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Rate and equilibrium constants for acid-catalyzed lactone hydrolysis of HMG-CoA reductase inhibitors

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Summary

The acid-catalyzed hydrolysis of mevalonolactone and several structurally related hypocholesterolemic agents was studied in a pH 2.0 buffer at 37°C. All of the reactions exhibited pseudo first-order kinetics from which the equilibrium constant and rate constants for hydrolysis and lactonization were derived. Except for mevalonic acid lactone, all of the compounds reacted at essentially the same rate. Mevalonolactone hydrolyzes at a rate similar to the other compounds but relactonizes at a substantially faster rate; variable temperature kinetic studies indicate that this difference is due to both enthalpic and entropic factors. The hydrolysis data are used to simulate the extent of drug degradation that occurs in acidic gastric fluids following oral administration of these drugs.

Introduction

Lovastatin and simvastatin are two members of the class of cholesterol lowering agents whose mechanism of action involves inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (Alberts et al., 1980; Krukemeyer and Talbert, 1987). This microsomal enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an important rate limiting step in cholesterol biosynthesis (Rodwell et al., 1976). Since cholesterol synthesis occurs mainly in the liver, it is advantageous for HMG-CoA reductase inhibitors to exhibit a high selectivity for distribution into the liver. To achieve this selectivity, both lovastatin and simvastatin are administered in the form of lactone prodrugs. The lactone rings are hydrolyzed in vivo to produce the corresponding hydroxy acid derivatives which are the pharmacologically active forms of these drugs, and this is believed to take place predominantly in the liver.

Since lactone hydrolysis reactions are strongly accelerated by general acid catalysis (Kirby, 1972), it is anticipated that conversion of lovastatin to its hydroxy acid derivative may occur in the strongly acidic gastric environment. Obviously, the desirable tissue selectivity of the lactone form is not realized if hydrolytic conversion in the G.I. tract occurs rapidly relative to lactone absorption. Thus, the goal of the study presented herein was to determine the rate and extent of lactone hydrolysis under pH conditions which are typical of stom-

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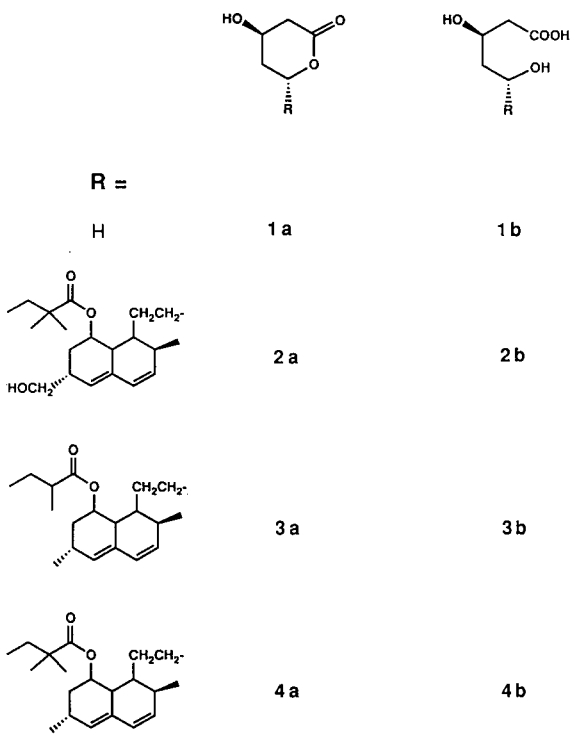


Fig. 1. Structures of lactones and hydroxy acids.

ach fluids. The structure of the compounds used in this study are shown in Fig. 1. Note that mevalonolactone (**1a**) was included in this study; although this compound is not an HMG-CoA reductase inhibitor, it does contain the same lactone ring as the inhibitor compounds. In this respect, mevalonolactone can be considered the 'parent' lactone in this series of structurally related inhibitors, and is useful as a reference compound for interpreting and comparing results.

Materials and Methods

Instrumentation

Analytical data were obtained by reversed phase HPLC using a Perkin Elmer system consisting of a Series 410 pump, ISS-100 autosampler, and a LC-235 UV detector. Chromatographic information was quantitated and stored with a Spectra Physics SP4270 computing integrator. An Orion Model 901 Research Meter was used for pH measurements. Temperature control was provided by a Laude RM6 recirculating temperature bath.

