Mechanism of Hydrolysis and Structure–Stability Relationship of Enaminones as Potential Prodrugs of Model Primary Amines

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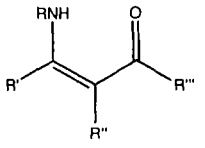
Abstract □ The objective of this work was to investigate the chemistry and the structure–stability relationship of enaminones (a class of enamines formed between a primary amine and a 1,3-dicarbonyl compound) and to evaluate their potential usefulness as prodrugs of primary amines. The acid-catalyzed degradation of the enaminones was found to be very sensitive to minor differences in the structure of the 1,3-dicarbonyl compound used to form the enaminone, but relatively insensitive to changes in the amine portion of the enaminones. A correlation was found between the rate of enaminone hydrolysis and the pK_a of the 1,3-dicarbonyl compound, suggesting that the rate-controlling step in the hydrolysis of the enaminones was the proton addition to the vinyl carbon of the enaminone. Enaminones formed with cyclic 1,3-dicarbonyl compounds were significantly more stable than those formed with structurally similar acyclic compounds. Based on chemical stability considerations alone, enaminones do not appear to be good candidates as prodrugs of primary amines. Evidence is presented, however, that enaminones formed between amines and 1,3-ketoesters or lactones may be subject to enyzme-catalyzed degradation. Further research on the design of enaminones destabilized by a triggering enzymatic event that results in the loss of conjugation (e.g., ester or lactone hydrolysis or an oxidation/reduction event) may prove worth pursuing.

The objective of this work was to investigate the chemistry and the structure–stability relationship of enaminones (**1**) a class of enamines formed between a primary amine and a 1,3-dicarbonyl compound. The ability of this class of primary amine derivatives to act as prodrugs of primary amines was evaluated. A similar study using only acyclic 1,3-dicarbonyl compounds and various amino acids has been recently published by Larsen and Bundgaard.[1]

The presence of a primary amine group in a drug can affect its physicochemical and biological properties in a deleterious manner. For example, drugs containing a primary amine group can undergo intra- or intermolecular aminolysis reactions leading to inactive and/or potentially toxic products. Examples are the hydrolysis of sodium ampicillin[2–4] and cycloserine.[5] When the primary amine group is present in a molecule with another ionizable functionality, like a carboxylic acid group, the molecule can have poor aqueous solubility and liposolubility due to the zwitterionic nature of the molecule in the physiologic pH range, thereby potentially limiting its dissolution rate and/or its passive permeability. The bioavailability of many peptides after oral administration is very poor, in part because of their enzymatic lability and their poor passive diffusion across the gastrointestinal mucosa. The terminal free amino group and the primary amine group on lysine are recognition sites for proteolytic enzymes like aminopeptidases and trypsin present in the gastrointestinal tract lumen, the brush border region, and the cytosol of the intestinal mucosa cells. The terminal and lysine residue amine groups can also contribute to the polarity of the peptides, thereby limiting their passive permeability characteristics.



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The aforementioned problems associated with drugs containing a primary amine group may be minimized by making prodrugs of the primary amine group. Potential strategies for prodrugs of primary amines have recently been reviewed by Bundgaard.[6] Except for a few unique cases, *N*-alkylation, *N*-acylation, and carbamate formation have been the most commonly used techniques to mask a primary amine group.

Enaminones, which are enamines formed from the reaction of an amine with a 1,3-dicarbonyl compound and are also called vinylogous amines, have not received much attention as prodrugs of amines, probably because of their acid instability and relatively good stability under simulated physiological pH conditions. A number of reviews have been published about the chemistry of enamines,[7,8] their physicochemical properties and uses, and their hydrolysis under a variety of conditions.[9–14] However, no extensive structure–stability relationships, except for the work of Larsen and Bundgaard,[1] have been established.

Presented here are the syntheses and a systematic evaluation of the chemical stability of various enaminones (**2a–2o**) of three model primary amines (aniline, phenethylamine, and D,L-valine), their structure–stability relationship, and an attempt to develop a rationale for the design of enaminones which could be potentially useful as prodrugs of amine drugs. Aniline was used as the principle model amine because the ionization properties of the amine had little effect on the relative stability of the enaminones and most of the aniline enaminones were solids and therefore easier to handle and purify than enaminones of simple alkyl amines. The 1,3-dicarbonyl compounds chosen were various 1,3-diketones, 3-ketoesters, and 3-ketolactones. The compounds **3a–3o** correspond to the dicarbonyl used to prepare enaminones, **2a–2o**, respectively, except that **2a–2c** were prepared from the same diketone, acetyl acetone (pentane-2,4-dione).

