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Reactivity of Lactones and GHB Formation

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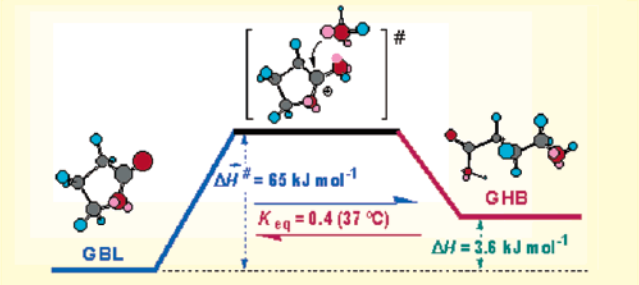

Reactivity of Lactones and GHB Formation

The behavior of lactones in their hydrolysis reactions is a good indicator of their reactivity as electrophilic molecules. The hydrolysis of four- to six-membered lactones was investigated in neutral (water) and slightly acid media and in water/dioxane media. The following conclusions were drawn: (i) The reactivity of β-propiolactone in neutral water is more than four times greater than that of β-butyrolactone, due to the flow of charge caused by the latter's methyl substituent. Reactivity is enthalpy-controlled. (ii) The reactivity of β-lactones diminishes in water/dioxane media when the percentage of dioxane increases. The increase in the dioxane percentage relaxing the intermolecular hydrogen bonds in the ordered structure of the water reduces $\Delta H^{\#}$ and simultaneously increases the $-\Delta S^{\#}$ value. (iii) An inverse solvent kinetic isotope effect in the acid-catalyzed hydrolysis of γ-butyrolactone and δ-valerolactone was observed, this being indicative of acyl cleavage. (iv) The $\Delta H^{\#}$ and $\Delta S^{\#}$ values permit discrimination between alkyl and acyl cleavage. (v) A correlation was found between the chemical reactivity of lactones and their carcinogenic activity. (vi) The results suggest that orally ingested γ-butyrolactone remains largely in its nonhydrolyzed form in the stomach before passing into the blood. (vii) The concentration equilibrium constant of GHB formation at human body temperature is K_{eq} (37 °C) = 0.40. (viii) Study of GHB formation shows that, contrary to earlier results, this is an endothermic process, with $\Delta_r H = 3.6$ kJ mol^{-1}.

Introduction

The reactivity of amino acids with an $-NH_2$ group in nitrosation reactions and its relationship with the alkylating potential of their products have been investigated in previous work.[1−3] Among other conclusions, it was observed that the species resulting from the nitrosation of these amino acids are the corresponding lactones.

Since some lactones give alkylating reactions with any of a number of nucleophilic sites in tissues,[4] the present investigation was performed with two main objectives: (i) To gain quantitative knowledge about the lactones' reactivity and to relate this to their carcinogenic activity and (ii) to gain insight into the formation of γ-hydroxy butyric acid (GHB) in the hydrolysis of γ-butyrolactone.

γ-Butyrolactone is used widely as an industrial solvent and is marketed as a dietary supplement. Since GHB is a drug of abuse and is also used as a dietary supplement claimed to improve physical performance and to reduce

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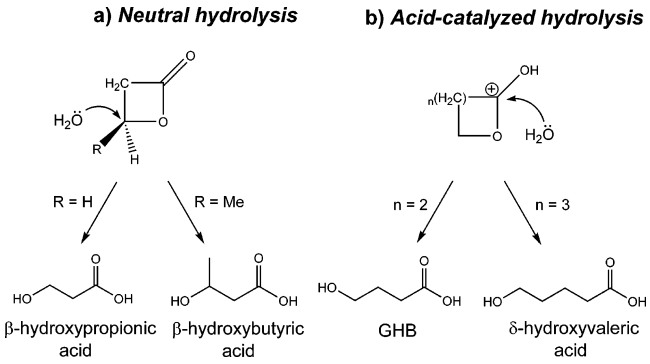
SCHEME 1. Hydrolysis of Lactones



stress,[5] investigation of its formation is of biological/biochemical interest.