Materials

All solvents were obtained from Fisher Scientific and were HPLC grade or equivalent. Distilled water was taken from a Millipore Milli-Q water purification system. Hydrochloric acid and potassium chloride were obtained from Mallinkrodt and used as received. Lovastatin (**3a**), simvastatin (**4a**), and compounds **2a**, **2b**, **3b**, and **4b** were obtained from the Merck, Sharp and Dohme Research Laboratories (Hoffman et al., 1986); each compound was at least 98% pure and used without further purification. Mevalonolactone (**1a**) was obtained from Sigma Chemicals and used as received.

Acidic buffers were prepared by adding hydrochloric acid dropwise to a 0.020 M potassium chloride solution until a pH of 2.00 ± 0.02 was measured.

Analytical methods

Except for mevalonolactone, all reversed-phase HPLC analyses used a Whatman Partisil 5ODS3 column (25 × 0.46 cm, 5 μm) with a mobile phase mixture of 0.02 M monobasic potassium phosphate/acetonitrile at a flow rate of 2 ml/min and UV detection at 240 nm. The percent composition of the mobile phase was approx. 50% aqueous/50% organic, but was adjusted for each compound so that retention times of 3–5 min for the hydroxy acids and 6–8 min for the lactones were achieved. Typical chromatograms are shown in Fig. 2.

Mevalonolactone is a much more polar compound than the other lactones and could not be analysed by the method described above. Adequate chromatography was achieved using a Keystone Hypersil ODS column (10 × 0.46 cm, 5 μm) eluted with 99% 0.05 M monobasic potassium phosphate and 1% acetonitrile at a flow rate of 1 ml/min. Detection was at 210 nm.

In separate control experiments, it was demonstrated that all the lactones and hydroxy acids are

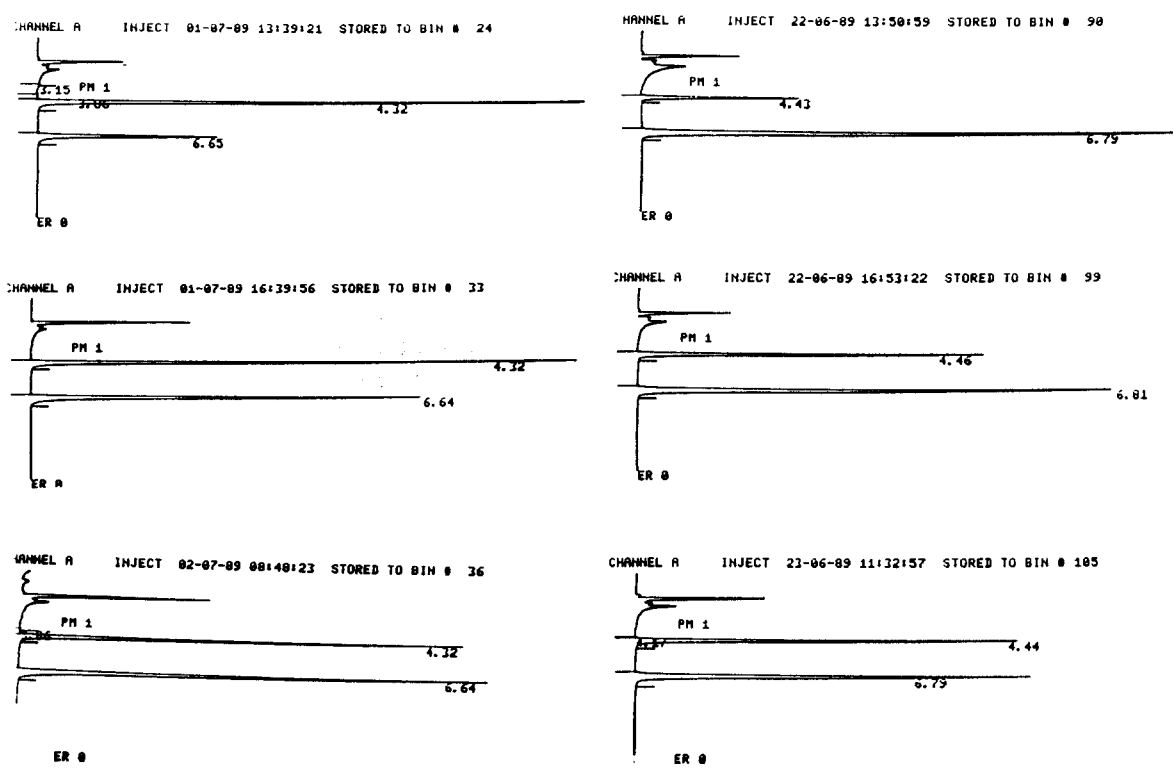


Fig. 2. Sample chromatograms showing the approach to equilibrium starting from lactone **3a** (right panels) and hydroxy acid **3b** (left panels). These chromatograms were obtained at 1 h, 3 h, and 15 h (equilibrium) into the kinetic run.

stable under the chromatographic conditions.

