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N-(Acyloxyalkoxycarbonyl) derivatives as potential prodrugs of amines. I. Kinetics and mechanism of degradation in aqueous solutions

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Summary

Four acetoxyethoxycarbonyl derivatives of closely related primary and secondary amines were synthesized as model prodrugs. The degradation kinetics of these compounds were studied in aqueous solutions as a function of pH and temperature to determine their stability and to assess their suitability as potential prodrugs of amines. At pH ≤ 4, the model prodrugs of primary amines degraded by specific acid-catalysed ester hydrolysis. At pH ≥ 5, the model prodrugs of primary amines degraded by parallel pathways involving hydroxide ion-catalyzed ester hydrolysis and intramolecular acyl transfer. The model prodrug derived from a secondary amine appeared to degrade by ester hydrolysis only, over the entire pH range studied ($1 \leq$ pH ≤ 9) and thus appeared to be a very promising prodrug form. The log rate constant vs pH profiles for all 4 compounds were very similar indicating that the hydrolytic rates were determined primarily by the nature of the common ester functional group. The amine substituents exhibited little influence on hydrolytic rates. Overall, the stability of these model prodrugs was adequate to allow them to be considered for pharmaceutical formulation either as solids or as solutions.

Introduction

Many drug substances contain primary and secondary amino groups as one of their most prominent functional groups. The presence of the amine function may give rise to certain problems in the formulation and the clinical effectiveness of the drug, e.g. inadequate chemical stability (Cohen et al., 1973; Higy et al., 1955), insufficient solubility (Mechlinski, 1972), unsatisfactory transport characteristics across biological membranes (Stella, 1975), etc. These stability, solubility and transport barriers to effective drug delivery might be overcome by preparing bioreversible derivatives or prodrugs. Except when sustained release of a drug is desired, the ideal bioreversible derivatives would be those that exhibit sufficient in vitro stability, yet provide very rapid and predictable in vivo release of the parent drug. This might be achieved if an enzymatic trigger mechanism is used for the

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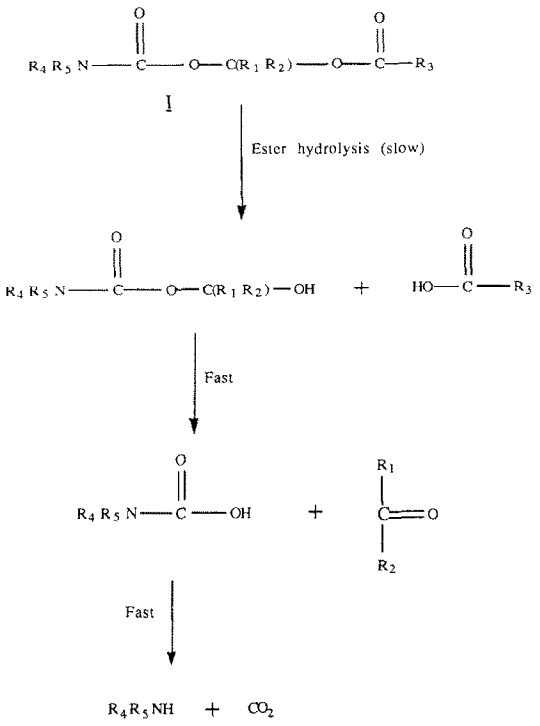


SCHEME 1 : Proposed Mechanism for the Release of the Parent Amine from the Acyloxyalkoxycarbamates.

release of the parent amine. Efforts to find prodrugs of amines which utilize enzymatic release mechanisms appear to have met with limited success (Gogate, 1987).

Modified carbamates with an enzymatically hydrolyzable ester function were suggested as prodrug candidates of amines by Alexander (1984). Specifically, the class of prodrug candidates suggested consisted of acyloxyalkoxycarbonyl derivatives of amines. The approach anticipates enzyme (esterase) catalyzed hydrolysis of the ester moiety to trigger the regeneration of the parent amine from such derivatives (I) as depicted in Scheme 1. The hydroxyalkoxycarbonylamine formed by the action of the esterases on I was expected to be very unstable and to release the corresponding carbamic acid derivative and the parent amine in quick succession.

No comprehensive studies dealing with stability have yet been undertaken for derivatives having structures such as I. Hence 4 acyloxyalkoxycarbonyl derivatives (II–V) of structurally similar primary and secondary amines were synthesized for investigation. The structural differences were carefully chosen to assess the effects of such differences on the prodrug stability. The names and chemical structures of these model prodrugs (II–V) are listed in Table 1 along with the names and structures of their parent amines. The degradation kinetics of the model prodrugs were studied in aqueous solutions as a function of pH and temperature to assess their stability and suitability as prodrugs of amines.

Materials and Methods

Materials and equipment

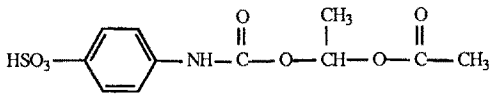
The reagents used in the various syntheses were of the highest purity commercially available. Silica gel (70-230 mesh from E. Merck) was used for the column chromatographic purification of the modified carbamates. Plastic-backed plates of silica gel 60 (F_{254}, 0.2 mm, from EM Reagents) were used for thin-layer chromatography (TLC) of compounds. The Chromatotron (model No-7924 from Harrison Research), a preparative, centrifugally accelerated, thin-layer chromatographic system (CA-TLC) was used for purification of some of the synthesized compounds. Silica gel (4 mm thickness)-coated glass discs (24 cm diameter) were used as stationary phase for purification of compounds by CA-TLC.

Water used for preparation of buffers was purified by first passing through mixed-bed ion exchange resin columns (Research System from Illinois Water Treatment Co.) and then by distillation from an all-glass apparatus. All the chemicals used for preparation of buffers were of analytical grade and used as received from commercially available sources.