In addition, the reactivity of the cyclic esters is of current interest because many biologically important molecules contain cyclic phosphate and lactone structures.[6]

The behavior of lactones in their hydrolysis reactions is a good indicator of their reactivity as electrophilic molecules. Most earlier investigations were carried out in alkaline media, and studies in neutral or slightly acid media have received scant attention,[7−12] even though they are the most relevant in biochemical terms.[4,13]

Previous research has shown that the hydrolysis of β-butyrolactone by molecular water yields bimolecular displacement reactions, with cleavage of the bond between the alcoholic carbon and oxygen.[10,14−17] Olson et al.[16,17] demonstrated that the hydrolysis of β-butyrolactone in basic and strongly acid solutions results in the expected acyl-oxygen cleavage.

In the chemistry of synthesis, different attempts have been made to find effective reagents for lactone ring-opening; e.g., the preparation of ω-alkylthio carboxylic acids through ω-carbon−oxygen bond cleavage.[18] β-Propiolactone has a curious dichotomy of reactivity; diethylaluminumethanethiolate has been reported[19] to cause both *O*-alkyl and *O*-acyl cleavage at the same time.

More recently, Moore and Schwab,[20] working with strongly acidic conditions (6 N HCl), concluded that five-membered lactones with bulky substituents at C-2 can be hydrolyzed by a previously unreported alkyl cleavage mechanism at the expense of the usually predominant acyl cleavage.

A review about the mechanisms of lactone hydrolysis was published by Kaiser and Kézdy.[6]

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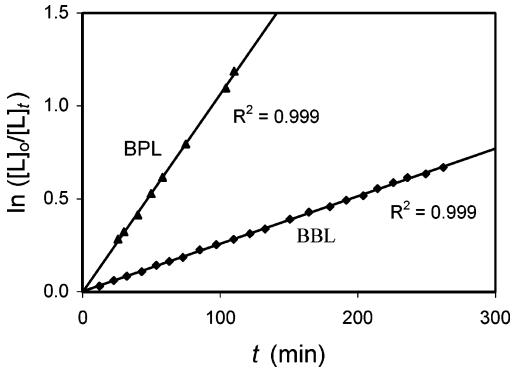

FIGURE 1. Integrated form of the pseudo-first-order rate equation (eq 1) for the hydrolysis of β-propiolactone and β-butyrolactone. $[L]_o = 0.08$ M, $T = 35$ °C.

TABLE 1. Rate Constants[a] as a Function of Temperature for the Neutral Hydrolysis[b] of β-Propiolactone and β-Butyrolactone

T (°C)	$10^7 k_{BPL}$	$10^7 k_{BBL}$
	(M^{-1} s^{-1})[c]	
15.0	3.54 ± 0.05	
17.5	4.30 ± 0.04	0.94 ± 0.01
22.5	7.6 ± 0.4	1.82 ± 0.01
25.0	10.6 ± 0.1	2.22 ± 0.03
27.5	14.9 ± 0.3	3.63 ± 0.02
30.0	20.2 ± 0.7	4.38 ± 0.05
32.5	27.4 ± 0.9	5.89 ± 0.08
35.0	31.9 ± 0.9	7.69 ± 0.07

[a] As k in eq 1. [b] $[BPL]_o = 0.08$ M; $[BBL]_o = 0.08$ M. [c] Values of rate constants are given within the 95% confidence interval.

The present investigation was carried out in two stages: (i) A kinetic study of the hydrolysis reactions of β-propiolactone (BPL), β-butyrolactone (BBL), γ-butyrolactone (GBL), and δ-valerolactone (DVL) and (ii) a kinetic and thermodynamic study of the formation of GHB.

Results and Discussion

Neutral Hydrolysis: Water Reactions. We investigated the hydrolysis of β-propiolactone and β-butyrolactone (Scheme 1a).

Experiments designed to determine the influence of the lactone concentration revealed the reactions to be first-order with respect to this reagent. Thus:

$$\text{rate} = k[H_2O][L] = k_1[L] \qquad (1)$$

where [L] represents the concentration of lactone and $k_1 = k[H_2O]$ is the pseudo-first-order rate constant.

Figure 1 represents the integrated form of eq 1 in terms of $[L]_t$, the concentration of lactone at time t, with $[L]_o$ being the initial concentration.

Table 1 gives the k_{BPL} and k_{BBL} rate constants (as k in eq 1) for the hydrolysis reactions of BPL and BBL, respectively, as a function of temperature (T).