Kinetic procedures

The procedure used for determining the hydrolysis kinetics varied slightly depending on the aqueous solubility of the compounds. The lactone/acid pairs **1a/b** and **2a/b** are sufficiently soluble in the aqueous buffer for studies without added organic cosolvents. Solubilization of compounds **3a/b** and **4a/b** required the addition of 20% v:v acetonitrile to the aqueous buffer. The two procedures are described in detail below. Unless otherwise noted, all kinetic runs were performed at 37.0 + 0.1°C.

Hydrolysis kinetics of 2a to 2b. Lactone **2a** (2.86 mg, 6.58 μmol) was dissolved in 50 ml of the HCl/KCl buffer which had been previously equilibrated to 37°C. Aliquots from this solution were transferred to HPLC sample vials (~ 1 ml capacity) which had also been pre-equilibrated to the temperature of the run, and the vials were crimp-sealed with teflon septa. The vials were placed in the thermostated sample tray of the HPLC autosampler and after 10 min an analysis was initiated by automatically injecting a 50 μl sample aliquot onto the chromatographic system. This first analysis was defined as the zero time point. Subsequent kinetic points were obtained by programming the autosampler to initiate a sample analysis every 20 min for the next 5 h. A final analysis was performed at 15 h and served as the infinite time point. A portion of leftover kinetic solution was used to confirm that the pH remained unchanged from its initial value to within ± 0.03 pH units.

Since the kinetic results depend only upon the change in relative concentration of a single species, the calculation of rate constants was performed directly from the peak areas without prior conversion to concentration units (see Eqns 1 and

2). However, since the molar absorptivities of the lactone and hydroxy acid forms were observed to differ by about 10%, the equilibrium constant cannot be directly equated to the peak area ratio. Thus, at the end of the kinetic run, freshly prepared standardized solutions of compounds **2a** and **2b** were analyzed, and the results were used to convert peak areas to molar concentrations for the purpose of calculating the equilibrium constant.

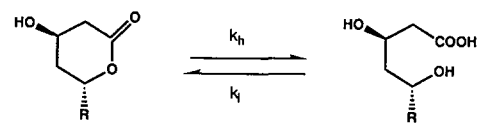
Hydrolysis kinetics of 3a to 3b. A stock solution was prepared from 2.31 mg (5.71 μmol) of lactone **3a** dissolved in 50.0 ml of acetonitrile. The solution was thermostated to 37°C and a 10 ml aliquot was quantitatively diluted to 50 ml with aqueous buffer (also equilibrated to 37°C). Aliquots of this solution were then transferred to thermostated HPLC vials and the run was then performed as described in the previous paragraph.

Results and Discussion

General kinetic features

Fig. 3 shows a plot of peak area vs time for the hydrolysis of lovastatin in 80% pH 2 buffer/20% acetonitrile at 37°C. An exponential decrease in peak area for the lactone and a corresponding increase in hydroxy acid peak area is observed throughout the entire kinetic run. However, the lactone concentration is not approaching zero, indicating that the hydrolysis is reversible under the conditions studied. A simple and direct confirmation of the reversibility of this reaction was obtained by repeating the experiment under identical conditions, but using the hydroxy acid as the starting material. As the chromatograms in Fig. 2 show, an identical equilibrium composition is achieved regardless of whether lactone **3a** or hydroxy acid **3b** is used as the reactant. Reversibility was also demonstrated in a similar manner for the other inhibitors used in this study.

Quantitation of the data was achieved by analyzing the reaction with a pseudo first-order, reversible kinetic model:



