bonate bands are compared (Table II). The carbonate bands observed in the calcium carbonate antacid are very similar to those of the unperturbed carbonate ion, indicating a symmetrical arrangement of carbonate in the crystal structure.

A significant perturbation of the carbonate IR absorption bands was observed in carbonate-containing aluminum hydroxide gel, reflecting the coordination of carbonate to aluminum in the amorphous structure. The splitting of the ν_3 vibration suggests a unidentate interaction with aluminum. Carbonate is perturbed to a greater degree in dihydroxyaluminum sodium carbonate, suggesting a bidentate coordination for the carbonate ion. This is in good agreement with the crystal structure of dawsonite.

IR evidence suggests that both carbonate and bicarbonate are interlayer anions in hydrotalcite.

Based on IR and X-ray analysis, magaldrate has a hydrotalcite-like structure with sulfate as the major interlayer anion and carbonate present in the interlayer space. The evidence does not support the present identification of magaldrate as a magnesium aluminum hydroxide.

The relationship between the carbonate-containing compounds used as antacids and natural minerals is also apparent based on this study. Amorphous carbonate-containing aluminum hydroxide gel can be classified mineralogically as amorphous aluminum hydroxycarbonate. The crystalline carbonate-containing antacids are somewhat poorly organized forms of the minerals calcite, $CaCO_3$; dawsonite, $NaAl(OH)_2CO_3$; and hydrotalcite, $Mg_6Al_2CO_3(OH)_{16}{\cdot}4H_2O$.

Based on these findings, it is suggested that the official definitions should recognize that: (*a*) carbonate is coordinated to aluminum in the aluminum hydroxide gel structure; (*b*) dihydroxyaluminum sodium carbonate is a synthetic form of the mineral dawsonite and the mineralogical formula $NaAl(OH)_2CO_3$ should be used rather than the present official formula, $CH_2AlNaO_5{\cdot}xH_2O$, since no molecular water is present and the official formula does not clearly indicate that the compound is a hydroxycarbonate; and (*c*) magaldrate has a hydrotalcite-like structure.

It is believed that an understanding of the structural relationship between carbonate-containing antacids will lead to improved methods for the production and control of the antacids and will provide a useful framework for the development of new antacids.

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Kinetics of Digoxin Stability in Aqueous Solution

LARRY A. STERNSON *x and RICHARD D. SHAFFER ‡

Received October 18, 1976, from the **Department of Pharmaceutical Chemistry, McCollum Laboratories, University of Kansas, Lawrence, KS 66044*, and the ‡*INTERx Research Corporation, Lawrence, KS 66044.* Accepted for publication June 13, 1977.

Abstract □ Digoxin hydrolysis was studied as a function of pH. Conversion of digoxin to digoxigenin was followed by high-pressure liquid chromatography and shown to proceed by the initial loss of one, two, or three sugars. The hydrolysis rate was directly proportional to parent drug concentration and hydrogen-ion activity. The individual hydrolysis rate constants of digoxin, digoxigenin bisdigitoxoside, and digoxigenin monodigitoxoside were determined by a simplex fitting procedure. Data are presented suggesting that at least some variation in the bioavailability of orally administered digoxin arises from observed variations in gastric pH; these variations influence the extent to which hydrolysis occurs and, thus, modify the composition of digoxin species available for absorption.

Keyphrases □ Digoxin—hydrolysis, kinetic study, effect of pH □ Hydrolysis—digoxin, kinetic study, effect of pH □ Kinetics—digoxin hydrolysis, effect of pH □ Stability—digoxin in aqueous solution, kinetic study of hydrolysis, effect of pH □ Cardiotonic agents—digoxin, hydrolysis, kinetic study, effect of pH

Digoxin is a widely prescribed glycoside used in the maintenance therapy of cardiac patients. Significant variability has been observed in the efficiency with which digoxin is absorbed from the GI tract among patients given the drug orally. Moreover, significant variations in bioavailability were noted within individual patients (1, 2).