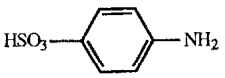
The HPLC system consisted of an Altex Model 110A pump, Altex fixed-volume injector (50 μl),

TABLE 1

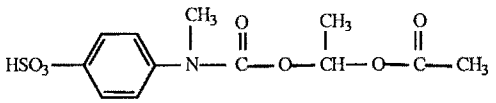
Structures of the 4 model prodrugs studied and their parent amines



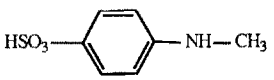
4-(N-(Acetoxyethoxycarbonyl)amino)benzenesulfonic acid (II)



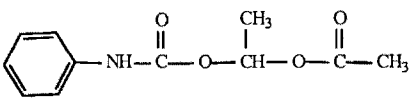
4-Aminobenzenesulfonic acid



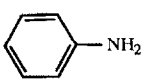
4-(N-(Acetoxyethoxycarbonyl)-N-methyl)benzenesulfonic acid (III)



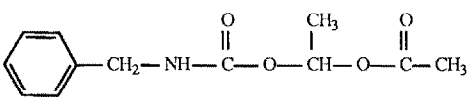
4-Methylaminobenzenesulfonic acid



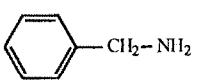
N-(Acetoxyethoxycarbonyl)aniline (IV)



Aniline



N-(Acetoxyethoxycarbonyl)benzylamine (V)



Benzylamine

and Beckman model 153 fixed wavelength detector. The detector response was recorded on a Fisher Recordall, series 5000, single pen variable speed recorder. ODS-Hypersil (Shandon Southern Instruments Inc.) columns (15 cm × 4.6 mm (i.d.)) were used with various mobile phases for HPLC analysis. The columns were slurry packed in-house, using a published procedure (Bristow et al., 1977). All mobile phases were filtered through 0.45 μm cellulose filters (Metricel GA-6).

A Corning Digital model 112 pH meter, equipped with a combination pH electrode (Cole-Parmer) was used for pH determination. Quantities > 5 mg were weighed using a Mettler Model H51AR balance, while those ≤ 5 mg using a Cahn Electrobalance (Model 21).

Methods

Synthesis

α-Chloroethyl chloroformate and *N*-acetylsulfanilic acid were synthesized according to the methods of Olofson et al. (1984) and Klotz and Melchior (1949), respectively.

The potassium salt of 4-(*N*-[acetoxyethoxycarbonyl]amino)benzenesulfonic acid (II) was synthesized as follows: sulfanilic acid (0.01 mol) and Proton Sponge (Aldrich Chemical Co., 0.011 mol) were placed in 1,4-dioxane (100 ml) which was previously dried by distilling from $LiAlH_4$ (Perrin et al., 1966). α-Chlorethyl chloroformate (0.01 mol) was added dropwise over a period of 10 min. After stirring for 2 h, the suspension was

filtered. The recovered solid was added to pentane-stabilized chloroform (100 ml). The unreacted sulfanilic acid present in the recovered solid remained undissolved and was separated by filtration. Chloroform was removed from the filtrate by evaporation under reduced pressure (0.25 mm Hg) at room temperature. The obtained solid was dissolved in glacial acetic acid (5 ml) and the solution was stirred for 2 h at room temperature. The solution was concentrated under reduced pressure (0.25 mm Hg) at 60 °C and the solid obtained was partitioned between chloroform (400 ml) and an aqueous solution (400 ml) of potassium iodide (2% w/v). The aqueous layer was recovered and the water removed at room temperature by evaporation under reduced pressure (0.25 mm Hg). The solid residue obtained was dissolved in methanol (5 ml) and fractionated by CA-TLC using an eluting solvent of chloroform: methanol (1 : 1). Fractions were collected and analysed by NMR spectroscopy for II. The fractions containing II were combined and the solvent evaporated at room temperature under reduced pressure (0.25 mm Hg). The product was a colorless solid which decomposed above 300 °C. The yield was ~ 18%. The elemental analysis was consistent with the composition of $C_{11}H_{12}NO_7KS$ and the 60 MHz NMR spectrum (in D_2O) was consistent with the structure of II. The characteristic NMR data were: 8.0–7.4 (4H, multiplet, aromatic), 6.9 (1H, quartet, CH), 2.0 (3H, singlet, CH_3CO), 1.5 (3H, doublet, CH_3CH).

The potassium salt of 4-[(*N*-acetoxyethoxycarbonyl-*N*-methyl)amino] benzenesulfonic acid (III) was synthesized by the above procedure from *N*-methylsulfanilic acid. The product obtained was a yellow solid which decomposed above 300 °C. The yield was ~ 17%. The elemental analysis was in agreement with the composition of $C_{12}H_{14}NO_7$ KS (III) as were the NMR data (in D_2O): 8.1–7.3 (4H, multiplet, aromatic), 6.9 (1H, quartet, CH), 3.4 (3H, singlet, CH_3N), 2.1 (3H, singlet, CH_3CO), 1.5 (3H, doublet, CH_3CH).

N-(Acetoxyethoxycarbonyl)aniline (IV) was synthesized as follows. Aniline (0.03 mol) and triethylamine (0.03 mol) were dissolved in 1,4-dioxane (20 ml) that was previously dried by distilling from $LiAlH_4$ (Perrin et al., 1966). To this solution α-chloroethyl chloroformate (0.03 mol) was added dropwise over a period of 10 min. The mixture was stirred for 3 h, during which period some aniline hydrochloride and triethylammonium hydrochloride separated as a white solid. The two hydrochloride salts were removed by filtration and the filtrate was dried under reduced pressure (0.25 mm Hg) at room temperature. Acetic acid (5 ml) and mercuric acetate (0.015 mol) were added to the solid obtained above. After stirring overnight, the mixture was filtered and the filtrate was dried at 60 °C under reduced pressure (0.25 mm Hg). The solid obtained was dissolved in chloroform (5 ml) and the solution was then applied to a silica gel (50 g) column (70 cm × 1.5 cm (i.d.)) and eluted with a mixture of chloroform and ethyl acetate (4 : 1). Fractions were analysed by NMR spectroscopy for compound IV. Fractions containing IV were combined and the solvent removed at room temperature under reduced pressure (0.25 mm Hg) to give IV as a yellowish powder. Sublimation of that powder under reduced pressure (0.5 mm Hg) at 40 °C gave white crystals. The yield was ~ 24%; m.p. 62 °C. The solid obtained was characterized as IV ($C_{11}H_{13}NO_4$) by elemental analysis and NMR data (in $CDCl_3$): 7.6–7.0 (6H, multiplet, aromatic and NH), 7.1–6.8 (1H, quartet, CH), 2.0 (3H, singlet, CH_3CO), 1.5 (3H, doublet, CH_3CH).

