
4.9	12.4 ± 0.1	9.0	78.9 ± 0.80
6.0	11.5 ± 0.1	9.4	168.7 ± 0.40
6.9	11.1 ± 0.1	9.8	372.7 ± 2.31
8.0	13.4 ± 0.1		
9.9	10.7 ± 0.1		

[a] Determined by extrapolation.

method of solubility determination. In that respect, it was found that the use of 30 times excess powder achieved a very fast dissolution rate compared with the degradation rate. At all pH values above 2.5, the equilibrium solubility was achieved almost instantly and maintained for periods long enough to allow reproducible measurements to be made. For example, at pH 6.9, the concentration found in the first analyzed sample at 2 min was the same as that obtained for consecutive samples up to 24 hr. The addition of extra powdered Progabide did not alter the solubility limit, indicating the true nature of the equilibrium achieved. However, for pH values below 2.5, the rate of degradation was comparable with the rate of dissolution, thus the solubility limit could not be maintained with time, as the available resource of powder was exhausted. Instead, the concentration in the solution showed a steady decline as shown in Fig. 5. To estimate the solubility, the data were extrapolated back to zero time, using linear regression analysis, and the value of the intercept was taken as the solubility limit. This assumption was justified on the basis that increasing the excess Progabide powder appeared to alter the slope of the line but not its intercept. The filtration procedure was superior to methods cited in the literature (11,12) in that the filtration was performed at the same temperature as that of the incubated suspension with minimal risk of precipitation prior to filtration.

The solubility data of Progabide and SL79.182 are presented in Table I, showing the low standard deviations obtained. The total solubility, S_t, of a weak base can be given by (13,14)

$$S_t = S_u \cdot (1 + [H^+]/K_1) \quad (2)$$

and that of a weak acid by

$$S_t = S_u \cdot (1 + K_1/[H^+]) \quad (3)$$

where S_u is the solubility of the nonionized species, $[H^+]$ is the hydrogen ion concentration, and K_1 is the dissociation constant. It can be seen from the solubility profiles in Figs. 6 and 7 that Progabide and SL79.182 are behaving, in the pH range investigated, as a typical weak base and weak acid, respectively. For Progabide, the intrinsic solubility of the free base can be directly estimated from the profile or, alternatively, in accordance with Eq. (2), a plot of $[H^+]$ against S_t should give a straight line of slope K_1/S_u and intercept $-K_1$. Similarly for SL79.182, and in accordance with Eq. (3), a plot of $1/[H^+]$ against S_t should give a straight line of slope $1/(K_1 \cdot S_u)$ and intercept $-1/K_1$. These plots were found to be linear and yielded values of 3.48 and 14.5 $\times 10^{-5}$ M for

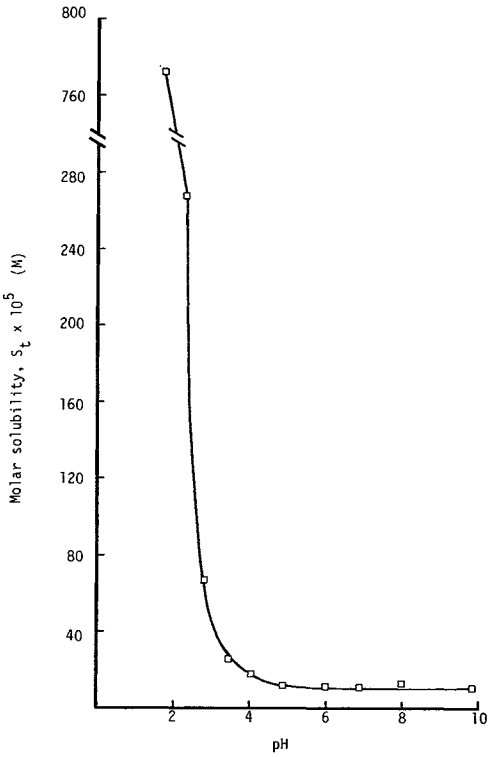

Fig. 6. Solubility–pH profile of Progabide at 37°C.

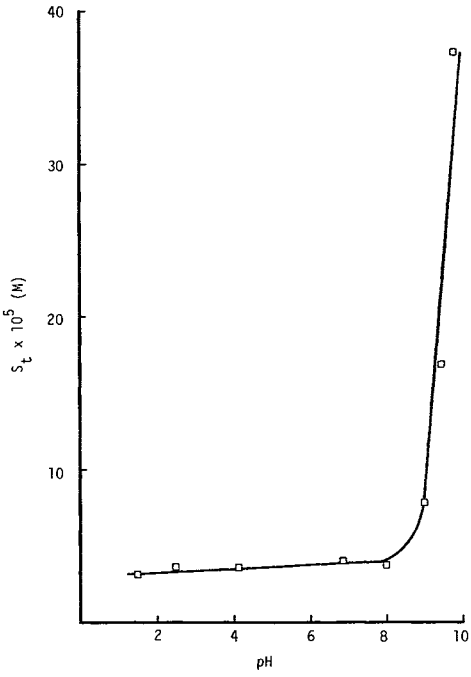

Fig. 7. Solubility–pH profile of SL79.182 at 37°C.