The results given in Table 1 show that the rate constants are more than four times shorter for β-butyrolactone than for β-propiolactone.

Table 3 shows the values of the activation parameters $\Delta H^{\#}$ and $\Delta S^{\#}$ for both hydrolysis reactions.

TABLE 2. Rate Constants[a] as a Function of the Composition of the Media and the Temperature for the Neutral Hydrolysis[b] of β-Propiolactone and β-Butyrolactone

	water/dioxane (volume ratio)							
	8/2		6/4		5/5		4/6	
T (°C)	10^7k_{BPL}	10^7k_{BBL}	10^7k_{BPL}	10^7k_{BBL}	10^7k_{BPL}	10^7k_{BBL}	10^7k_{BPL}	10^7k_{BBL}
	$(M^{-1}\ s^{-1})$[c]							
25.0	10.3 ± 0.3	1.75 ± 0.01	6.84 ± 0.07	0.87 ± 0.01	5.1 ± 0.1	0.61 ± 0.01	3.43 ± 0.04	0.387 ± 0.008
27.5	13.40 ± 0.06	2.34 ± 0.01	8.46 ± 0.07	1.20 ± 0.01	7.02 ± 0.03	0.81 ± 0.02	4.50 ± 0.01	0.500 ± 0.007
30.0	17.5 ± 0.4	3.10 ± 0.03	11.8 ± 0.2	1.55 ± 0.02	8.6 ± 0.3	1.09 ± 0.01	5.66 ± 0.08	0.67 ± 0.02
32.5	23.2 ± 0.6	4.10 ± 0.04	14.7 ± 0.4	2.09 ± 0.03	11.0 ± 0.5	1.39 ± 0.01	7.31 ± 0.03	0.87 ± 0.01
35.0	30.4 ± 0.9	5.41 ± 0.05	18.9 ± 0.5	2.63 ± 0.06	14.6 ± 0.3	1.85 ± 0.01	9.3 ± 0.1	1.11 ± 0.03

[a] As k in eq 1. [b] $[BPL]_o = 0.08$ M; $[BBL]_o = 0.08$ M. [c] Values of rate constants are given within the 95% confidence interval.

TABLE 3. Activation Parameters as a Function of the Composition of the Media for the Neutral Hydrolysis of β-Propiolactone and β-Butyrolactone

water/dioxane (volume ratio)	$\Delta H^{\#a}$ (kJ mol^{-1})		$-\Delta S^{\#a}$ (J K^{-1} mol^{-1})	
	BPL	BBL	BPL	BBL
10/0	83 ± 2	88 ± 3	80 ± 8	78 ± 10
8/2	80 ± 1	84 ± 1	90 ± 3	94 ± 1
6/4	76 ± 3	82 ± 2	106 ± 10	105 ± 7
5/5	75 ± 3	82 ± 1	112 ± 9	109 ± 4
4/6	73 ± 1	79 ± 2	123 ± 3	122 ± 6

[a] Values are given with their standard deviations.

With the aim of gaining deeper insight into the transition state, the hydrolysis of BPL and BBL in water/dioxane was investigated. Tables 2 and 3 show the results obtained.

The very similar $\Delta S^{\#}$ values found for the hydrolysis of BPL and BBL (Table 3) are consistent with the analogous geometry of these lactones. They also show that the reactivity of these β-lactones is mainly enthalpy-controlled.

The greater $\Delta H^{\#}$ value obtained for the hydrolysis of β-butyrolactone must be caused by the methyl group as a donor of charge on the β-carbon, with a decrease in its electrophilic character (Scheme 1a). Since in general terms substitution on carbon atoms 2 or 3 in lactones results in a decrease or loss in carcinogenic activity,[21] the steric hindrance of the BBL methyl group must also contribute to the higher value of $\Delta H^{\#}$.

It can be observed that when the percentage of dioxane increased: (i) the rate constant decreased appreciably, (ii) the enthalpy of activation became smaller, and (iii) the absolute value of the entropy of activation increased.