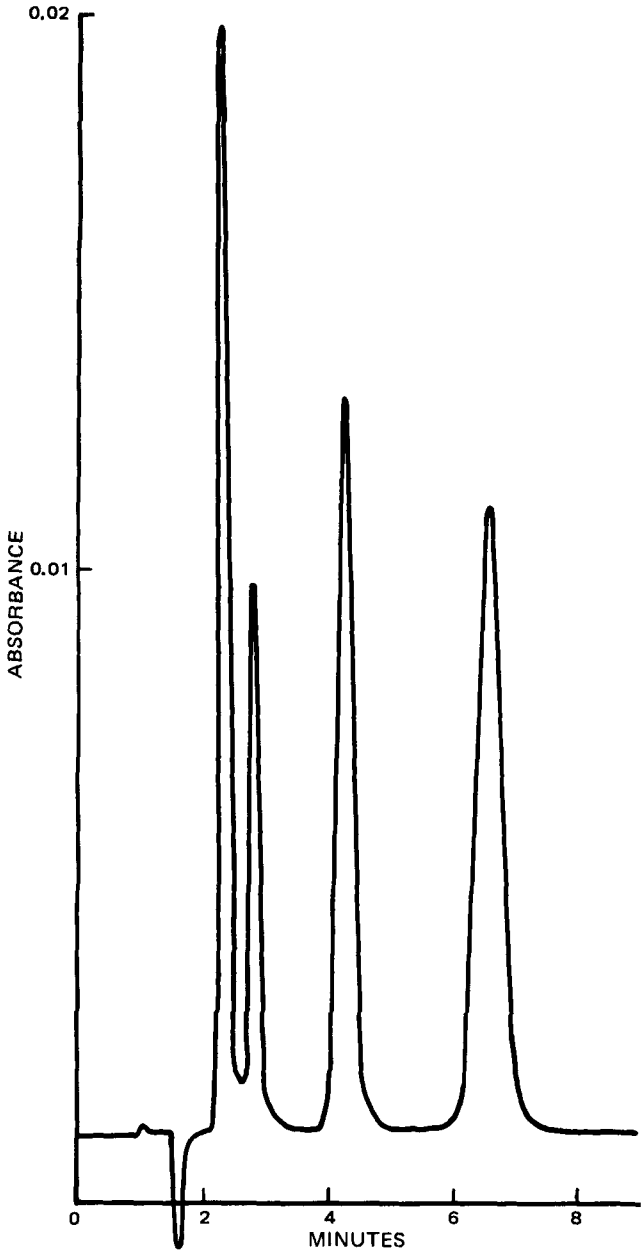


Figure 1—*Chromatogram of digoxigenin species separated by reversed-phase HPLC, obtained from an aqueous solution containing IV (t_R = 2.2 min; 12.2 μg/ml), III (t_R = 2.6 min; 10.2 μg/ml), II (t_R = 4.1 min; 30 μg/ml), and I (t_R = 6.4 min; 4.16 μg/ml).*

Potential sources for this variability have been postulated to involve dosage formulation and inconsistencies in administration (3–7). However, investigations of these parameters have failed to explain many observations associated with oral digoxin absorption.

The glycosidic nature of the digoxin molecule makes it susceptible to hydrolysis, especially in the acidic environment of the stomach. If hydrolysis is rapid enough to compete with absorption, fluctuations in bioavailability may reflect alterations in the amount and composition of the absorbed species. The hydrolysis products have been shown (8) to exhibit different pharmacological and toxicological activity than digoxin but are not distinguished from the parent drug using presently available clinical analytical methods.