N-(Acetoxyethoxycarbonyl)benzylamine (V) was synthesized by the procedure used for the synthesis of IV with the exception that benzylamine was used instead of aniline. The product was obtained as a yellow oily liquid at a yield of ~ 56%. The oily liquid was characterized as V by elemental analysis and by NMR data (in D_2O): 7.5–7.2 (5H, multiplet, aromatic), 6.9 (1H, quartet, CH), 6.5–6.2 (1H, multiplet, NH), 4.4 (2H, doublet, CH_2), 2.0 (3H, singlet, CH_3CO), 1.5 (3H, doublet, CH_3CH).

Kinetic studies

The kinetics of degradation of the model prodrugs and of *N*-acetylsulfanilic acid were studied in aqueous buffer solutions at 37.0 ± 0.1 °C. Hydrochloric acid, acetate, phosphate, and borate solutions were used as buffers. A constant ionic strength was maintained at 0.5 M for each of the

TABLE 2

Mobile phases used in HPLC analysis of various compounds

Mobile phase	Compounds analysed (retention volumes in ml)
CH_3CN : acetate buffer (0.1 M, pH 4.6) (55 : 100) (0.05% THABr [a])	II (5.6), III (4.6) [b]
CH_3CN : phosphate buffer (0.1 M, pH 6.3) (55 : 100) (0.05% THABr)	II (5.6), III (4.6) [c]
CH_3CN : acetate buffer (0.1 M, pH 4.6) (40 : 100) (0.05% THABr)	sulfanilic acid (4.5), *N*-acetylsulfanilic acid (5.5)
40% CH_3CN : water [d]	IV (7.5), V (7.5)
35% CH_3CN : water [d]	benzylamine (4.1), *N*-acetylbenzylamine (3.0)
10% CH_3CN : water [d]	aniline (10.0), acetanilide (13.0)

[a] THABr, tetrahexyl ammonium bromide
[b] When pH of the buffer solution ≤ 5.4.
[c] When pH of the buffer solution > 5.4.
[d] Organic solvent brought to volume with water.

buffers by adding a calculated amount of sodium chloride. The kinetics of degradation of compound II were studied at four different buffer concentrations for each pH value (except pH 1.0, where only HCl was used to control pH), whereas kinetics of degradation of compounds III, IV, and V were studied only at one buffer concentration (0.1 M).

The kinetics of degradation were studied by analyzing the solutions with high-performance liquid chromatography (HPLC) using ODS Hypersil columns. The various mobile phases used in the analysis of designated compounds are listed in Table 2 along with their retention volumes. A mobile phase flow rate of 1 ml/min was used and the column effluent was monitored at 254 nm. The quantitation of the compounds was achieved by measuring the peak heights in relation to those of standard solutions chromatographed under the same conditions.

A common procedure was used for studying the kinetics of degradation of compounds II, III, and *N*-acetylsulfanilic acid. In a typical degradation experiment, an accurately weighed sample was placed in a 10 ml volumetric flask. A sufficient quantity of buffer solution at 37 °C was added to bring the volume to 10 ml. The initial concentration of the compound under study was 10–30 μM. The buffer solutions were then placed in a thermostated waterbath maintained at 37 °C. The loss of the sulfonic acid derivative was followed by withdrawing samples from the reaction mixture at various time points and analyzing by HPLC. The apparent first order rate constants for degradation were obtained by linear regression analysis ($r > 0.97$ for all systems) of natural log of concentration vs time.

The procedure used for studying the kinetics of degradation of compounds IV and V was similar to the one described above with the exception that weighed samples were dissolved in 0.5 ml acetonitrile immediately prior to the addition of buffer solutions.

Product analysis

Following the completion of the hydrolysis reactions, aqueous buffer solutions were analysed for the amounts of the various degradation products generated. Analytical procedures used were identical to those described above.

Results and Discussion

Stability of compounds II–V in aqueous buffer solutions

Compounds II–V exhibited pseudo-first-order kinetics in the aqueous media at the temperatures and pH values studied. The kinetics of degradation of compound II were studied at 4 different buffer concentrations for each pH value (except pH 1.0) and the rate constants for zero buffer concentration were obtained by extrapolation of plots of k_{obs} vs buffer concentration. The values of the rate constants for degradation of II at zero buffer concentration (i.e. $k_{0,II}$) and those of the catalytic rate constants (k_{cat}) are reported in Table 3 as a function of pH. The effect of buffer concentration on rates of degradation of II appeared to be insignificant, as was evident from relatively low values of k_{cat} (see Table 3). Accordingly, only one buffer concentration (0.1 M) was