Mixed solvents behave regularly only insofar as the components are similar; thus, they can be characterized by the bulk relative permittivity, which is a monotonic function of component permittivities. If one solvent is protic and highly structured, its structure is broken by a foreign solvent and the solvation power diminishes rapidly,[22] increasing the disorder of the protic solvent. This latter being the current case: (i) the increase in the dioxane percentage relaxing the intermolecular O···H hydrogen bonds in the ordered structure of the water increases its hydrolytic activity by reducing $\Delta H^{\#}$ (see Table 3); (ii) as a consequence of the considerable disorder induced by the dioxane in the reagent water, $-\Delta S^{\#}$ values

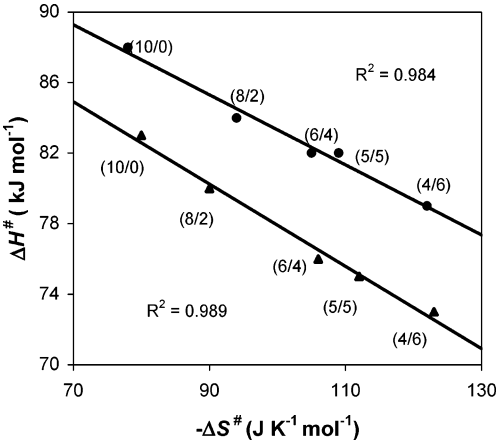

FIGURE 2. Isokinetic relationship in the hydrolysis of β-propiolactone (▲) and β-butyrolactone (●) in water/dioxane media.

should decrease when increasing those of the water/dioxane ratio, as was also observed (Table 3).

Since the strong effect of the dioxane on the kinetic parameters is a consequence of a strong lactone−water interaction, the enthalpy and entropy will both tend to be linear functions of the volume fraction of that solvent, and an isokinetic relationship should result.[23] Figure 2 shows that this effect was indeed observed.

Because the tumorigenicity of lactones would be correlated with their alkylating capacity on nucleophilic substrates,[4,21] the greater reactivity of β-propiolactone would imply a greater carcinogenic potential. The data reported in Table 4 are consistent with such a conclusion.

Acid-Catalyzed Hydrolysis. Under the same conditions as for BPL and BBL, no neutral hydrolysis reactions of GBL and DVL were observed.

The loss of reactivity of GBL and DVL at neutral pH is in agreement with their inactivity as carcinogens,[4] as well as with our previous results concerning the reactivity of lactones resulting in the nitrosation of amino acids.[1−3] The alkylation time of 4-(*p*-nitrobenzyl)pyridine (a trap for alkylating agents[27,28] with similar nucleophilic char-

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TABLE 4. Dose and Tumorigenicity of Lactones by Subcutaneous Injection in Mice and Rats[21,24–26]

	dose in mg	number of malignant tumors at injection site/number of animals
	subcutaneous injection in mice[a]	
β-propiolactone	0.73	18/30
β-butyrolactone	10	18/30
	subcutaneous injection in rats[b]	
β-propiolactone	4	13/20
β-butyrolactone	100	9/20

[a] Performed with 0.05 mL of tricaprylin as a vehicle. [b] Performed with 0.1 mL of tricaprylin as a vehicle.

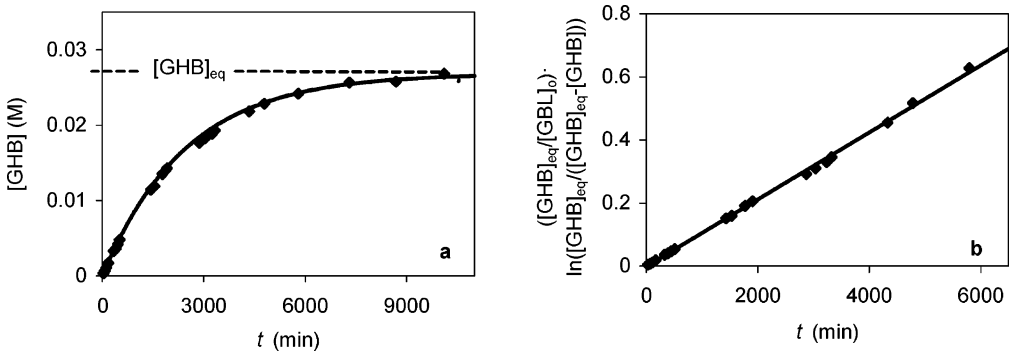

FIGURE 3. Formation of GHB in the acid hydrolysis of γ-butyrolactone. (a) Variation in [GHB] with the reaction time. (b) Integrated form of the rate equation (eq 1). $[GBL]_o = 0.1$ M, $[HCl] = 1.18 \times 10^{-2}$ M, $T = 20$ °C.

