Acetaminophen Prodrugs III: Hydrolysis of Carbonate and Carboxylic Acid Esters in Aqueous Buffers

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Abstract □ The hydrolysis rates of four carbonate and five carboxylic acid ester prodrugs of acetaminophen were determined in aqueous buffers at various pH's. The hydrolysis reactions of all the compounds except the hemisuccinate were first order in ester and in hydroxyl ion over the relatively alkaline pH ranges studied. The apparent enthalpies of activation were between 18 and 23 kcal./mole. The results suggest that it should be possible to formulate pharmaceutically stable suspensions of this type of acetaminophen prodrugs.

Keyphrases □ Acetaminophen prodrugs—hydrolysis □ Carbonate, carboxylic acid esters of prodrugs—hydrolysis, aqueous buffers □ Hydrolysis rates—acetaminophen prodrug esters □ UV spectrophotometry—analysis

The synthesis, physicochemical properties, and analgesic activities in rats of several prodrug esters of acetaminophen have been previously reported (1–3). These studies showed that significant differences in analgesic potency and duration of action might be expected following the oral administration of carbonate and carboxylic acid esters of various structures. The expected differences were attributable primarily to differences in the rates of dissolution and absorption of the esters following oral administration. In the case of 4-acetamidophenyl 2,2,2-trichloroethyl carbonate, the peak heights and rates of decline of blood levels of acetaminophen in humans depended upon the particle size of the administered powder (4).

Thus, the dose–time–action profiles of drugs that can be converted into carbonate or carboxylic acid esters might be modified at will by: (*a*) selecting appropriate ester structures, and (*b*) controlling the prodrug's particle size in pharmaceutical formulations. The selection of ester structures on the basis of hydrolysis catalyzed by various body enzymes has been discussed (3), but relatively little information on the chemical stability of drugs of this type has been reported. Therefore, the base-catalyzed hydrolysis of several acetaminophen prodrugs was studied to gain information on their relative hydrolytic stability, which would be useful in the formulation of pharmaceutical dosage forms, and to further elucidate their hydrolysis by esterolytic enzymes.

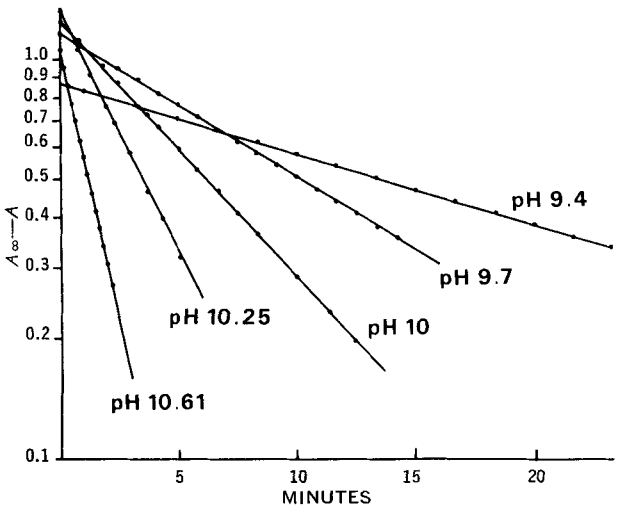


Figure 1—*Plots showing the pseudo-first-order nature of the hydrolysis of 4-acetamidophenyl 2,2,2-trichloroethyl carbonate (II) over the pH range 9.40–10.61 at 25°. The half-lives are shown in Table I.*

EXPERIMENTAL

Materials—The synthesis and physical properties of the prodrug esters have been described (2). All other chemicals were reagent grade. A Leeds & Northrup model 7401 pH meter and a Cary model 15 spectrophotometer were used.

Buffer Solutions—*Carbonate (pH 10.61, 10.25, 10.0, 9.7, and 9.4)*—These buffers were 0.1 *M* with respect to carbonate ion and were adjusted to ionic strength 0.5 with KCl. The buffers were prepared by dissolving sodium bicarbonate and potassium chloride in distilled water and by adjusting the pH by the dropwise addition of sodium hydroxide.

Phosphate (pH 7.40 and 6.81) and Succinate (pH 5.85 and 5.4)—The method of preparation was similar to that described for the carbonate buffer.