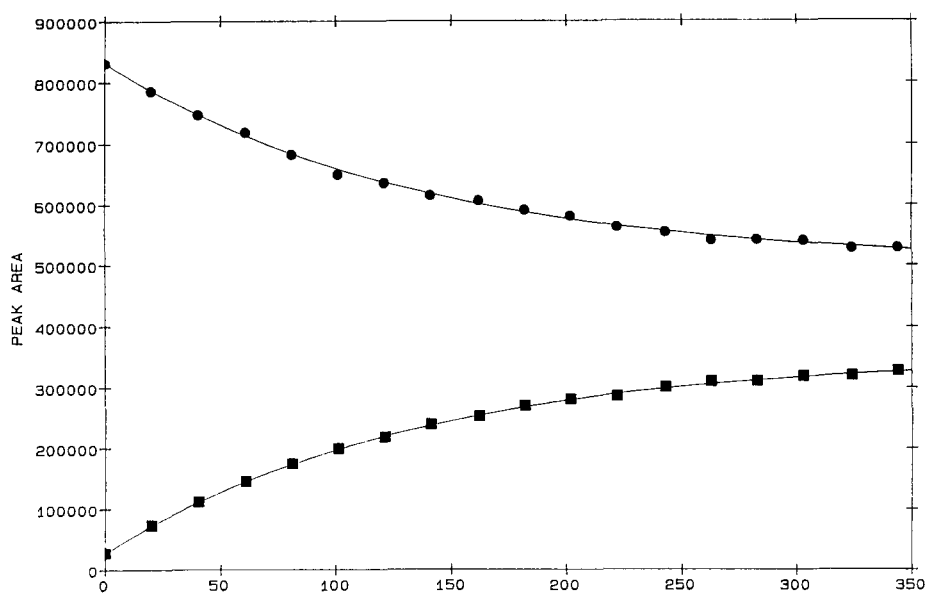


Fig. 3. Plot of integrated HPLC area vs time for the hydrolysis of lovastatin (**3a**) at pH 2.0, 37°C. The circles and squares are experimental data for the lactone and hydroxy acid forms, respectively, and the curves are the least squares fit of Eqns 1 and 2 (see text) to the data.

The integrated rate law for this scheme is described by Eqn 1 for the loss of lactone and by Eqn 2 for the growth of hydroxy acid:

$$A_t = (A_0 - A_c)\, e^{-k_{obs}t} + A_c \quad (1)$$

$$A_t = A_c - (A_c - A_0)\, e^{-k_{obs}t} \quad (2)$$

In Eqns 1 and 2, A_0, A_c, and A_t are the peak areas measured initially, at equilibrium, and at any time t, and k_{obs} is the observed rate constant for the reaction. For each kinetic run, the observed rate constants were obtained by nonlinear least-squares curve fitting of the experimental data to Eqns 2 and 3 using A_0, A_c and k_{obs} as adjustable parameters.

Before considering the data further, it is worthwhile to consider the precision of the results. In particular, note that each kinetic run provides two independent measurements of the observed rate constant (one each from the forward and reverse reaction). In all the kinetic runs, the rate constants derived from following the loss of reactant and the growth of product differed by no more than ± 3%. The good agreement indicates that no side reactions are competing with the hydrolysis/lactonization scheme. In accord with this, it was found that the sum molar concentration of lactone and hydroxy acid remains constant to within ± 5% throughout the duration of a kinetic run. Finally, it is notable that the initial and equilibrium concentrations of both species as calculated by least-squares fitting differed by no more than 2% from the observed values, and that the correlation coefficients for the regressions varied from 0.996 to greater than 0.999. It is concluded from these facts that the rate and equilibrium data are precise to no worse than ± 5%.

Hydrolysis/lactonization kinetics and equilibria

The rate constants for hydrolysis and lactonization of compounds **1–4** are shown in Table 1. These values were derived from the observed rate and equilibrium constants by the simultaneous solution of Eqns 3 and 4:

$$k_{obs} = k_H + k_L \quad (3)$$

$$K_{eq} = k_H/k_L \quad (4)$$

Both rate constants have been corrected for the constant catalyst (proton) concentration. In addition, the rate constants for hydrolysis have been corrected for the water concentration (55.5 M); although this is an unusual procedure, it will facilitate comparison of the data for purely aqueous and mixed aqueous/organic systems. The concentration of water in 80% aqueous acetonitrile was taken as 44.4 M which is equivalent to ignoring the small difference in partial molar volume of the two liquids.

Table 1 also gives the equilibrium constants for these reactions as defined by:

$$K_{eq} = [\text{hydroxy acid}]/[\text{lactone}][H_2O]$$

As described for the kinetics, the water concentration has been left as an explicit term in the equilibrium expression to facilitate comparison between the two solvent systems.