Therefore, a study was initiated to determine if the

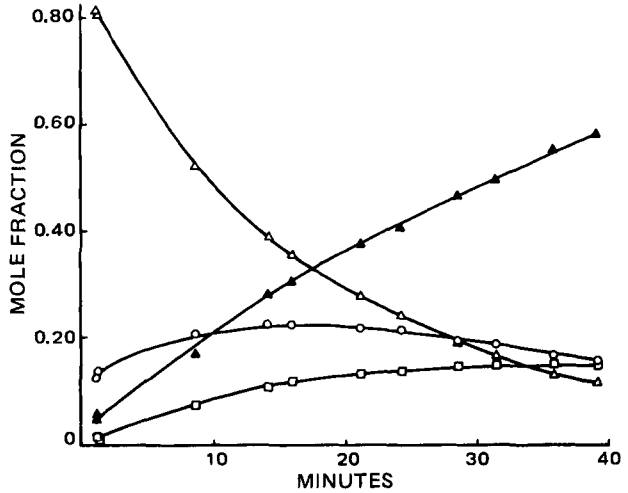


Figure 2—*Time-concentration profile of digoxigenin species [I (△), II (○), III (□), and IV (▲)] present in an aqueous solution of I at pH 1 incubated at 37°.*

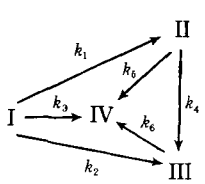
variability in digoxin absorption could arise from variations in the extent to which hydrolysis occurs among patients and, thus, competes with drug absorption. An analytical method was developed to quantitate digoxin and its three potential hydrolysis products simultaneously. This method was used to study the hydrolysis kinetics of digoxin species under acid conditions, similar to those found during oral absorption.

RESULTS AND DISCUSSION

Digoxin (digoxigenin trisdigitoxoside) hydrolysis was studied at 37° at pH 1, 2, 4, and 7. At pH 7, no appreciable hydrolysis was observed over 48 hr. In all other media, four-component mixtures were generated, corresponding to the cleavage of one, two, or three digitoxose residues. Separation and simultaneous quantitation of each of the four components in the hydrolysis mixtures were affected by reversed-phase high-pressure liquid chromatography (HPLC) (Fig. 1). Twenty-five nanograms of digoxigenin species could be determined by monitoring the eluent spectrophotometrically at the λ_{max} (225 nm) for digoxin species.

Digoxin (I) hydrolysis is a complex combination of parallel reactions that can proceed initially by three alternative pathways to yield digoxigenin (IV), which is unreactive, and the bisglycoside (II) and monoglycoside (III), which may undergo further hydrolysis (Scheme I). Figures 2 and 3 show the variation in the composition of digoxin hydrolysis mixtures as a function of time at pH 1 and 2, respectively. Each possible hydrolysis product is observed in the hydrolysate. In a series of parallel experiments, the hydrolysis of the reactive products, II and III was studied similarly.

As shown in Fig. 4, for reactions carried out at pH 1, substrate (I, II, or III) disappearance follows pseudo-first-order kinetics. The magnitude of the pseudo-first-order rate constants obtained from these graphs is shown in Table I and reflects the number of alternative pathways available for hydrolysis. These rate constants represent the sum of the individual rate constants describing the disappearance of parent species, and their magnitude increases as the number of available degradation pathways increases. Therefore, I, which can react by three alternative paths ($k_D = k_1 + k_2 + k_3 = 0.053\ min^{-1}$) has a shorter half-life (13.5 min) than II (16.5 min), which can be consumed *via* two pathways ($k_B = k_4 + k_5 = 0.040\ min^{-1}$); III is the most stable ($t_{1/2}$ = 24.3 min) species, since there is only one route available for its breakdown ($k_M = k_6$). Thus, differences in disappearance rates for I–III appear to reflect both probability factors