Procedure for Hydrolysis Studies—The hydrolysis rates of the acetaminophen prodrugs in suitable buffers at constant ionic

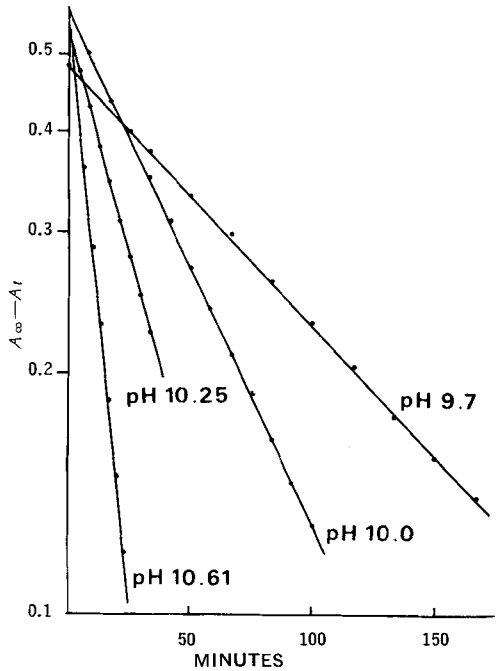


Figure 2—*Plots showing the pseudo-first-order nature of the hydrolysis of 4-acetamidophenyl butyrate (VI) over the pH range 9.70–10.61 at 25°. The half-lives are shown in Table II.*

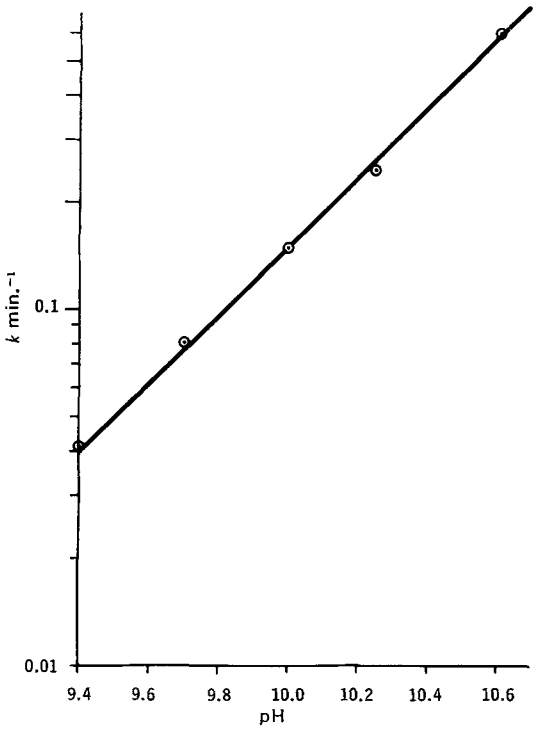


Figure 3—*Plot of pseudo-first-order rate constants* versus *pH for 4-acetamidophenyl 2,2,2-trichloroethyl carbonate (II) over the pH range 9.4–10.61 at 25°.*

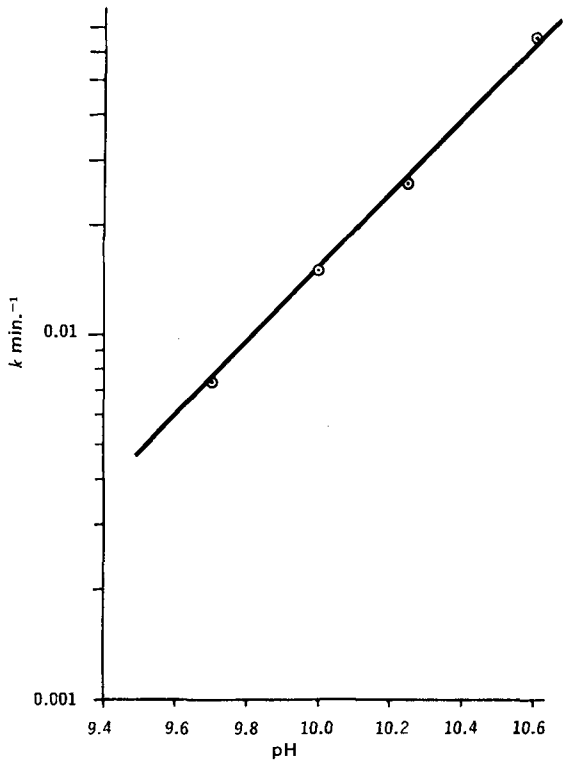
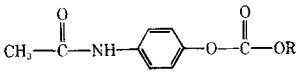