With the exception of mevalonolactone, the compounds in Table 1 exhibit quite similar kinetics towards acid-catalyzed hydrolysis. The average rate constants for lactonization are 0.536 M^{-2} s^{-1} for compounds **3b** and **4b** in 20% aqueous acetonitrile, and 0.574 M^{-2} s^{-1} for **2b** in the same medium. In the purely aqueous system, the lactonization rate constant for **2b** is 0.577 M^{-2} s^{-1},

TABLE 1

Rate constants for hydrolysis (k_H) and lactonization (k_L) at pH 2.0 and 37.0°C[a]

Reactant	Medium	$10^3\, k_H$	$10\, k_L$	K_{eq}
1a	aqueous[b]	8.68	35.1	0.247
2a	aqueous[b]	10.7	5.69	1.87
2b	aqueous[b]	11.8	5.85	2.02
2a	20% acetonitrile[c]	8.86	5.42	1.63
2b	20% acetonitrile[c]	9.64	6.07	1.59
3a	20% acetonitrile[c]	8.50	5.50	1.55
3b	20% acetonitrile[c]	8.33	5.22	1.59
4a	20% acetonitrile[c]	8.19	5.13	1.60
4b	20% acetonitrile[c]	8.39	5.59	1.50

[a] k_H in units of M^{-2} s^{-1}; k_L in units of M^{-1} s^{-1}; K_{eq} in units of M^{-1}.
[b] HCl/KCl buffer.
[c] 80% HCl/KCl buffer, 20% acetonitrile.

indicating that the addition of 20% acetonitrile has little if any effect on the ring closure reaction. Note, however, that a small solvent effect is evidenced by comparison of the hydrolysis rates of **2a** in water and 20% acetonitrile. The solvent effect is in the direction anticipated based on polarity considerations. The hydrolysis produces a relatively polar hydroxy acid from a relatively nonpolar lactone, and a more polar reaction medium should provide greater electrostatic stabilization of the transition state compared to the ground state reactants; this leads to a more facile reaction. Accordingly, lactone **2a** hydrolyzes about 20% faster in pure water relative to the mixed solvent system. Aside from this small solvent effect, the rate constants for the inhibitors do not differ by more than the combined experimental error of the measurements. Obviously, since the forward and reverse rate constants are virtually the same, the equilibrium constants are also quite similar.

The near constancy in kinetic parameters is easily rationalized by mechanistic considerations. The mechanism of ester hydrolysis reactions has been extensively studied and can be applied to lactone hydrolysis. The compounds used in this study presumably hydrolyze in acidic solution by the $A_{ac}2$ mechanism (Zimmerman and Rudolph, 1965). This mechanism involves rate-determining nucleophilic attack on a protonated lactone to form an intermediate which breaks down to product. Structural features which may influence the rate of nucleophilic attack include steric and inductive effects. The structural variation among the compounds used in this study are such that no differential effects due to steric factors are likely since the substituents are far from the reactive carbonyl center. Similarly, the inductive effect of the substituents will be greatly attenuated due to their remoteness from the reaction center.

More difficult to rationalize is the large difference in reactivity exhibited by mevalonolactone compared to the other compounds. Specifically, the hydrolysis rate of mevalonolactone is comparable to the other compounds, but the rate constant for relactonization is approximately 6-fold higher. The origin of this rate enhancement may be due to a combination of electronic and steric effects as described in the previous paragraph. Alternatively, the faster lactonization may be the result of a more favorable orientation of the reacting functional groups during ring closure. This orientation effect has been studied by Koshland and coworkers who have concluded that small changes in orientation during lactonization can have dramatic effects on reaction rates (Stern and Koshland, 1972). Due to its small size and lack of bulky ring substituents, mevalonic acid may be freer than the other hydroxy acids to adapt a favorable reaction conformation during ring closure.

Temperature dependence of rate and equilibrium constants

To gain a more complete understanding of the energetics of these reactions, the hydrolysis of mevalonolactone and lactone **2a** was studied as a function of temperature. Rate and equilibrium constants for **2a** were obtained in the temperature range of 10–50°C, whereas the corresponding measurements with **1a** were limited to a smaller temperature range due to experimental limitations.

The kinetic data obtained in these experiments are shown in Table 2. The activation enthalpies and entropies (and the overall reaction enthalpy and entropy) were obtained from log k (and log K) vs $1/T$ plots. Satisfactory linear fits were obtained in all cases as illustrated by the data for lactonization of **1a** and **2a** (Fig. 4). Table 3 summarizes the derived enthalpy and entropy values for these reactions.

In considering the overall reaction, the enthalpy

TABLE 2

Rate constants for hydrolysis and lactonization of compounds ***1a*** *and* ***2a*** *as a function of temperature*[a]

Compound	T (°C)	$10^3\,k_H$	$10\,k_L$	K_{eq}
1a	9.9	1.20	4.92	0.239
	24.9	4.32	17.8	0.243
	37.0	8.68	35.2	0.247
2a	10.5	1.25	0.417	3.00
	24.9	3.86	1.78	2.16
	37.0	10.7	5.69	1.87
	50.0	22.5	13.1	1.72

[a] K_{eq} in units of M^{-1}. Units for rate constants as defined in footnote a, Table 1.