Scheme I

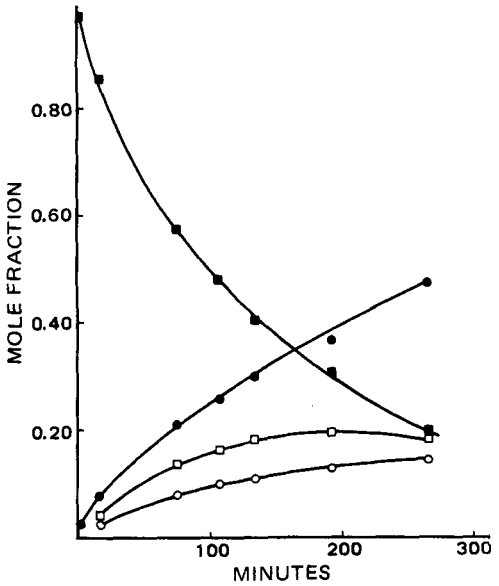


Figure 3—*Time-concentration profile of digoxigenin species [I (△), II (○), III (□), and IV (▲)] present in an aqueous solution of I at pH 2 incubated at 37°.*

as well as variations in stereochemical restrictions imposed at specific glycosidic linkages. Over the pH range studied (1–4), disappearance of substrate showed a first-order dependence on a_{H^+} (Fig. 4). At pH 2, k_D (0.0053 min^{-1}) is one-tenth the magnitude observed at pH 1.

The digoxin hydrolysis mixture was further analyzed to determine which reaction pathways (Scheme I) were operative and to get an indication of the significance of each route. In the experiments in which digoxin was the parent, its disappearance can be described as a pseudo-first-order process, expressed by:

$$-\frac{dD}{dt} = (k_1 + k_2 + k_3)D = k_D D \qquad \text{(Eq. 1)}$$

where k_D is the sum of k_1, k_2, and k_3. Rate expressions describing the appearance of bisglycoside (B), monoglycoside (M), and genin (G) take similar forms, expressed by:

$$\frac{dB}{dt} = k_1 D - (k_4 + k_5)B \qquad \text{(Eq. 2)}$$

$$\frac{dM}{dt} = k_2 D + k_4 B - k_6 M \qquad \text{(Eq. 3)}$$

$$\frac{dG}{dt} = k_3 D + k_5 B + k_6 M \qquad \text{(Eq. 4)}$$

These differential equations can be solved[1] and the amount of each component in the mixture can be expressed as the mole fraction of all digoxin species present:

$$D/D_0 = e^{-k_D t} \qquad \text{(Eq. 5)}$$

$$\frac{B}{D_0} = Ae^{-k_D t} + \left(\frac{B_0}{D_0} - A\right) e^{-\alpha t} \qquad \text{(Eq. 6)}$$

where $A = k_1/(\alpha - k_D)$ and $\alpha = k_4 + k_5$, and:

$$\frac{M}{D_0} = A\left\{Fe^{-k_D t} + \left(1 - \frac{B_0}{D_0 A}\right)Ce^{-\alpha t} - \left[F + \left(1 - \frac{B_0}{D_0 A}\right)C - \frac{M_0}{D_0 A}\right] e^{-k_6 t}\right\} \qquad \text{(Eq. 7)}$$

where $F = [k_4 + (k_2/A)]/(k_6 - k_D)$ and $C = k_4/(\alpha - k_6)$, and:

$$\frac{G}{D_0} = \frac{G_0}{D_0} + \frac{1}{k_D}(k_3 + k_5 A + k_6 AF)(1 - e^{-k_D t}) + \frac{1}{\alpha}\left[k_5\left(\frac{B_0}{D_0} - A\right) + k_6 A\left(1 - \frac{B_0}{D_0 A}\right)C\right](1 - e^{-\alpha t}) + \frac{1}{k_6}\left\{k_6 A\left[\frac{M_0}{D_0 A} - F - C\left(1 - \frac{B_0}{D_0 A}\right)\right]\right\}(1 - e^{-k_6 t}) \qquad \text{(Eq. 8)}$$

[1] Complete derivation of all equations is available from the authors on request.