TABLE 3

The rate constants at various pH values for degradation of II at zero buffer concentration (k_0) and catalytic rate constants (k_{cat})

pH (buffer species)	$k_{0,II}$ [a] (h^{-1})	k_{cat} [b] ($h^{-1}\ M^{-1}$) ± S.D.
1.0 (HCl)	6.25×10^{-2}	–
2.0 (Phosphate)	6.12×10^{-3}	$(9.0\ \pm0.4)\times10^{-3}$
2.6 (Tartrate)	1.94×10^{-3}	$(3.5\ \pm2.0)\times10^{-4}$
3.2 (Tartrate)	4.68×10^{-4}	$(8.6\ \pm2.0)\times10^{-4}$
4.6 (Acetate)	3.24×10^{-4}	$(5.4\ \pm0.3)\times10^{-4}$
5.4 (Acetate)	1.51×10^{-3}	$(1.9\ \pm0.3)\times10^{-3}$
6.0 (Phosphate)	3.44×10^{-3}	$(8.8\ \pm1.2)\times10^{-3}$
6.7 (Phosphate)	1.57×10^{-2}	$(2.4\ \pm0.5)\times10^{-2}$
7.5 (Phosphate)	9.06×10^{-2}	$(7.0\ \pm3.3)\times10^{-2}$
9.0 (Borate)	2.94	0.78 ± 0.14

[a] Obtained as intercept from linear regression of k_{obs} vs [buffer].
[b] Obtained as slope from linear regression of k_{obs} vs [buffer].

used while studying kinetics of degradation of compounds III–V. The values of observed rate constants for the degradation (k_{obs}) of compounds III–V are reported in Table 4 as a function of pH. Fig. 1 shows the plot of log k_0 vs pH for compound II and the plots of log (k_{obs}) vs pH for compounds III–V in aqueous buffer solutions (0.1 M) at 37 °C.

The log rate constant–pH profiles for degradation of compounds II–V (Fig. 1) were V-shaped, as is also observed with many esters (Siegel et al., 1959; Patel et al., 1968), and appear to be composed of two distinct portions; one at pH ≤ 4 with slope = −1 and the other at pH ≥ 5 with slope = +1.

TABLE 4

The rate constants for degradation of the compounds III, IV, and V as a function of pH at 37 °C

pH	k_{obs} (h^{-1}) ± S.D.		
	III	IV	V
1.0	$(7.2\ \pm0.3)\times10^{-2}$	$(7.2\ \pm0.2)\times10^{-2}$	$(6.7\ \pm0.1)\times10^{-2}$
2.0	$(6.6\ \pm0.4)\times10^{-3}$	n.d.	$(6.5\ \pm0.3)\times10^{-3}$
3.2	n.d.	$(4.3\ \pm0.3)\times10^{-4}$	n.d.
5.4	n.d.	$(2.7\ \pm0.1)\times10^{-3}$	$(3.0\ \pm0.1)\times10^{-3}$
6.7	$(1.5\ \pm0.1)\times10^{3}$	$(4.5\ \pm0.2)\times10^{-2}$	$(5.3\ \pm0.4)\times10^{-2}$
9.0	0.30 ± 0.01	7.91 ± 0.12	7.71 ± 0.30

n.d., not determined.

Degradation of compounds II–V at pH ≤ 4

It was anticipated that the products of degradation of II–V which contained a benzene nucleus would be detected by the UV detector (λ = 254 nm) that monitored the HPLC effluent. In the descending portions of the log (rate constant)–pH profiles, only one degradation product containing a benzene nucleus was observed for each of the compounds II–V and it was identified as the parent amine in each case, e.g. sulfanilic acid for compound II (Table 1). The identification of the degradation products was based on observing identical HPLC retention times for the products of degradation and the authentic samples of the parent amines. Additionally, stopped-flow UV spectra of the peaks on HPLC system for sulfanilic acid and for the degradation product of compound II (at pHs 1.0, 2.6 and 3.2) were found to be identical, thus confirming the identity of the degradation product of compound II in the descending portion of log rate-pH profile. The formation of the respective parent amines from compounds II–V can be accounted for by a reaction sequence similar to that represented in Scheme 1, where the rate-determining ester hydrolysis step is catalyzed by hydrogen ions.

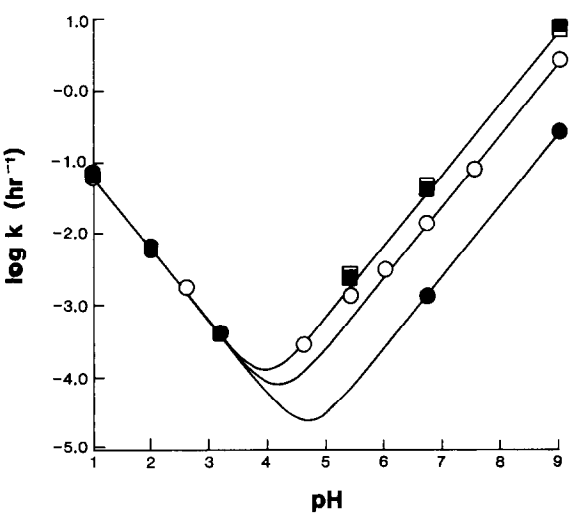


Fig. 1. Log rate constant–pH profiles for degradation (at 37 °C) of compounds II–V. Log k_0 is plotted for compound II (○), whereas log k_{obs} (buffer concentration 0.1 M) is plotted for compounds III (●), IV (□), and V (■).

Aqueous solutions of II in pH 1.0 buffer were analysed for sulfanilic acid following the completion of the hydrolysis reaction. Sulfanilic acid accounted for $100.0 \pm 0.5\%$ of the initial concentration of II in pH 1.0 buffer. Also, the rate constant for formation of sulfanilic acid from compound II at pH 1.0 (6.09×10^{-2} h^{-1}) was found to be nearly identical with that obtained for the degradation of compound II under similar conditions (6.25×10^{-2} h^{-1}, see Table 3). These data indicate that the reactions involving elimination of acetaldehyde and carbon dioxide (second and third steps in Scheme 1) occurred rapidly following the slower rate-determining ester hydrolysis.

The descending portions of log rate–pH profiles for compounds II–V were essentially identical to one another. Since the ester portion of compounds is identical and since the differing amine portion is distant from the reactive ester portion, it is reasonable that the rate constants for degradation due to ester hydrolysis would be very similar for these compounds.