Experimental Section

Materials—Unless otherwise stated, all chemicals were at least ACS reagent grade and used without further purification. Aniline was purified by distillation under reduced pressure and was stored under nitrogen and protected from light. The water used in the kinetic studies was deionized and freshly distilled from an all-glass still.

Synthesis of Enaminones and Intermediates—All compounds not commercially available were synthesized by standard techniques.[15,16] The structure and purity of intermediates and final products were confirmed by ^1H NMR (Varian FT-80A or Varian T-60) and combustion analysis (Medicinal Chemistry Department, University of Kansas) for C, H, and N. All compounds gave elemental analyses within

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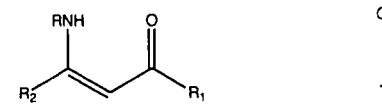
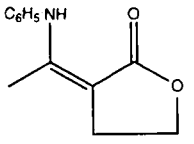
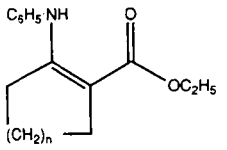


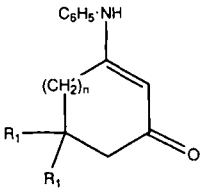
R = -C_6H_5; R_1 = R_2 = -CH_3 2a
R = -$(CH_2)_2C_6H_5$; R_1 = R_2 = -CH_3 2b
R = -CH(COOH)$CH(CH_3)_2$; R_1 = R_2 = -CH_3 2c
R = -C_6H_5; R_1 = -OC_2H_5; R_2 = -CH_3 2d
R = -C_6H_5; R_1 = OCH_3; R_2 = -$CH_2CH(OH)CH(CH_3)_2$ 2e



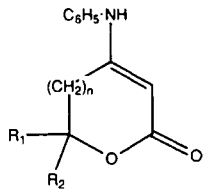
2f



n = 1 2g
n = 2 2h



n = 0; R_1 = -H 2i
n = 1; R_1 = -H 2j
n = 1; R_1 = -CH_3 2k



n = 0; R_1 = R_2 = -H 2l
n = 0; R_1 = R_2 = -CH_3 2m
n = 1; R_1 = -CH_3; R_2 = -H 2n
n = 1; R_1 = -$CH(CH_3)_2$; R_2 = -H 2o

±0.4% unless otherwise stated. The ^{1}H NMR shifts for vinyl protons and other relevant protons are given to confirm the presence of the enamine versus the imine structure and the oxo versus the enol structure where relevant.

4-(N-Phenylamino)-3-pentene-2-one (2a)—Compound **2a** was prepared by the general method for enamine synthesis of Stork et al.[17] by the reaction of aniline with pentane-2,4-dione (**3a**). The product was purified by recrystallization from petroleum ether (40–60 °C); yield, 94%; mp, 42–43 °C; ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3C=C), 2.89 (t, I2H, CH_2C=C, J = 7.8 Hz), 4.34 (t, 2H, CH_2O, J = 7.8 Hz), 7.17 (m, 5H, Ar—H), and 9.97 ppm (broad s, 1H, NH).

4-(2-N-Phenethylamino)-3-pentene-2-one (2b)—δ-Phenylethylamine was reacted with pentane-2,4-dione by the method referenced for **2a**. The product was purified by vacuum distillation (bp 125–130 °C at 5 mmHg); yield, 80%; ^{1}H NMR ($CDCl_3$): δ 1.74 (s, 3H, CH_3C=C), 1.96 (s, 3H, CH_3C=O), 2.8 (t, 2H, CH_2—Ar, J = 7.0 Hz), 3.40 (m, 2H, CH_2—N, J = 6.8 Hz), 7.22 (broad s, 5H, Ar—H), and 10.88 ppm (broad s, 1H, NH).