TABLE 3

Thermodynamic and activation parameters for compounds 1a and 2a[a]

		1a	2a
Equilibrium	ΔH	0.1	−2.5
	ΔS	−12	−16
Hydrolysis	$\Delta H^{\ddagger}$	12	13
	$\Delta S^{\ddagger}$	−37	−34
Lactonization	$\Delta H^{\ddagger}$	12	15.5
	$\Delta S^{\ddagger}$	−25	−18

[a]Enthalpies in units of kcal mol^{-1}, entropies in units of cal K^{-1} mol^{-1}.

and entropy changes for the hydrolysis of lactone **2a** are $\Delta H = -2.51$ kcal mol^{-1} and $\Delta S = -15.9$ cal K^{-1} mol^{-}. It is interesting to note that the hydrolysis reaction is strongly favored by enthalpic factors, but that this is more than offset by the large and unfavorable entropy of reaction. For instance, at 37°C the entropy factor $T\Delta S$ is −4.93 kcal mol^{-1}, and the overall free energy change for hydrolysis is thus 2.42 kcal mol^{-1}. Mevalonolactone provides an even more striking example of the key role of entropy in these reactions. Within experimental error, the reaction enthalpy for mevalonolactone hydrolysis is zero and the position of equilibrium is consequently determined solely by the reaction entropy. The large and negative entropy values for these reactions are undoubtedly due in part to the fact that there is one less product molecule than reactant in the hydrolysis, although differences in solvation may also contribute to the entropy change.

For the hydrolysis of both mevalonolactone and **2a**, the activation parameters shown in Table 3 are remarkably similar; this suggests that the basic reaction geometries and dynamics of the two reactions are similar despite the large structural difference between the two lactones. In contrast, the reverse reaction (lactonization) displays significant differences in both the enthalpy and entropy of activation. Particularly noteworthy is the 3.3 kcal mol^{-1} difference in activation enthalpy which may be due to a steric repulsion in **2b** which may develop as the acyclic reactant moves toward the cyclic transition state. However, much of this 3.3 kcal mol^{-1} difference in activation enthalpy is compensated for by the large difference in activation entropy; consequently, the overall differ-

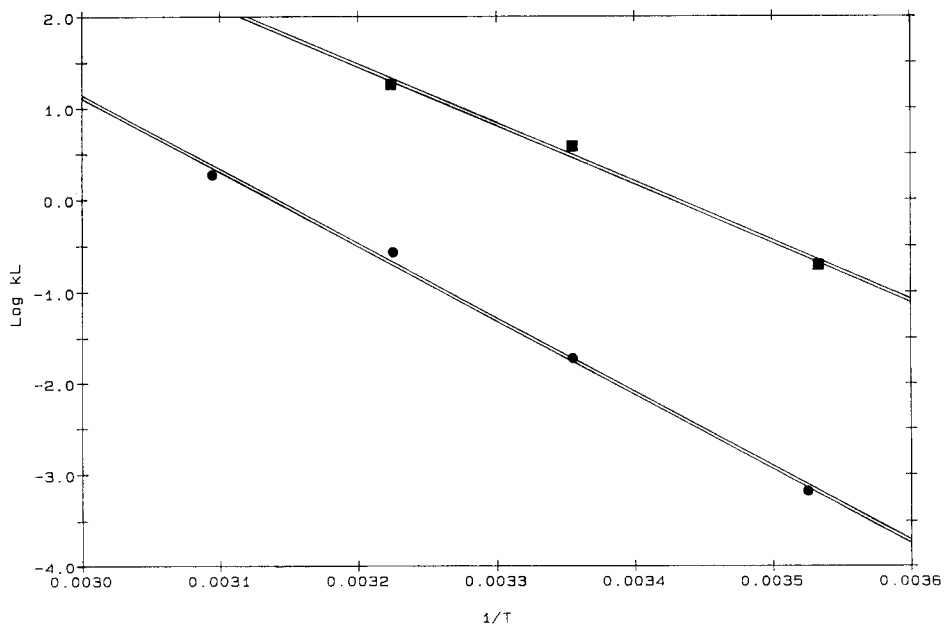


Fig. 4. Plot of the log of the lactonization rate constant vs reciprocal temperature (in K^{-1}) for compounds **1b** (squares) and **2b** (circles). The data are from Table 2.

ence in free energy of activation for lactonization of **1b** and **2b** is only 1.1 kcal mol^{-1} at 37°C.