Figure 4—*Plot of pseudo-first-order rate constants* versus *pH for 4-acetamidophenyl butyrate (VI) over the pH range 9.7–10.61 at 25°.*

strength were determined spectrophotometrically by direct UV analysis in the thermostated cell compartment. Fifty milliliters of buffer solution was equilibrated at the approximate temperature in a 50-ml. mixing cylinder. One-half milliliter of anhydrous methanol, containing approximately 0.25 mg. of prodrug, was pipeted into the cylinder, which was then shaken thoroughly. A portion of this mixture was transferred to a 10-cm. sample cell of the spectrophotometer, and the absorbance at 300 mμ (the absorbance maximum of acetaminophen) was followed until no further change in absorbance could be observed (3). All hydrolysis reactions followed pseudo-first-order kinetics, and plots of log $(A_\infty - A_t)$ *versus* time were used to determine the first-order rate constants.

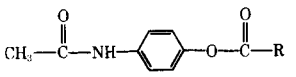
Table I—Hydrolysis Data for Carbonate Ester Prodrugs of Acetaminophen, 25°



R	pH	Buffer System[a]	$t_{1/2}$, min.	$k_{obs.}$ min.$^{-1}$	k_{OH} l. mole^{-1} min.$^{-1}$[b]
I					
$-CH_2-CH_3$	10.61	Carbonate	28	0.025	61
	10.25	Carbonate	56	0.012	68
	10.00	Carbonate	103	0.0067	67
	9.70	Carbonate	183	0.0038	76
					Av. 68
II					
$-CH_2-C-Cl_3$	10.61	Carbonate	1.1	0.62	1.5×10^3
	10.25	Carbonate	2.7	0.25	1.4×10^3
	10.00	Carbonate	4.6	0.15	1.5×10^3
	9.70	Carbonate	8.3	0.082	1.6×10^3
	9.40	Carbonate	17	0.041	1.6×10^3
					Av. 1.5×10^3
III					
$-CH_2-CH(CH_3)_2$	10.59	Carbonate	26	0.027	69
	10.31	Carbonate	48	0.015	74
	9.98	Carbonate	108	0.0064	67
	9.76	Carbonate	168	0.0041	71
					Av. 70
IV					
$-(CH_2)_2-N(CH_3)_2 \cdot HCl$	7.40	Phosphate	1.7	0.40	1.6×10^6
	6.81	Phosphate	5.8	0.12	1.9×10^6
	5.85	Succinate	55	0.013	1.8×10^6
					Av. 1.7×10^6

[a] Buffers were 0.1 *M* and adjusted to ionic strength 0.5 with KCl. [b] Calculated fom $k_{obs.}/[OH^-]$, where $[OH^-] = 10^-(pk_w - pH)$ and the pk_w = 13.9965 at 25°.

Table II—Hydrolysis Data for Carboxylic Acid Ester Prodrugs of Acetaminophen, 25°



R	pH	Buffer System[a]	$t_{1/2}$, min.	$k_{obs.}$ min.$^{-1}$	k_{OH} l.mole^{-1} min.$^{-1}$[b]
V					
$-CH_3$	10.59	Carbonate	6	0.12	320
	10.31	Carbonate	12	0.059	300
	9.98	Carbonate	26	0.026	290
	9.76	Carbonate	42	0.016	320
					Av. 310
VI					
$-(CH_2)_2CH_3$	10.61	Carbonate	11	0.066	170
	10.25	Carbonate	27	0.026	150
	10.00	Carbonate	47	0.015	150
	9.70	Carbonate	93	0.0074	150
					Av. 155
VII					
	10.61	Carbonate	17	0.041	100
	10.31	Carbonate	35	0.020	100
	9.98	Carbonate	78	0.0089	100
	9.76	Carbonate	130	0.0053	110
					Av. 103
VIII					
$-C(CH_3)_3$	10.59	Carbonate	72	0.0097	25
	10.31	Carbonate	148	0.0047	24
	9.98	Carbonate	380	0.0018	20
					Av. 23

[a] Buffers were 0.1 *M* and adjusted to ionic strength 0.5 with KCl. [b] Calculated from $k_{obs.}/[OH^-]$, where $[OH^-] = 10^{-(pk_w - pH)}$ and the pk_w = 13.9965 at 25°.