acteristics to DNA) by β-lactones generated in the nitrosation of β-amino acids was $\vec{k}_{alkylation} = 2$ to more than 70 h, with $t_{alkylation} > 150$ h for the γ-butyrolactone derived from the nitrosation of γ-amino butyric acid.[1]

Upon carrying out the reactions in weak hydrochloric acid solutions, the following results were obtained.

Since the hydrolysis reaction of GBL is reversible, the first-order rate constant for the forward reaction ($\vec{k}_1 = \vec{k}_{GBL}[H_3O^+]$, $\vec{k}_{GBL}$ being the catalytic constant) is given by

$$\vec{k}_1 = \frac{1}{t}\frac{[GHB]_{eq}}{[GBL]_o}\ln\frac{[GHB]_{eq}}{[GHB]_{eq} - [GHB]} \qquad (2)$$

where $[GBL]_o$ represents the initial lactone concentration, and $[GHB]_{eq}$ and [GHB] are the concentrations of the hydroxy acid at equilibrium and at time t, respectively.

Figure 3a shows a typical kinetic run, allowing one to know $[GHB]_{eq}$ as the plateau value of [GHB]. Figure 3b shows the good fit of the results to eq 2.

To check the hydrolysis of GBL occurring through an acid-catalyzed mechanism, experiments at different pH values were carried out. The slope value of $\alpha = 1.00$ in Figure 4 is evidence of this.[29]

The values of $\vec{k}_{GBL}$ at different temperatures are reported in Table 5. With the experimental value of $[GHB]_{eq}$, knowledge of K_{eq}, the concentration equilibrium constant of the hydrolysis of GBL,

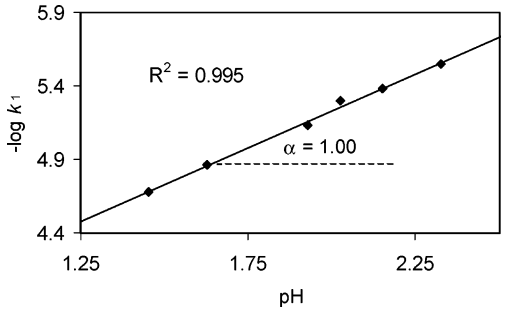

FIGURE 4. Variation in the GBL hydrolysis rate constant with the acidity of the medium. $[GBL]_o = 0.1$ M, $T = 35$ °C.

$$K_{eq} = \vec{k}_{GBL}/\overleftarrow{k}_{GHB} = [GHB]_{eq}/[GBL]_{eq} = [GHB]_{eq}/([GBL]_o - [GHB]_{eq}) \qquad (3)$$

is straightforward ($\vec{k}_{GBL}$ and $\overleftarrow{k}_{GHB}$ represent the second-order forward and backward rate constants, respectively).

Regarding DVL, the experiments revealed that the reactions were first-order with respect to this reagent: rate $= k_{DVL}[H_3O^+][DVL]$. The results obtained with this lactone are summarized in Tables 5 and 6 and show the following: (i) The rate constant of the acid-catalyzed hydrolysis of DVL (k_{DVL}) is two-orders of magnitude higher than that ($\vec{k}_{GBL}$) of GBL hydrolysis. (ii) The Gibbs energy of activation for DVL hydrolysis (81 kJ mol^{-1}; 25 °C) is lower than that seen for GBL (94 kJ mol^{-1}; 25 °C). The data reveal that the activity of DVL as an electrophilic reagent is greater than that of GBL, so much so that the hydrolysis of the latter is a clearly reversible reaction.