Biopharmaceutical implications

As was highlighted in the Introduction, the premature conversion of HMG-CoA reductase inhibitors from the lactone to hydroxy acid form is undesirable due to the less favorable tissue selectivity of the latter. The in vitro kinetic experiments described in this paper indicate that lactone hydrolysis occurs reversibly with an observed half life of approx. 1 h. This chemical half life is on the same order of magnitude as the mean residence time of gastric contents in humans (Gruber et al., 1987); it therefore appears plausible that several biopharmaceutical parameters including bioavailability and tissue distribution are influenced by the lactone hydrolysis reaction.

A semi-quantitative evaluation of the effect of lactone hydrolysis on gastrointestinal absorption can be made with reference to the model shown in Fig. 5. This model is designed to estimate the rela-

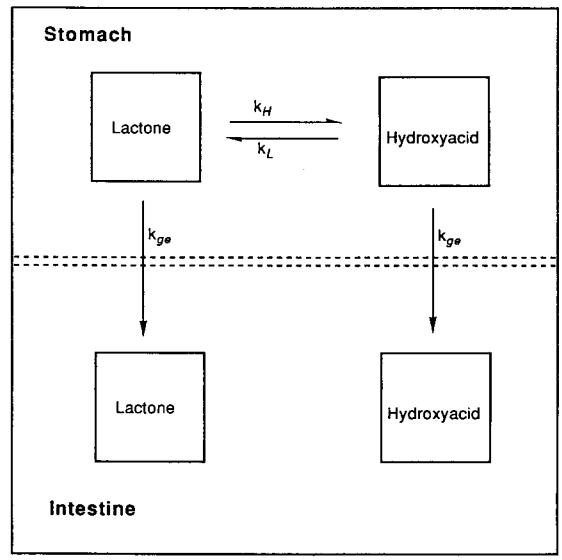


Fig. 5. Schematic model used to estimate the effect of hydrolysis on the gastrointestinal concentrations of lactone and hydroxy acid forms.

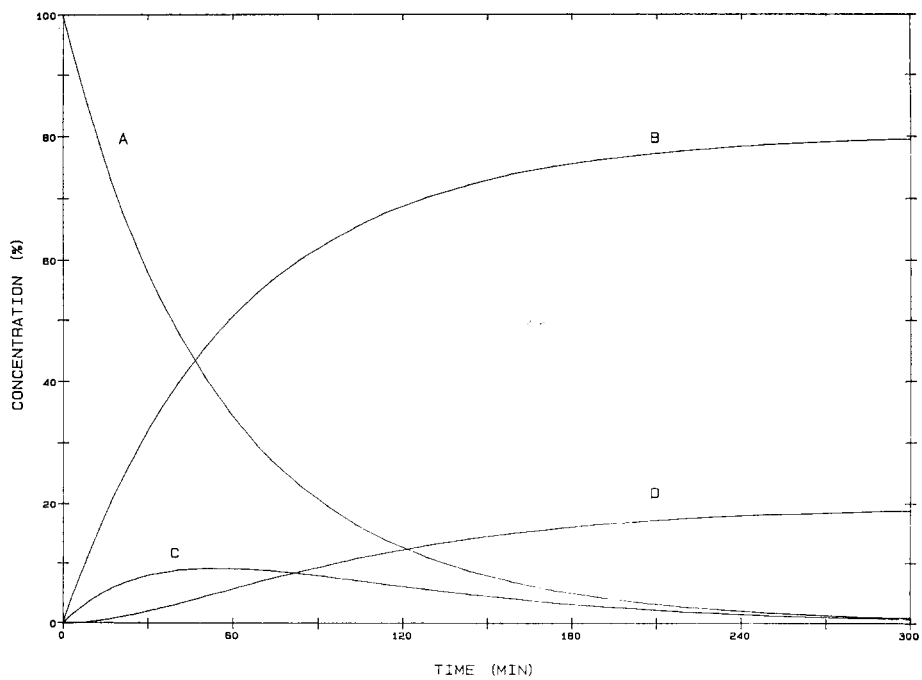


Fig. 6. Simulated concentration profiles calculated from the model shown in Fig. 5. (A) Lactone concentration in stomach; (B) lactone concentration in intestine; (C) hydroxy acid concentration in stomach; (D) hydroxy acid concentration in intestine.