The log rate–pH profiles for compounds II–V at pH ≤ 4 can be represented by Eqn. 1(for zero buffer concentration).

$$\text{Rate} = k_H[H^+][C] \quad (1)$$

where $k_H (= 0.62\ h^{-1})$ is the hydrogen ion-catalysed rate constant, $[H^+]$ is the hydrogen ion concentration and [C] is the molar concentration of the particular compound (II–V) under study.

Degradation of compounds II–V at pH ≥ 5

For compounds II, IV and V, two detectable degradation products containing the benzene nucleus* were observed at pH ≥ 5, whereas only one such detectable degradation product was detected for III. The respective parent amine and the *N*-acetylated amine were observed as degradation products for compounds II, IV and V, while only the parent amine (*N*-methylsulfanilic acid) was detected as the degradation product of III at pH ≥ 5. The identification of the degradation products was based on identical HPLC retention times and UV spectra (obtained by stopped-flow spectrophotometry of the HPLC peaks) of the products and of the authentic samples of parent amines and *N*-acetylated parent amines.

Following the completion of hydrolysis reactions, aqueous buffer solutions of II–V were analysed for the amounts of various degradation products generated. In Table 5 are listed the percent products generated by the degradation in phosphate buffer solutions of compounds II–V at pH 7.4 and of compound II at pH 6.7. From the results it appeared that the relative amounts of the products formed from degradation of II did not change with pH in the ascending portion of the log (rate constant)–pH profile.

Hydroxide ion-catalysed ester hydrolysis could explain the formation of the parent amines from compounds II–V and would also be consistent with the slope of +1 observed for the log rate constant vs pH profiles at pH ≥ 5. The proposed reaction scheme for hydroxide ion-catalysed ester hydrolysis is in accord with Scheme 1 where the first step of ester hydrolysis involves hydroxide ion catalysis.

Formation of the *N*-acetylated parent amines from compounds II, IV and V can be attributed to ionization of the carbamate group followed by an O → N intramolecular acyl transfer reaction as shown in Scheme 2. Such a reaction involves intramolecular nucleophilic attack on the carbonyl carbon by the negatively charged carbamate nitrogen through formation of a 6-membered transition state.

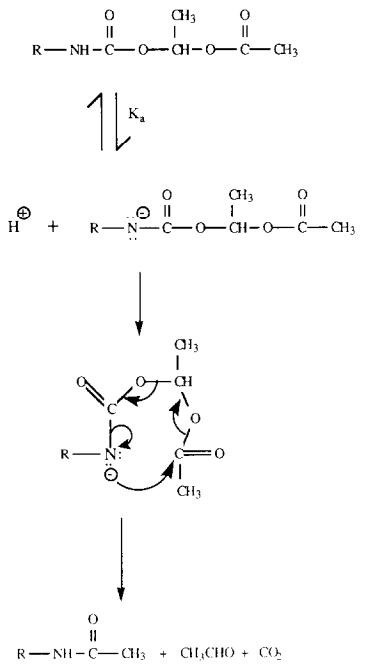
Using linear free-energy relationships, the ionization constants of the carbamate groups in

TABLE 5

Percent products formed from compounds II–V in phosphate buffers at 37 °C

Compound	pH	% Amine formed	% *N*-Acetylated amine formed
II	7.4	10.0 ± 1.9	90.0 ± 2.0
	6.7	10.4 ± 2.0	89.6 ± 2.1
III	7.4	100.0 ± 1.8	0
IV	7.4	2.5 ± 0.2	97.5 ± 0.4
V	7.4	2.2 ± 0.3	97.8 ± 0.5

* Based on UV absorption at $\lambda = 254$ nm.

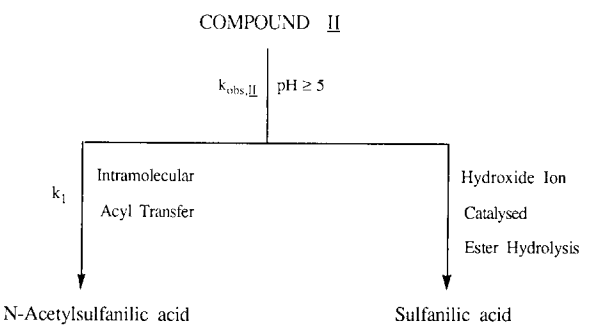


SCHEME 2: Proposed Mechanism for Formation of N-Acetylated Parent Amines from II, IV, and V.

compounds, II, IV, and V were estimated to be in the range of 15.2 and 21.3 (see Appendix). Thus, in the pH range under study (pH 5–9), the fraction of those compounds present as anionic carbamate is expected to be very low. However, the intramolecular acyl transfer reaction could still account for formation of *N*-acetylated parent amines from II, IV, and V, because of the expected high reactivity of generated anions. Such intramolecular nucleophilic attack by an amidate anion, which is structurally similar to carbamate anion, has been observed previously in the pH range where the fraction present in the ionized form is very low (Stella, 1971). The mechanism involving nucleophilic attack by the anionic carbamate (Scheme 2) is consistent with the slope of +1 of the log (rate constant)–pH profiles of II, IV, and V at pH ≥ 5.

The formation of the parent amine and *N*-acetylated parent amine accompanying the degradation of II, IV, and V at pH ≥ 5 could be due either to two parallel degradation pathways, i.e., intramolecular acyl transfer and hydroxide ion-catalysed ester hydrolysis (as shown in Scheme 3), or to a sequential scheme involving formation of *N*-acetylated amine followed by its hydrolysis to yield the parent amine. The latter possibility was ruled out when *N*-acetylsulfanilic acid was found to be very stable (at pH 7.4 and 37 °C) with no detectable degradation occurring over a period of 18 h. Additionally, the rate constants for the formation of sulfanilic acid ($11(\pm 2) \times 10^{-2}$ h^{-1}) and *N*-acetylsulfanilic acid ($10(\pm 1) \times 10^{-2}$ h^{-1}) from degradation of II at pH 7.5 were observed to be very similar to the rate constant for degradation of II (9.1×10^{-2} h^{-1}, Table 3) under similar conditions. Thus, compound II appears to degrade by two parallel pathways at pH ≥ 5, as depicted in Scheme 3. Since the structures of II, IV and V are similar, and since their degradation products are similar (i.e. parent amines and *N*-acetylated parent amines), compounds IV and V might also be expected to degrade by parallel pathways, i.e. intramolecular acyl transfer and hydroxide ion-catalysed ester hydrolysis.