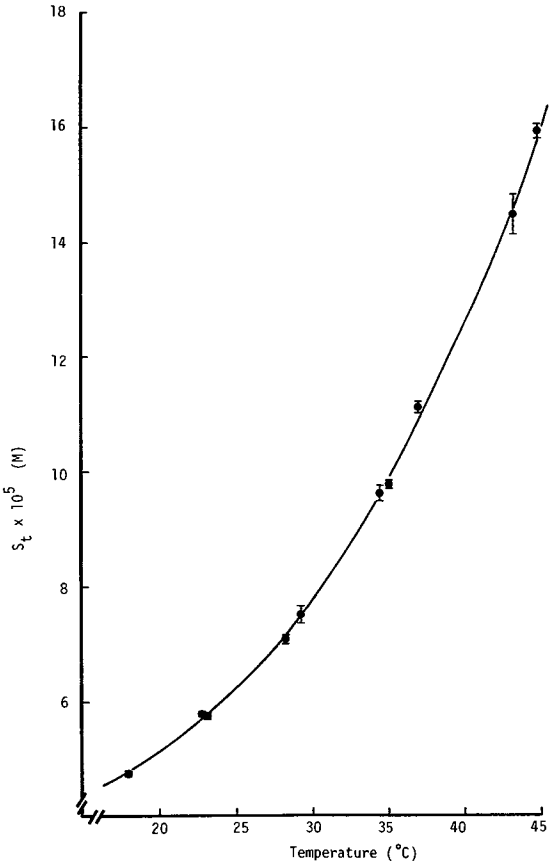

Fig. 8. Variation of the solubility of Progabide with temperature, at pH 7.0, I = 0.1. Each point represents the mean ± SD.

the pK_a and S_u of Progabide and 8.80 and 33.4 × 10^{-6} M for the pK_a and S_u of SL79.182, respectively.

The aqueous solubility will also vary with temperature. As a general rule, the solubility of many drugs in water increases with increasing temperatures (15). This may obey the van't Hoff plot where the natural logarithm of the mole fraction (X) of the drug is plotted against the reciprocal of the absolute temperature (T) in accordance with (16)

$$\ln X = (\Delta S/R) - (\Delta H/R) \cdot 1/T \quad (4)$$

where ΔH and ΔS are the change in enthalpy and entropy, respectively. The solubility of Progabide increased with an increase in temperature (Fig. 8). From the van't Hoff plot, the value of ΔH was found to be 3.49 × 10^4 J mol^{-1}, a positive value indicating that the system absorbed heat from its surroundings as would be expected (17) for a nonpolar molecule with low aqueous solubility. Further, ΔS was found to be 3.45 J $°K^{-1}$ mol^{-1}.

Overall, the *in vivo* implication of the data obtained is that Progabide will be expected to dissolve rapidly in the stomach and slowly in the intestine. Its stability is expected to be good in the intestine but poor in the gastric environment. It is therefore expected that the gastric pH and gastric emptying rate will have a profound effect on the oral bioavailability of this compound. Further, any SL79.182 that is produced within the gastrointestinal tract is expected to show a low pH-independent solubility throughout the upper part of the tract.

REFERENCES

1. A. A. Sinkula. In E. B. Roche (ed.), *Design of Biopharmaceutical Properties Through Prodrugs and Analogs,* American Pharmeutical Association, Washington, D.C., 1977, pp. 1–17.
2. G. Bartholini, B. Scatton, B. Zivkovic, and K. G. Lloyd. In P. Krogsgaard-Larsen, J. Scheel-Krager, and H. Kofod (eds.), *GABA-Neurotransmitters,* Munksgaard, Copenhagen, 1979, pp. 326–339.
3. J. P. Kaplan, B. M. Raizon, M. Desarmenien, P. Feltz, P. M. Headley, P. Worms, K. G. Lloyd, and G. Bartholini. *J. Med. Chem.* 23:702–704 (1980).
4. N. F. Farraj, S. S. Davis, G. D. Parr, and H. N. E. Stevens. *Pharm. Res.* 4:28–32 (1987).
5. N. F. Farraj, S. S. Davis, G. D. Parr, and H. N. E. Stevens. Submitted for publication (1987).
6. R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones. *Data for Biochemical Research,* Clarendon Press, Oxford, 1986, pp. 417–448.
7. B. Maupas, S. Letellier, M. B. Fleury, and B. Mompon. *Analysis* 12:122–128 (1984).
8. D. G. Pope. *Drug Cosmet. Ind.* 127:48–66, 110–116 (1980).
9. B. Ferrandes, A. Durand, P. Padovani, J. T. Burke, D. Garrigou, J. Fraisse-Andre, P. Hermann, and J. Allen. In G. Bartholini, L. Bossi, K. G. Lloyd, and P. L. Morselli (eds.), *Epilepsy and GABA Receptor Agonists, L.E.R.S. Monograph Series, Vol. 3,* Raven Press, New York, 1985, pp. 217–230.
10. N. F. Farraj, S. S. Davis, G. D. Parr, and H. N. E. Stevens. *Int. J. Pharm.,* in press (1988).
11. S. Miyazaki, H. Inoue, T. Nadai, T. Arita, and M. Nakano. *Chem. Pharm. Bull.* 27,1441:1447 (1979).
12. Z. T. Chowhan. *J. Pharm. Sci.* 67:1257–1260 (1978).
13. H. A. Krebs and J. C. Speakman. *J. Chem. Soc.* 593–595 (1945).
14. S. Niazi. *Textbook of Biopharmaceutics and Clinical Pharmacokinetics,* Appleton–Century–Crofts, New York, 1979.
15. B. E. Ballard. *J. Pharm. Sci.* 63:1345–1358 (1974).
16. M. A. Lauffer. *Entropy Driven Processes in Biology,* Springer-Verlag, New York, 1975.
17. K. Embil and G. Torosian. *J. Pharm. Sci.* 71:191–193 (1982).