tive proportion of lactone and hydroxy acid that is delivered from the stomach to the small intestine assuming the following conditions: (1) no drug absorption occurs directly from the stomach; (2) gastric emptying is an irreversible first order process characterized by a single rate constant which is independent of the drug form; and (3) interconversion of lactone and acid forms is insignificant at intestinal pH (6–8) on the time scale of the simulation (Garrett and Won, 1971). The possible effects of enzymatic hydrolysis cannot be assessed from the available data and are not considered in the model. The hydrolysis and lactonization rate constants were taken from the data in Table 1 for lovastatin. For the rate of gastric emptying, a value of $k_{gc} = 0.014\ min^{-1}$ ($t_{1/2} = 50$ min) obtained in studies with healthy, fasted human volunteers was used (Evans et al., 1981). It should be noted that gastric emptying is generally slower in the non-fasted state, but postprandial gastric pH levels are significantly higher (Dressman, 1986) than the pH of 2 used for the hydrolysis experiments. Thus, the longer gastric residence time of the drug is offset by the rise in pH, and overall the data for fasted state gastric emptying should be most suitable to this model. The concentrations of the various species were then calculated from the model and these rate constants using the GEAR kinetic simulation package (Weigert, 1987); the results of the calculation are shown graphically in Fig. 6.

After 6 h, transit of total inhibitors (i.e. lactone plus hydroxy acid) from the stomach to the small intestine is essentially complete. The curves in Fig. 6 show that the corresponding total inhibitor concentration in the intestine after this time is composed of 80% lactone and 20% hydroxy acid. At earlier time points, the ratio of lactone to hydroxy acid in the intestine is greater than the equilibrium ratio of 4:1. For instance, the ratio is approx. 10:1 after 1 h, 6:1 after 2 h, and 5:1 after 3 h. The significance of these ratios is that they represent the instantaneous relative proportions of the two forms that are available for intestinal absorption into the circulatory system. The actual systemic concentrations will depend on the rate constants for absorption of the two forms. These rate constants are not known and, in any case, the current model is far too crude to be used for predicting systemic drug levels. Nonetheless, the kinetic simulation does suggest that the 'pool' of intestinal drug at any given time after oral dosing is predominantly composed of lactone. In fact, the lactone/hydroxy acid ratio may be greater than the values derived from the simulation since a slow rate of dissolution for these poorly water soluble prodrugs would tend to decrease the amount of gastric hydrolysis. Accordingly, it is concluded that the favorable tissue selectivity of the prodrug can be exploited despite the acid lability of the lactone ring.

Acknowledgements

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References

Alberts, A.W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Curries, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogstein, K., Liesch, J. and Springer, J., Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol lowering agent. *Proc. Natl. Acad. Sci. USA*, 77 (1980) 3957–3968.

Dressman, J.B., Comparison of canine and human gastrointestinal physiology. *Pharm. Res.*, 3 (1986) 123–131.

Evans, M.A., Triggs, E.J. and Cheung, M., Gastric emptying rate in the elderly: implications for drug therapy. *J. Am. Geriatr. Soc.*, 29 (1981) 201–205.

Garrett, E.R. and Won, C.M., Prediction of stability in pharmaceutical preparations. XVI. Kinetics of hydrolysis of canrenone and lactonization of canrenoic acid. *J. Pharm. Sci.*, 60 (1971) 1801–1809.

Gruber, P., Rubinstein, A., Li, V.H.K., Bass, P. and Robinson, J.R., Gastric emptying of non-digestible solids in the fasted dog. *J. Pharm. Sci.*, 76 (1987) 117–122.

Hoffman, W.F., Alberts, A.W., Anderson, P.S., Chen, J.S., Smith, R.L. and Willard, A.K., 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. 4. Side chain derivatives of mevinolin. *J. Med. Chem.*, 29 (1986) 849–852.

Kirby, A.J., Ester hydrolysis. In Bamford, C.F.H. and Tipper, C.H. (Eds), *Comprehensive Chemical Kinetics*, Elsevier,

Amsterdam, 1972, pp. 57–207.
Krukemeyer, J.J. and Talbert, R.L., Lovastatin: a new cholesterol lowering agent. *Pharmacotherapy*, 7 (1987) 198–210.
Rodwell, V.W., Nordstrom, J.L. and Mitschelen, J.J, Regulation of HMG-CoA Reductase. *Adv. Lipid Res.*, 14 (1976) 1–14.
Stern, D.R. and Koshland, D.E. Jr, Effect of small changes in orientation on reaction rates. *J. Am. Chem. Soc.*, 94 (1972) 5815–5825.
Weigert, F.J., The gear iterator. *Comput. Chem.*, 11 (1987) 273–280.
Zimmerman, J.H., *Chemical Kinetics and Reaction Mechanisms*, McGraw-Hill, New York, 1981, pp. 42–45.