Another piece of information consistent with the notion of acylic cleavage is the solvent kinetic isotope effect,

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TABLE 5. Rate Constants as a Function of Temperature for the Acid-Catalyzed Hydrolysis[a] of γ-Butyrolactone and δ-Valerolactone

T (°C)	$10^4\vec{k}_{GBL}$	10^2k_{DVL}
	$(M^{-1}\ s^{-1})^b$	
15.0	0.99 ± 0.01	1.97 ± 0.03
17.5	1.27 ± 0.01	2.35 ± 0.06
20.0	1.55 ± 0.03	2.76 ± 0.05
22.5	1.98 ± 0.03	3.24 ± 0.05
25.0	2.40 ± 0.02	4.06 ± 0.07
27.5	3.11 ± 0.05	4.7 ± 0.2
30.0	3.82 ± 0.04	5.4 ± 0.1
32.5	4.95 ± 0.07	6.7 ± 0.2
35.0	6.22 ± 0.06	7.3 ± 0.2

[a] $[GBL]_o = 0.1$ M; $[DVL]_o = 0.1$ M; $[HCl]_{GBL} = 1.18 \times 10^{-2}$ M; $[HCl]_{DVL} = 4.72 \times 10^{-3}$ M. [b] Values of rate constants are given within the 95% confidence interval.

TABLE 6. Activation Parameters for the Acid-Catalyzed Hydrolysis of γ-Butyrolactone and δ-Valerolactone

lactone	$\Delta\vec{H}^{\neq a}$ ($\Delta\overleftarrow{H}^{\neq b}$) (kJ mol^{-1})	$-\Delta\vec{S}^{\neq a}$ ($-\Delta\overleftarrow{S}^{\neq b}$) (J K^{-1} mol^{-1})
γ-butyrolactone	65 ± 1 (61 ± 1)	96 ± 3 (100 ± 4)
δ-valerolactone	47 ± 1	113 ± 5

[a] Values are given with their standard deviations. [b] Magnitudes within parentheses correspond to the back reaction.

TABLE 7. Solvent Kinetic Isotope Effect in the Acid-Catalyzed Hydrolysis of Lactones

lactone	k_{H_2O}/k_{D_2O}
γ-butyrolactone	0.67
δ-valerolactone	0.41

SKIE.[30] Table 7 shows this effect to be inverse. This is indicative of preequilibrium protonation of the lactone molecule prior to the reaction, as would be anticipated for an acid-catalyzed mechanism.[31] Brown et al.[32] reported a number of examples of transition states for acid acyl cleavage mechanisms with inverse solvent effects (it should be noted that SKIE was not observed in the noncatalyzed hydrolysis of BPL and BBL).

Comparison of the results from the study of neutral and acid-catalyzed reactions provided the following conclusions:

(i) Lower $\Delta H^{\#}$ values for the acid-catalyzed reactions. The acyl-oxygen fission route being the normal path, the presence of H^+ ions enhancing the electrophilic character of the carbonyl carbon should imply lower $\Delta H^{\#}$ values, as was observed.

(ii) More negative $\Delta S^{\#}$ values for the acid-catalyzed reactions. In the acyl fission mechanism, cleavage of the C−O bond is not required in the transition state. In addition, nucleophilic attack by water on the lactone molecule occurs on the previously protonated carbonyl C-atom, i.e., a C^+-atom. This involves a compact tetrahedral intermediate in the case of the acid-catalyzed hydrolysis, i.e., a more negative $\Delta S^{\#}$ value (Table 6).

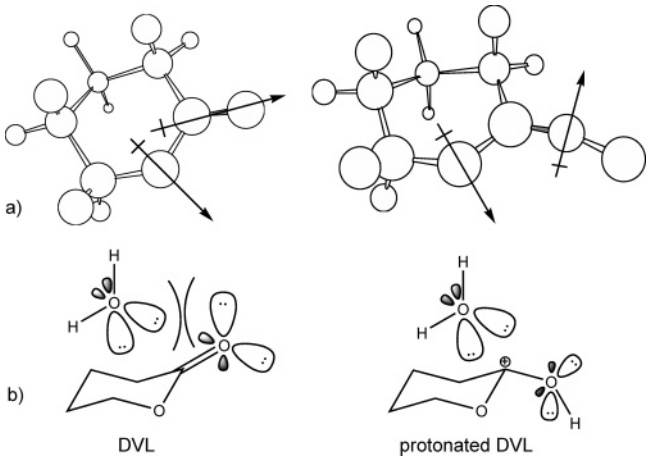

FIGURE 5. (a) Orientation of the dipoles at oxygen for unprotonated and protonated δ-valerolactone. In the protonated molecule, the dipoles' orientation is more favorable. (b) Contraction of the lone pair orbitals leading to decreased lone pair−lone pair repulsion.