*N-(1-Methyl-3-oxo-1-butenyl)-*D,L*-valine (2c)*—A suspension of D,L-valine and pentane-2,4-dione was reacted by the method referenced for **2a**. Compound **2c** was isolated from the filtrate after the removal of unreacted D,L-valine; yield, 88%; mp, 134–135 °C; ^{1}H NMR ($CDCl_3$): δ 1.05 (d, 6H, $(CH_3)_2C$, J = 6.6 Hz), 1.92 (s, 3H, CH_3C=C), 2.05 (s, 3H, CH_3C=O), 2.25 (m, 1H, C—CH—C, J = 6.6 Hz), 3.9 (m, 1H, CH—CH(NH)—CO—), 5.04 (S, 1H, CH=C), 9.28 (broad s, 1H, NH), and 10.96 ppm (broad s, 1H, COOH).

Ethyl 3-(N-Phenylamino)-2-butenoate (2d)—Compound **2d** was prepared according to the method of Reynolds and Hauser.[18] A solution of 6.4 mL (0.05 mol) of ethyl acetoacetate (**3d**), 4.6 mL (0.05 mol) of freshly distilled aniline, and 0.1 mL of glacial acetic acid was refluxed in a 50-mL round-bottomed flask attached to a Dean and Stark constant water separator. Refluxing was continued until no more water separated (3 h). Benzene was then removed by rotary evaporation and the product was purified by vacuum distillation (bp 110 °C at 5 mmHg); yield, 7.5 g (73%); ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H, CH_3C, J = 7 Hz), 1.96 (s, 3H, CH_3C=C), 4.14 (q, 2H, CH_2—C, J = 7 Hz), 4.69 (s, 1H, CH=C), 7.21 (m, 5H, Ar—H), and 10.38 ppm (broad s 1H, NH).

Methyl 5-Hydroxy-6-methyl-3-(N-phenylamino)-2-heptene-1-carboxylate (2e)—Aniline was reacted with methyl 5-hydroxy-6-methyl-3-oxoheptane-1-carboxylate (**3e**, prepared from isobutyraldehyde and diketene by the method reported by Izawa and Mukaiyama[19]) in dry ether according to the method referenced for **2a**. The reaction was incomplete even after 40 h. Thin-layer chromatography (silica, diethyl ether) showed two products apart from the reactants. The mixture of products was separated from the reactants by chromatography (model 7924 Chromatotron, Harrison Research, Palo Alto, CA; silica, 2 mm; methylene chloride). The two products could not be further separated. The ^{1}H NMR analysis indicated **2e** as the major component; it was used as such in the kinetic studies.

3-[1-(N-Phenylamino)-ethylidene]-4,5-dihydro-2(3H)-furanone (2f)—Compound **2f** was synthesized by the general method referenced for **2a** by reacting aniline with 3-acetyl-4,5-dihydro-2(3*H*)-furanone (**3f**), except that glacial acetic acid was excluded from the reaction. The product was recrystallized from cyclohexane; yield, 80%; mp 87–88 °C; ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3C=C), 2.89 (t, 2H, CH_2C=C, J = 7.8 Hz), 4.34 (t, 2H, CH_2O, J = 7.8 Hz), 7.17 (m, 5H, Ar—H), and 9.97 ppm (broad s, 1H, NH).

Ethyl 2-(N-Phenylamino)-1-cyclopentene-1-carboxlyate (2g)—A procedure similar to that described for **2d** was used by reacting aniline with ethyl 2-oxo-cyclopentane-1-carboxylate (**3g**). The viscous liquid product was purified by column chromatography (alumina, 20% ethylacetate in *n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, CH_3C, J = 7 Hz), 1.93 (m, 2H, C—CH_2—C, J = 7 Hz), 2.69 (m, 4H, CH_2—C=C, J = 7 Hz), 4.2 (q, 2H, OCH_2, J = 7 Hz), 7.06 (m, 5H, Ar—H), and 9.59 ppm (broad s, 1H, NH). Hydrogen analysis was within 0.41%.

Ethyl 2-(N-Phenylamino)-1-cyclohexene-1-carboxylate (2h)—Compound **2h** was synthesized by the method reported by Taguchi and Westheimer[16] by the reaction of aniline with ethyl 2-oxo-cyclohexane-1-carboxylate (**3h**). The initial product was a thick reddish residue. The residue was purified by treatment with decolorizing charcoal and crystallization from petroleum ether (20–40 °C) to give white crystals; Yield, 40.8%; mp, 57–58 °C; ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, CH_3—C, J = 7 Hz), 1.59 (m, 4H, C—CH_2CH_2—C), 2.35 (broad s, 4H, CH_2—C=C—), 4.17 (q, 2H, CH_2O), 7.14 (m, 5H, Ar—H), and 10.73 ppm (broad s, IH, NH). Compound **3h** was prepared by reacting 1-(*N*-morpholino-1-cyclohexene), prepared by the reaction of morpholine and with cyclohexanone using the literature procedure of Stork et al.[15] with ethyl chloroformate to obtain **3h** in an overall yield of 40%. The structure was confirmed by ^{1}H NMR.