RESULTS AND DISCUSSION

Log ($A_\infty - A_t$) *versus* time plots for 4-acetamidophenyl 2,2,2-trichloroethyl carbonate (II) and 4-acetamidophenyl butyrate (VI) at various pH's are shown in Figs. 1 and 2, respectively. These figures are typical of the hydrolysis behavior of all the prodrug esters and show that the reactions followed pseudo-first-order kinetics at constant pH. Plots of log *k* (the pseudo-first-order rate constant) *versus* pH were straight lines with slopes essentially equal to 1, showing that the hydrolysis reactions were first order in hydroxyl ion as well as in ester (Figs. 3 and 4). The results of the hydrolysis studies at 25° for the carbonate ester prodrugs are summarized in Table I; those for the carboxylic acid ester prodrugs are summarized in Table II. Essentially the same half-lives as those shown in Tables I and II were obtained in buffers ranging from 0.03 to 0.5 *M* and in solutions of ionic strength ranging from 0.1 to 0.5. Thus, specific buffer catalysis and salt effects are apparently negligible for these hydrolytic reactions.

The results in Table I show that chlorine substitution in the aliphatic alcohol portion of the carbonate ester (II) markedly

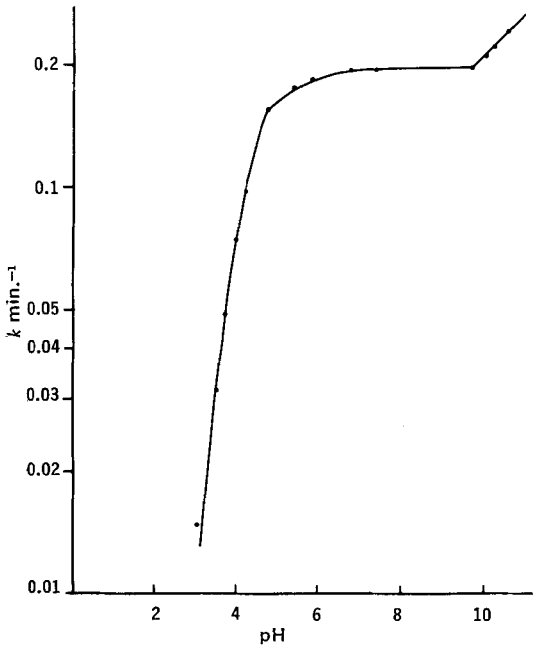


Figure 5—*Plot of pseudo-first-order rate constants* versus *pH for 4-acetamidophenyl hemisuccinate (IX) over the pH range 3.08–10.60 at 25°. The half-lives are shown in Table III.*

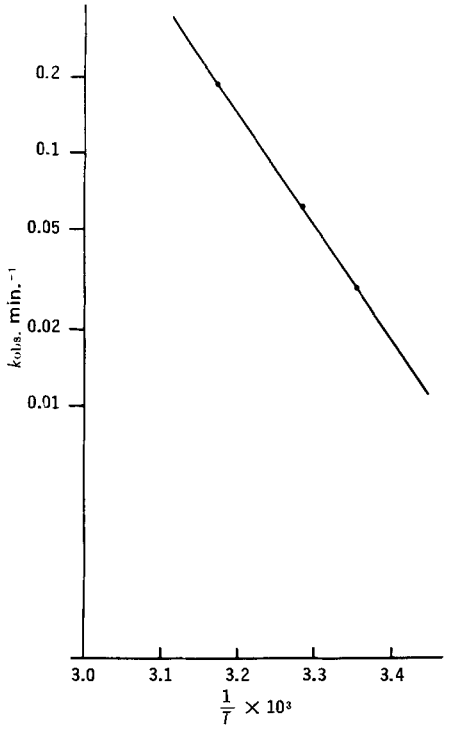
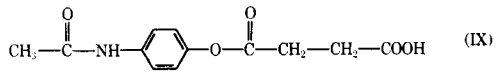


Figure 6—*Arrhenius plot for the hydrolysis of 4-acetamidophenyl ethyl carbonate (I) at pH 10.61 over the temperature range 25–42°. Apparent energy of activation* (ΔH) = *21 kcal./mole (Table IV).*

Table III—Hydrolysis Data for Hemisuccinate Ester of Acetaminophen, 25°



(IX)

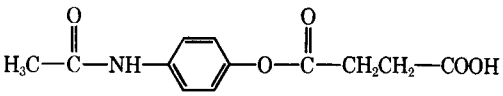
pH	Buffer System[a]	$t_{1/2}$, min.	$k_{obs.}$ min.$^{-1}$
3.08	Citrate	47	0.015
3.5	Citrate	22	0.032
3.7	Citrate	14	0.049
4.0	Acetate	9	0.074
4.25	Acetate	7	0.098
4.75	Acetate	4.5	0.16
5.4	Succinate	3.9	0.18
5.85	Succinate	3.8	0.18
6.81	Phosphate	3.6	0.19
7.41	Phosphate	3.6	0.19
9.72	Carbonate	3.5	0.19
10.05	Carbonate	3.3	0.21
10.27	Carbonate	3.1	0.22
10.60	Carbonate	2.9	0.24