TABLE 8. Variation in the Equilibrium Constant with Temperature in the GHB Formation[a]

T (°C)	$10K_{eq}$[b]
15.0	3.66 ± 0.04
20.0	3.73 ± 0.04
25.0	3.79 ± 0.04
30.0	3.93 ± 0.04
35.0	4.03 ± 0.04

[a] $[GBL]_o = 0.1$ M; $[HCl] = 1.18 \times 10^{-2}$ M. [b] Mean values of three experiments.

At the molecular level, there are arguments that rationalize the kinetic and thermodynamic parameters found in the acid-catalyzed hydrolysis of GBL and DVL. Some of the major effects of protonation are a change in the direction of the dipole component at the carbonyl oxygen (Figure 5a) and a contraction of the lone pair orbitals[33] (Figure 5b). Accordingly, the hydrolytic encounter between the protonated lactones and water molecules will be favored by diminishing intermolecular lone pair−lone pair repulsion. This should cause a lower enthalpy of activation and a more negative entropy of activation in the reactions occurring with protonation of the carboxyl carbon, as was observed (Table 6).

Study of the reactions of triethyloxonium ion with GBL and DVL to form the ethylated lactone has shown[33] that the basicity of DVL is 13 kJ mol^{-1} greater than that of GBL. Ab initio calculations[33] also reveal that the proton affinity of DVL is 8 kcal mol^{-1} greater than that of GBL. This allows one to assume a lower Gibbs energy of activation for DVL-catalyzed hydrolysis reaction than that for GBL, as was in fact observed.

It may be concluded that the kinetic and thermodynamic results can be used as a criterion to discern between mechanisms occurring through alkyl-oxygen or acyl-oxygen cleavage.

Finally, it must be pointed out that when we studied the hydrolysis of BPL and BBL, the possibility of acid-catalyzed hydrolysis was also investigated. Results showed

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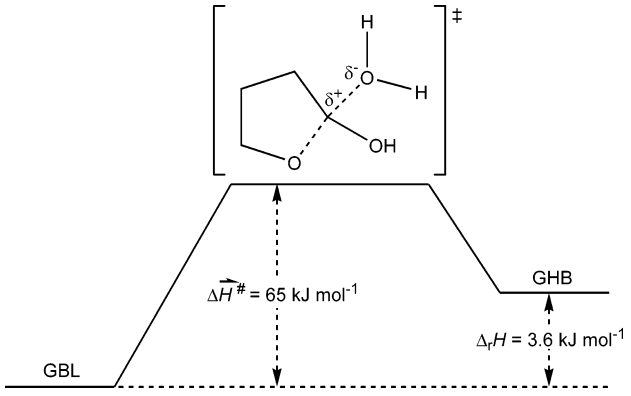
SCHEME 2. Reaction Path of the GHB Formation in the Acid-Catalyzed Hydrolysis of γ-Butyrolactone



that, in the working conditions, catalyzed hydrolysis can be discarded.

Formation of GHB: Heat of Reaction. Since the fraction of lactone and hydroxy acid existing at equilibrium is dependent on the relative thermodynamic stability of the acid and its precursor lactone, the concentration equilibrium constant K_{eq} was measured.

With eq 3, K_{eq} values were determined at different temperatures. Table 8 shows the results obtained by using the van't Hoff equation[34]

$$\frac{\mathrm{d}\ln K_{eq}}{\mathrm{d}(1/T)} = -\frac{\Delta_r H}{R} \qquad (4)$$

From the equilibrium constants of Table 8, $\Delta_r H$ for the hydrolysis reaction is calculated to be $\Delta_r H = 3.6 \pm 0.3$ kJ mol^{-1}. This result shows that the acid-catalyzed hydrolysis of GBL to form GHB is an endothermic reaction (Scheme 2). Such a conclusion is contrary to that of Coffin and Long,[12] who reported a value of $\Delta_r H = -710$ cal ($\simeq -3$ kJ mol^{-1}), implying that the reaction would be exothermic.