3-(N-Phenylamino)-2-cyclopentene-1-one (2i)—Compound **2i** was prepared from aniline and cyclopentan-1,3-dione (**3i**) using the method referenced for **2h**. Compound **2i** was recrystallized from methanol:ethylacetate; yield, 57%; mp, 235–236 °C; ^{1}H NMR (DMSO-d_6): δ 2.00 (s, 2H, CH_2CO), 2.83 (s, 2H, CH_2C=C), 5.93 (s, 1H, CH=C), 7.20 (broad s, 5H, Ar—H), and 8.93 ppm (broad s, 1H, NH).

3-(N-Phenylamino)-2-cyclohexene-1-one (2j)—Compound **2j** was synthesized by the same method as that described for **2f** by reacting aniline with cyclohexane-2,4-dione (**3j**). The product was recrystallized from methanol; yield, 94%, mp, 181–182 °C; ^{1}H NMR ($CDCl_3$): δ 2.02 (m, 2H, C—CH_2—C, J = 5.8 Hz), 2.31 (t, 2H, CH_2C=C, J = 6.0 H), 2.5 (t, 2H, CH_2C=O, J = 5.9 Hz), 5.53 (s, 1H, CH=C), 7.17 (m, 5H, Ar—H), and 7.43 ppm (broad s, 1H, NH).

5,5-Dimethyl-3-(N-phenylamino)-2-cyclohexene-1-one (2k)—Compound **2k** was prepared from aniline and 5,5-dimethylcyclohexane-1,3-dione (**3k**) using the method referenced for **2f**. Compound **2k** crystallized out of the reaction mixture in 91% yield; mp, 186–188 °C; ^{1}H NMR ($CDCl_3$): δ 1.04 (s, 6H, $(CH_3)_2C$), 2.16 (s, 2H, CH_2C=C), 2.34 (s, 2H, CH_2C=O), 5.53 (s, 1H, CH=C), 7.15 (m, 5H, Ar—H), and 7.30 ppm (broad s, 1H, NH).

4-(N-Phenylamino)-2(5H)-furanone (2l)—Compound **2l** was prepared according to the method of Greenhill and Tomassini[20] from dimethyl 1-(*N*-phenylamino)-ethene-1,2-dicarboxylate. A solution of 3.64 g (0.04 mol) of aniline in 20 mL of 90% ethanol was added to a solution of 4.92 mL (0.04 mol) of dimethylacetylene dicarboxylate in 20 mL of 90% ethanol at 20 °C. Diethyl 1-(*N*-phenylamino)-ethene-1,2-dicarboxylate was isolated in 85% yield after removing the solvent by rotary evaporation. A solution of 2 g (0.085 mol) of diethyl 1-(*N*-phenylamino)-ethene-1,2-dicarboxylate in 10 mL of dry tetrahydrofuran (THF) was added in a dropwise manner to a suspension of 0.4 g (0.0105 mol) of lithium aluminum hydride in 20 mL of THF, and the mixture was maintained under gentle reflux. The isolation of the product involved addition of 0.4 mL of water, 0.8 mL of 10% sodium hydroxide, and 1 mL of water in the given order to destroy the excess lithium aluminum hydride. The suspension was

filtered and 2l was isolated after removal of the solvent by rotary evaporation. The product was recrystallized from methanol; yield, 0.4 g (29%); mp, 220–221 °C; ^{1}H NMR ($CDCl_3$): δ 3.33 (s, 2H, $-CH_2O$), 5.30 (s, 1H, —CH=C), 7.30 (m, 5H, Ar—H), and 9.55 ppm (broad s, 1H, NH).