The ascending portions of log rate constant–pH profiles (i.e. pH ≥ 5) for degradation of compounds II, IV and V could be accounted for by



SCHEME 3: Proposed Parallel Degradation Pathways for Compound II at pH ≥ 5.

the following rate expression (neglecting the expected minimal buffer effects).

$$\text{Rate} = k_{OH}[OH^-][C] + k_0'[C^-] \quad (2)$$

where, k_{OH} = hydroxide ion-catalysed hydrolysis rate constant, [C] = total molar concentration of carbamate II, IV, or V; k_0' = intrinsic rate constant of carbamate anion of II, IV, or V; and $[C^-]$ = concentration of II, IV, or V present as carbamate anion.

The two terms used in this rate expression are kinetically equivalent and cannot be distinguished from one another based on the kinetic data only, and either term could account for the slope of +1 at pH ≥ 5. However, because of the observed formation of the two degradation products, the inclusion of both terms in the rate expression appears warranted.

As stated earlier, *N*-methylsulfanilic acid was the only degradation product detected for compound III at pH ≥ 5. Since III does not have a dissociable hydrogen on the carbamate nitrogen, no carbamate anion can be formed. Accordingly, intramolecular acyl transfer reaction cannot occur for III and at pH ≥ 5, degradation of III occurs only by hydroxide ion-catalysed ester hydrolysis. The following rate expression accounts for degradation of III at pH ≥ 5.

$$\text{Rate} = k_{OH}[OH^-][III] \quad (3)$$

where, k_{OH} = hydroxide ion catalysed hydrolysis rate constant,

[III] = molar concentration of III.

Relative stability of II–V at pH ≥ 5

At any given pH value in the ascending portion of the profile, the rate constant for degradation of III was one-tenth that for the degradation of II under the same experimental conditions (see values at pH 6.7 and 9.0 in Tables 3 and 4). According to the hypothesis of parallel degradation pathways for degradation of II at pH ≥ 5, the ratio of the rate constant for degradation of II due to hydroxide ion-catalysed ester hydrolysis (term k_2 in Scheme 3) to the overall rate constant for degradation of II (term $k_{obs,II}$ in Scheme 3) would be equal to the fraction of II that degrades to sulfanilic acid. At pH 7.4, 10% of II degraded to sulfanilic acid (Table 5). Thus, the value of the ratio $k_2/k_{obs,II}$ would be equal to 0.1, i.e. $k_{obs,II} = 10\ k_2$. Since the ester portions of II and III are identical and the degradation of III occurs solely by hydroxide ion-catalysed ester hydrolysis, the rate constant for degradation of III might be expected to be approximately equal to the value of k_2 (Scheme 3). Hence, the expected value of $k_{obs,II}$ would also be 10 times the rate constant for degradation of III. The good agreement of the calculated and the observed values of the relative rate constants for degradation of II and III at pH ≥ 5 supports the hypothesis of two parallel pathways for degradation of II at pH ≥ 5.

The structures of the compounds IV and V are closely related to those of the compounds II and III and were chosen to evaluate the effects of the amine structure on their stability. It was anticipated that by studying the kinetics of degradation of these compounds the effects of the acidic pK_a value of the carbamate and the effect of the nucleophilicity of the generated anion might be better defined. Intramolecular acyl transfer reaction is the major route of degradation for compounds IV and V as indicated by their nearly quantitative conversion (97.5% and 97.8% respectively, Table 5) to the corresponding *N*-acetylated parent amines.

The rate constants for degradation of IV and V were determined to be essentially identical at pH ≥ 5 and were typically 2.75 times greater than those for II and 28 times greater than those for III as the same pH. Thus the two carbamate derivatives, IV and V, whose acidic pK_a values estimated by linear free-energy relationships (see Appendix) differed by 4.7 units ($pK_{a,IV} = 16.6$, $pK_{a,V} = 21.3$), still exhibited very similar rate constants for degradation by intramolecular acyl transfer. This observation can be rationalized by considering the effects of structure on various terms in the rate expression (Eqn. 2) which can be expressed as Eqn. 4:

$$\text{Rate} = k_{OH}[OH^-][C] + k_0' f_{N^-}[C] \quad (4)$$

where f_{N^-} = fraction of total carbamate present as carbamate anion.

Eqn. 4 can also be written as

$$\text{Rate} = k_{OH}[OH^-][C] + k_0' K_a[C]/([H^+] + K_a) \quad (5)$$

The acidic pK_a values of the 3 ionizable carbamates under study are estimated to be in the range of 15.2–21.3 (see Appendix). Therefore, at $5 \leq pH \leq 9$, $[H^+] \gg K_a$.
Hence, Eqn. 5 is reduced to