[a] Buffers were 0.1 *M* and adjusted to constant ionic strength 0.5 with KCl.

increased the lability of the ester group to base-catalyzed hydrolysis. The effect was probably due to the electron-withdrawing properties of the chlorine atoms. Chain branching in this alcohol moiety (III) had relatively little influence on the rate of the hydrolysis reaction. Addition of a β-dimethylamino group to the alcohol moiety (IV) caused a dramatic increase in the lability of the ester to base attack. The hydrolysis of IV was first order in hydroxyl ion within the pH region studied (Table I) and can be assigned to an hydroxyl-ion attack on the protonated species.

The results in Table II show that the acetate ester (V) was more labile to base-catalyzed hydrolysis than the ethyl carbonate ester (I). Lengthening the aliphatic chain (VI) or substituting an aromatic hydrocarbon (VII) in the carboxylic acid moiety slightly decreased the lability of the ester group to hydrolysis. Branching of the aliphatic chain near the carboxyl group (VIII) significantly slowed the reaction.

The hydrolysis behavior of the hemisuccinate ester of acetaminophen (IX) was studied extensively because it represents a type of



IX

compound that might display a higher aqueous solubility, especially at higher pH's, than some other prodrug esters. The results shown in Table III and Fig. 5 are very similar to those reported by Gaetjens and Morawetz (5) for phenyl acid succinates. As might be expected, this compound was more stable at low pH's where the free carboxyl group is essentially completely unionized, but it is too labile to be formulated into a suitable pharmaceutical solution under any conditions.

A brief study of the temperature dependency of the hydrolysis reactions showed that, in all cases, the Arrhenius law was obeyed at the pH's studied. A typical Arrhenius plot for Compound I is shown in Fig. 6, and the results for all compounds are summarized in Table IV. The apparent energies of activation are consistent with values previously reported for base-catalyzed ester hydrolyses (5). The conclusion might be drawn from this brief study that stable pharmaceutical suspensions might be formulated from aliphatic carbonate and aliphatic and aromatic carboxylic acid prodrug esters of acetaminophen. A rough extrapolation from pH 10 to pH 5.5, the pH of maximum stability of the acetaminophen amide group

Table IV—Temperature Dependency of the Hydrolysis Reactions of Carbonate and Carboxylic Acid Prodrugs of Acetaminophen

R	pH	$k_{obs.}$ min.$^{-1}$	Temperature	ΔH (kcal./mole)
I	10.61	0.029	25°	21
		0.062	31.5°	
		0.19	42°	
II	9.35	0.044	25°	19
		0.091	31.5°	
		0.022	42°	
III	10.61	0.027	25°	23
		0.055	31°	
		0.024	43°	
IV	5.85	0.013	25°	22
		0.028	31°	
		0.11	43°	
V	10.00	0.026	25°	20
		0.051	31°	
		0.23	45°	
VI	10.61	0.063	25°	19
		0.12	31.5°	
		0.33	42°	
VII	10.25	0.019	25°	18
		0.033	31°	
		0.14	45°	
VIII	10.61	0.0097	25°	20
		0.018	31°	
		0.060	42°	

toward hydrolysis (6), yields t_{90} values of approximately 1, 1, 0.2, 0.4, 0.7, and 3.5 years for Compounds I, III, V, VI, VII, and VIII, respectively. These relatively high t_{90} values for the compounds *in solution*, coupled with their relatively low aqueous solubilities (2) and high doses, suggest that it might be possible to formulate pharmaceutical suspensions with some of the prodrug esters that would retain 90% of their potencies for at least 2 years at room temperature.

The more water-soluble prodrugs, *i.e.*, the *N,N*-dimethylethanolamine carbonate (IV) and the hemisuccinate (IX), are too labile to be formulated in liquid dosage forms under any conditions since they hydrolyze very rapidly at both slightly acidic and slightly basic pH's.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 16, 1970, from *Smith Kline and French Laboratories, Philadelphia, PA 19101* and the †*College of Pharmacy, University of Kentucky, Lexington, KY 40506*

Accepted for publication June 16, 1970.

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