Since (i) the Coffin and Long $\Delta_r H$ value was calculated by working only at three temperatures and (ii) the K_{eq} values used by those authors were mean values from experiments carried out under nonhomogeneous conditions (in a 9-fold [H$^+$] range), the Coffin and Long $\Delta_r H$ value would be affected by an appreciable error.

Because the kinetic experiments were carried out under pH conditions (pH $\sim$ 2) comparable to those existing in the stomach, from the data in Tables 5 and 6 it can be deduced that at human body temperature the amount of GBL converted into GHB is less than 3% after 2 h (as shown in Figure 3a, 1 week is necessary for the equilibrium situation to be reached). This means that when ingested (see Introduction), GBL flows into the blood in the nonhydrolyzed, nonopened form.

Although GBL is rapidly hydrolyzed in the blood by a γ-lactonase (with a half-life of less than 1 min[35−37]), it is more liposoluble than GHB, which helps it to pass through the lipid layers of tissues before it can be hydrolyzed. Such tissues may act as a GBL reservoir and extend the duration of its action as compared to GHB.[38−40]

These results would also help to explain the fact that the oral bioavailability of GBL (the degree to which it becomes available to target tissues) in rats is 85%, higher than that of GHB[40] and also explain why GBL has a longer duration of action than GHB, possibly due to differences in drug distribution.[41−43]

Conclusions

(i) β-Propiolactone and β-butyrolactone show hydrolysis reactions in neutral (water) media, while γ-butyrolactone and δ-valerolactone need acid media to allow their hydrolysis to occur. (ii) The reactivity of the β-lactones is enthalpy-controlled; that of β-propiolactone is more than four times greater than that of β-butyrolactone, due to the flow of charge caused by the latter's methyl substituent. (iii) The reactivity of β-lactones diminishes in water−dioxane media when the percentage of dioxane increases. The increase in the dioxane percentage relaxing the intermolecular hydrogen bonds in the ordered structure of the water reduces $\Delta H^{\#}$ and simultaneously increases the $-\Delta S^{\#}$ value. (iv) The inverse solvent kinetic isotope effect in the acid-catalyzed hydrolysis of γ-butyrolactone and δ-valerolactone points to an acyl-cleavage mechanism. (v) The $\Delta H^{\#}$ and $\Delta S^{\#}$ values permit discrimination between alkyl and acyl cleavage. (vi) A correlation was found between the chemical reactivity of the lactones and their carcinogenic activity. (vii) The results suggest that orally ingested γ-butyrolactone remains largely in its nonhydrolyzed form in the stomach before passing into the blood, where it is rapidly hydrolyzed to GHB by the action of a γ-lactonase. (viii) The concentration equilibrium constant of GHB formation at human body temperature is K_{eq} (37 °C) = 0.40. (ix) Contrary to earlier results, this is an endothermic process, with $\Delta_r H = 3.6$ kJ mol^{-1}.

Experimental Section

β-Propiolactone, β-butyrolactone, and γ-butyrolactone were obtained from Sigma. δ-Valerolactone was a Fluka product. Since the commercial δ-valerolactone may be polymerized up to 25%, its purity was checked periodically, and when appropriate, it was reconstituted by distillation under reduced pressure.

The heavy water used to study the solvent isotope effect was an Aldrich product (99.9% D), and the dioxane used in the study of the influence of the reaction medium was from Panreac.

To monitor the reaction kinetics, hydroxy acids resulting in hydrolysis of the lactones were used as control species. The concentration of the hydroxy acid was determined by titration with NaOH, this latter being titrated with potassium hydrogen phthalate. In the titration procedure, 1 mL aliquots of the

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reacting mixture (lactone and unbuffered CO_2-free water in the case of neutral hydrolysis; lactone and diluted HCl solutions for acid-catalyzed reactions) were removed from time to time, added to 9 mL of ice water, and titrated immediately to a bromthymol blue endpoint (the hydroxy acid pK_a values are in the range 4.5−4.7[44]). Kinetic runs were performed in triplicate. Temperatures were kept constant to ±0.04 °C using a Lauda-Ecoline RE 120 thermostat.

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