$$\text{Rate} = k_{OH}[OH^-][C] + k_0' K_a[C]/[H^+] \quad (6)$$

Since the first term in this simplified rate expression (Eqn. 6) represents the hydroxide ion-catalysed ester hydrolysis and the ester portions of II, IV, and V are identical, the numerical value of the first term might be expected to be quite similar for all 3 compounds. Therefore, the overall rates of degradation of II, IV, and V at $pH \geq 5$ would be expected to differ in proportion to the contribution of the second term in the rate expression (which represents the rate of intramolecular acyl transfer reaction). Any differences in the values of the acidic dissociation constants (K_a) of the carbamates under study, caused by inductive effects of substituents on the carbamate –NH, are expected to be accompanied by offsetting differences in the values of k_0', the intrinsic reactivity of the generated anions. For example, the presence of an electron-donating substituent adjacent to the carbamate –NH is expected to lower the value of its K_a. At the same time, the resulting free electron pair is expected to be more available for nucleophilic attack, thereby increasing the value of k_0'. Accordingly, the magnitude of K_a for the benzylamine derivative, V, is expected to be lower than that for the aniline derivative, IV, while the magnitude of k_0' for anion of V is expected to be higher than that of IV. These compensatory shifts in the values of K_a and k_0' could explain the similar rate constants of degradation for IV and V at $pH \geq 5$. Intramolecular nucleophilic attack by carbamate anions observed for II, IV, and V is similar to that by amidate anions observed previously (Stella, 1971; Shafer and Morawetz, 1963). The values of k_0' and K_a for amides also have been observed to shift in a compensatory manner (Shafer and Morawetz, 1963). From the above discussion, it appears that the reactivity of carbamates II, IV and V with respect to intramolecular acyl transfer reaction is independent of the structure of the parent primary amine. As stated earlier, the rate constants for degradation of acetoxyethoxycarbonyl derivatives due to ester hydrolysis also are largely independent of the structure of the parent amines. Accordingly, the stability characteristics of acetoxyethoxycarbonyl derivative of any primary amine are likely to be similar to those of compounds II, IV and V.

Description of log k_{obs}–pH profiles for compounds II–V

Based on Eqns. 1 and 6, the rate constants for degradation of compounds II, IV and V over the pH range $1 \leq pH \leq 9$ can be described by Eqn.7.

$$k_{obs} = k_H[H^+] + k_{OH}[OH^-] + k_0' K_a/[H^+] \quad (7)$$

Eqn. 7 can also be written as Eqn. 8.

$$k_{obs} = k_H[H^+] + (k_{OH} + k_0' K_a/K_w)[OH^-] \quad (8)$$

Based on Eqns. 1 and 3, the rate constants for degradation of compound III in the pH range $1 \leq pH \leq 9$ can be described by Eqn. 9.

$$k_{obs} = k_H[H^+] + k_{OH}[OH^-] \quad (9)$$

The solid lines in Fig. 1 were obtained using Eqn. 8 (for II, IV and V) and Eqn. 9 (for III) and the rate constants listed in Table 6.

Effect of temperature on the stability of II

The kinetics of degradation of compound II were studied in buffer solutions having pH values of 1.0 and 9.0 at 0 °C, 26 °C and 37 °C. The rate constants for degradation of compound II in solutions with different buffer concentrations were determined and the rate constants at zero buffer concentration (k_0) under those pH and temperature conditions were obtained by extrapolation. Fig. 2 shows the Eyring plots for the degradation

TABLE 6

Values [a] of the various rate constants for degradation of compounds II–V in aqueous solutions at 37 °C and $\mu = 0.5$

Compound	Rate constant (h^{-1})		
	k_H	$(k_{OH} + k_0' K_a / K_w)$	k_{OH}
II	0.62	3×10^5	[b]
III	0.62	[c]	3×10^4
IV, V	0.62	8×10^5	[b]

[a] These values were used along with either Eqn. 8 or Eqn. 9 to generate the solid lines in Fig. 1.
[b] Included in the term $(k_{OH} + k_0' K_a / K_w)$.
[c] Not applicable.

of compound II at pH 1.0 and pH 9.0. The apparent values of enthalpy and entropy of activation obtained from these plots are listed in Table 7. For the degradation of II at pH 1.0, $\Delta S = -26.5$ cal · deg^{-1} · mol^{-1}. Since degradation of II occurs only by hydrogen ion-catalysed ester hydrolysis at pH 1.0, the ΔS value is that for ester hydrolysis only and is similar in magnitude to those reported for simple esters such as aspirin ($\Delta S = -28.8$ cal · deg^{-1} · mol^{-1}; Garrett, 1957), and benzocaine ($\Delta S = -26.4$ cal · deg^{-1} · mol^{-1}; Marcus and Baron, 1959). The value of ΔS (−9.5 cal · deg^{-1} · mol^{-1}, Table 7) obtained for degradation of II at pH 9.0 is a mixed parameter since II degrades by parallel routes involving intramolecular acyl transfer reaction and hydroxide ion-catalysed ester hydrolysis. The information regarding the effect of temperature on the product distribution for II at pH 9.0 would be helpful in relating this value of ΔS with the mechanism of degradation of II. Since the related experiments were not

TABLE 7

Apparent enthalpies and entropies of activation for degradation of compound II at pH 1.0 and at pH 9.0 [a]

pH	ΔH (kcal mol^{-1})	ΔS (cal · deg^{-1} · mol^{-1})
1.0	16.7	−26.5
9.0	19.6	−9.5

[a] Calculated from Fig. 2.

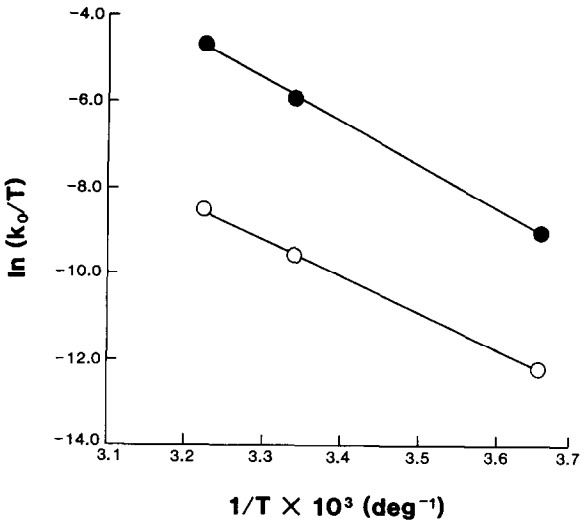


Fig. 2. Eyring plots of the rate constants for degradation of II at pH 1.0 (○) and 9.0 (●).

undertaken as a part of the present studies, no meaningful conclusions can be drawn based on the limited data available.