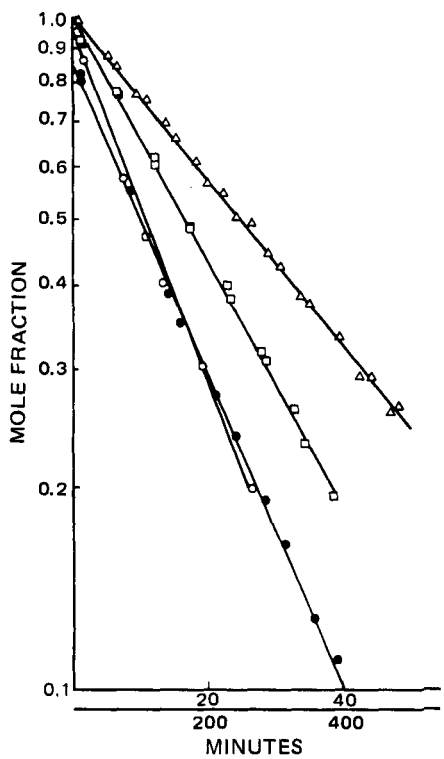


Figure 4—*Disappearance of digoxigenin species from acid solution as a function of time. In the pH 1 (0–60-min time scale) solution, the parent compound was III (▲), II (□), or I (○). In the pH 2 (0–600-min time scale) solution, the parent compound was I (●).*

Initial conditions were defined such that at $t = 0$, the amount of component J present in the mixture was J_0.

Imposing these boundary conditions provides general expressions in which the concentration of digoxin species can be determined in samples initially containing more than one digoxigenin component. Such manipulations were necessary because samples contained small amounts of impurities formed from prior hydrolysis of the parent compound.

From the HPLC tracings obtained during hydrolysis experiments, the amount of each component in the mixture could be expressed as the mole fraction of all digoxin species present as a function of time. Expressing the composition in this form eliminates error due to variations in the HPLC injection volume. When digoxin was the parent compound, these data were fit simultaneously to Eqs. 5–8 by an adaptive simplex procedure similar to that proposed by Nelder and Mead (9).

The quality of the fit at each iteration was determined by using Eqs. 5–8 to predict concentrations for each component at each time point, calculating the difference between these theoretical concentrations and the experimentally determined levels, and then summing the squares of all differences. In this way, all six rate constants were evaluated in one fitting procedure (Table I). The accuracy of this procedure is demonstrated by comparing the pseudo-first-order rate constant (k_D), determined experimentally for digoxin disappearance, with the sum of the individual rate constants (k_1, k_2, and k_3) it represents, determined by computer fit (Table I).

Table I—Rate Constants for Digoxin Hydrolysis at pH 1 [a]

Constant	Time, min^{-1}
k_1	0.0263
k_2	0.0089
k_3	0.0193
k_4	0.0207
k_5	0.0211
k_6	0.0301
k_M [b]	0.029
$(k_4 + k_5)$ [c]	0.042
k_B [b]	0.040
$(k_1 + k_2 + k_3)$ [d]	0.0545
k_D [b]	0.053

[a] Determined by the simplex fitting procedure as described in the text. [b] Determined experimentally from pseudo-first-order plots (Fig. 4). [c] Sum equivalent to k_B. [d] Sum equivalent to k_D.

Table II—Rate Constants for Hydrolysis of II and III at pH 1

Constant	Time, min^{-1}
k_4[a]	0.0232
k_5[a]	0.0165
k_6[a]	0.0368
$k_4 + k_5$[a,b]	0.040
k_B[c]	0.040
k_M[c]	0.029
k_6[d]	0.0290

[a] Determined from the simplex fit of data obtained for II hydrolysis. [b] Sum equivalent to k_B. [c] Determined experimentally from pseudo-first-order plots (Fig. 4). [d] Determined from the simplex fit of data obtained for III hydrolysis.