The observed energies of activation (E_a) for degradation reactions of II at pH 1.0 and pH 9.0 were calculated using the Arrhenius equation. At pH 1.0, the value of E_a was 17.3 kcal · mol^{-1} and at pH 9.0, it was 20 kcal · mol^{-1}. The observed value of E_a for hydrogen ion catalysed ester hydrolysis of II (i.e. 17.3 kcal · mol^{-1} at pH 1.0) is again similar to those reported for simple esters (e.g., aspirin, $E_a = 16.7$ kcal · mol^{-1} (Garrett, 1957) and benzocaine, $E_a = 18.6$ kcal · mol^{-1} (Marcus and Baron, 1959). The observed value of E_a at pH 9.0 is again a mixed constant because of parallel pathways of degradation for II as shown in Scheme 3. Accordingly, the Arrhenius plot for the degradation of II at pH 9.0 might not be expected to be linear. However, it is observed to be linear (as judged by the value of the correlation coefficient, $r = 0.9997$, for linear regression analysis of ln k vs T), probably due to the predominance of the intramolecular acyl transfer reaction as evidenced by the fact that *N*-acetylsulfanilic acid accounts for ~ 90% of the degradation of II.

Using the observed values of E_a and considering the reactions taking place at pH 1.0 and 9.0

for compound II, its shelf life (i.e. the time required for loss of 10% potency) under optimal conditions was estimated to be ~ 4.2 years when stored at 4° C as a pH 4.0 solution. The shelf life for III under the same conditions was estimated to be ~ 5.2 years. Assuming that the temperature dependency of intramolecular acyl transfer reactions for compounds IV and V was similar to that for II, the shelf life for the compounds IV and V stored at 4° C as a solution with pH 4.0 is estimated to be ~ 3.1 years.

Summary and Conclusions

The log rate constant–pH profiles for degradation of acetoxyethoxycarbonyl derivatives of amines (i.e. modified carbamates) were V-shaped as is observed for many esters. From the results obtained, it appears that for acetoxyethoxycarbonyl derivatives of primary amines, the rate constants for degradation at $1 \leq pH \leq 9$ would be similar to those for II, IV and V, barring a significant steric influence. In the case of other acyloxyalkoxycarbonyl derivatives of primary amines, their stability in the pH range below 4 would be largely dependent upon the stability of the ester function. However, at $pH \geq 5$, their stability will depend both on the stability of the ester function and on the facility with which the intramolecular acyl transfer reaction can take place. In the case of acyloxyalkoxycarbonyl derivatives of secondary amines, the stability over the entire pH range will depend only upon the hydrolytic stability of the ester portion. Thus, the stability of prodrugs of secondary amines could be controlled by a judicious choice of the acyl group in the promoiety.

The shelf life values for aqueous solutions of compounds II–V were estimated to be > 3 yrs under optimal conditions. If formulated as solids, these derivatives would be expected to be much more stable (Carstensen, 1977). Thus, acetoxyethoxycarbonyl derivatives of amines of the type studied here might yield prodrug candidates with sufficient stability for formulation as solids or as aqueous solutions. Studies evaluating the rates of reversion of these model prodrugs to yield the parent amines in the presence of biological component in the subject of a continuing study.

Appendix

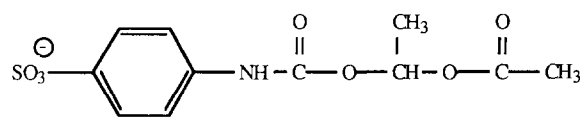
Estimation of the values of the acidic pK_a of the carbamate group in compounds II, IV and V

The acidic pK_a of a carbamate can be represented as follows:

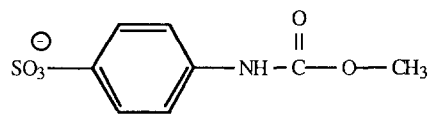
$$R\text{–}NH\text{–}COOR' \overset{K_a}{\rightleftarrows} R\text{–}N^-\text{–}COOR' + H^+$$

For estimation of the acidic pK_a value of the carbamate group in II, the reported basic pK_a values for the parent amines of compounds VI and VII and the reported value for the carbamate group in VII were used (Scheme IV).

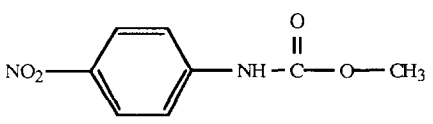
The acidic pK_a of the carbamate group in VII was determined by Hegarty and Frost (1973) to be 13.0. The basic pK_a of *p*-nitroaniline, the parent amine for the compound VII, was determined by Bascombe and Bell (1959) to be 1.02 and that of sulfanilic acid, the parent amine for the compound VI, was determined to be 3.23 (McLaren and Swinehart, 1951). Thus the basic pK_a of sulfanilic



II



VI



VII

Scheme 4.

acid is ~ 2.2 units higher than that of *p*-nitroaniline. Assuming that the sensitivity of acidic pK_a values of carbamates to the changes in electronic surroundings is similar to that of the basic pK_a values of amines, the acidic pK_a of VI might be expected to be ~ 2.2 units higher than that of VII. The above-mentioned assumption is a reasonable one considering that the sensitivities of the basic pK_a values of amines and of acidic pK_a values of amides to electronic effects have been shown to be similar (Barlin and Perrin, 1966) and that carbamates are structurally similar to amides. Thus the acidic pK_a value of the carbamate group in VI is expected to be (13.0 + ~ 2.2) = ~ 15.2. Since the structures and the electronic surroundings of the carbamate groups in II and VI are similar, the acidic pK_a value of the carbamate group in II is also expected to be ~ 15.2.

The acidic pK_a of carbamate groups in compounds IV and V can be estimated in a similar manner. The basic pK_a of aniline, the parent amine of IV, has been reported to be 4.59 (Bryson, 1960) and the basic pK_a of benzylamine, the parent amine of V, has been reported to be 9.35 (Robinson and Kiang, 1956). Therefore, acidic pK_a of the carbamate group in IV is expected to be ~ 16.6 and that of V is expected to be ~ 21.3.

Acknowledgements

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