In experiments where II served as a substrate, kinetics can be described by the differential Eqs. 9–11 which are integrated to give Eqs. 12–14:

$$-\frac{dB}{dt} = (k_4 + k_5)B \quad \text{(Eq. 9)}$$

$$\frac{dM}{dt} = k_4B - k_6M \quad \text{(Eq. 10)}$$

$$\frac{dG}{dt} = k_5B + k_6M \quad \text{(Eq. 11)}$$

$$\frac{B}{B_0} = e^{-\alpha t} \quad \text{(Eq. 12)}$$

$$\frac{M}{B_0} = -Ce^{-\alpha t} + \left(\frac{M_0}{B_0} + C\right)e^{-k_6t} \quad \text{(Eq. 13)}$$

$$\frac{G}{B_0} = \frac{1}{\alpha}(k_5 - k_6C)(1 - e^{-\alpha t}) + \frac{1}{k_6}\left[k_6\left(\frac{M_0}{B_0} + C\right)\right](1 - e^{-k_6t}) + \frac{G_0}{B_0} \quad \text{(Eq. 14)}$$

Similar treatment for III hydrolysis yields:

$$-\frac{dM}{dt} = k_6M = \frac{dG}{dt} \quad \text{(Eq. 15)}$$

$$\frac{M}{M_0} = e^{-k_6t} \quad \text{(Eq. 16)}$$

$$\frac{G}{M_0} = \left(\frac{1}{M_0} - e^{-k_6t}\right) \quad \text{(Eq. 17)}$$

Simplex fit of the data obtained from II and III hydrolysis to Eqs. 12–14 and 16 and 17, respectively, gave the results shown in Table II. The goodness of fit is shown by comparison of k_B and k_M, determined experimentally, with the individual rate constants [$(k_4 + k_5)$ and k_6, respectively] they represent.

These results suggest that all possible hydrolysis routes are operative and approximately equally weighted, except for the direct cleavage of two digitoxose residues from digoxin (k_2) to form III, which occurs to a lesser extent. This observation may reflect stereochemical restrictions imposed at the glycosidic linkage or could be an artifact of the computer-fitting procedure.

The results presented also suggest that the variation in digoxin bioavailability after oral administration may be explained by the pH dependence of its hydrolysis. The digoxin half-life decreased from 131 min at pH 2 to 13.5 min at pH 1. Thus, the composition and amount of digoxin species available for absorption from the stomach should vary significantly with gastric acidity. The stomach pH varies widely among individuals (pH 0.5–4) and also within a patient as influenced by emotional and physical stresses, diet, and other factors (10). Thus, the variability in oral digoxin absorption may arise from variations in gastric pH, which influence the extent to which hydrolysis occurs and effectively competes with absorption of the active drug.

EXPERIMENTAL

Kinetic Experiments—The parent compound was dissolved in aqueous solution of appropriate pH and adjusted to an ionic strength of 1.0 with solid potassium chloride. The final concentration of substrate in all solutions was approximately 10 γ/ml. Ten milliliters of solution was incubated at 37°. At timed intervals, 25-μl samples were withdrawn[2] and subjected directly to HPLC analysis.

HPLC Analysis—Chromatography was performed on a component system[3]; the eluent was monitored spectrophotometrically at 225 nm. The separation utilized a column[4] (4 mm × 30 cm) operating at 2.0 ml/min with methanol-water (55:45) as the mobile phase. The retention volumes for IV, III, II, and I were 4.4, 4.2, 8.2, and 12.8 ml, respectively. The practical detection limit for digoxin was 25 ng from 25-λ injections. Components were quantitated by measuring peak heights and comparing the height with an external standard containing a mixture of known amounts of the four components.

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[2] Hamilton syringe.

[3] The chromatographic system consisted of a Waters M6000A solvent delivery system and a U6K universal injector coupled with a Varian Vari-Chrom detector.

[4] μBondapak C_{18}, Waters Associates, Inc.