5,5-Dimethyl-4-(N-phenylamino)-2(5H)-furanone (2m)—Compound 2m was prepared by the reaction of 5,5-dimethyl-2,4(3*H*,5*H*)-furandione with aniline using the method referenced for 2h. Chloroform was used as the solvent and the product was recrystallized from acetone; yield, 95%, mp, 253 °C; ^{1}H NMR (DMSO-d_6): δ 1.55 (s, 6H, $(CH_3)_2C$), 5.10 (s, 1H, CH=C), 7.20 (broad s, 5H, Ar—H), and 9.55 ppm (broad s, 1H, NH). Carbon analysis was within 0.64%. 5,5-Dimethyl-2,4(3*H*,5*H*)-furandione was synthesized from 3-hydroxy-3-methyl-2-butanone by the method of Jerris et al.[21] in 27% yield; mp, 140–141 °C. The structure was confirmed by ^{1}H NMR.

5,6-Dihydro-6-methyl-4-(N-phenylamino)-2-pyrone (2n)—Compound 2n was prepared by the reaction of aniline with 5,6-dihydro-4-hydroxy-6-methyl-2-pyrone using the method referenced for 2h in 63% yield; mp, 190–191 °C; ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, CH_3C, J = 7 Hz), 2.46 (m, 2H, $-CH_2-C=C$), 4.40 (m, 1H, CH—O), 5.17 (s, 1H, CH=C), 7.15 (m, 5H, Ar—H), and 7.27 ppm (s, 1H, NH). 5,6-Dihydro-4-hydroxy-6-methyl-2-pyrone was prepared from paraldehyde and diketene by the method reported by Izawa and Mukaiyama.[19] The yield was 75%, the mp was 122–123 °C, and the structure was confirmed by ^{1}H NMR.

5,6-Dihydro-6-(1-methylethyl)-4-(N-phenyl-amino)-2-pyrone (2o)—Compound 2o was prepared identically to 2n, except that aniline was reacted with 5,6-dihydro-4-hydroxy-6-(1-methylethyl)-2-pyrone, using the method referenced for 2h in 70% yield; mp, 195–196 °C; ^{1}H NMR ($CDCl_3$): δ 1.03 (d, 6H, $(CH_3)_2C$, J = 3 Hz), 1.81 (m, 1H, CHC_3), 2.52 (m, 2H, $CH_2-C=C$), 3.69 (m, 1H, CH—O), 5.23 (s, 1H, C=CH—C=O), 6.82 (s, 1H, NH), and 7.25 ppm (m, 5H, Ar—H). Carbon analysis was within 0.60%.

5,6-Dihydro-4-hydroxy-6-(1-methylethyl)-2-pyrone was prepared from isobutyraldehyde and diketene by the method reported by Izawa and Mukaiyama[19] in 25.6% yield (mp, 78.5–79.5 °C). The structure was confirmed by ^{1}H NMR.

Hydrolysis Kinetics—Hydrolysis of the enaminones was carried out in aqueous buffer solutions of pH 1–9 (model 701/Digital pH meter, Orion Research, Cambridge, MA) that were thermostatically maintained in a waterbath at 25 ± 0.05 °C (Lauda RC20 Brinkman, West Germany). The buffers used were HCl (pH 1–3), acetate (pH 3–6), and phosphate (pH 6–9), and the ionic strength was adjusted to 0.5 M by the addition of KCl. Studies were carried out at various buffer concentrations (0.01–0.1 M) in order to determine rate constants at zero buffer concentration as well as the buffer catalysis constants at various pH values.

The wavelength for maximum absorption (λ_{max}) was determined for all the enaminones by scanning their UV-vis spectra (Carry 118, Varian, Palo Alto, CA). The rates of decomposition were followed spectrophotometrically (PM6, Carl Ziess, New York) by observing the decrease in absorbance at the λ_{max} of individual enaminones. In some cases, complete spectral scans (Carry 118, Varian, Palo Alto, CA) were recorded to confirm the presence of a single isosbestic point during the hydrolysis and to help confirm the final products of reactions. In a typical hydrolysis experiment, 3 mL of an appropriate buffer at 0.02, 0.05, 0.07, and 0.10 M were placed in four cuvettes and the temperature was equilibrated in the cell compartment, which was maintained at 25 ± 0.05 °C, of the spectrophotometer. Stock solutions of the enaminone in dry acetonitrile were introduced (25 μL) by a Hamilton syringe into each cuvette and agitated, resulting in initial enaminone concentrations of 30–100 μM. The pseudo first-order rate constants for the disappearance of enaminones were determined from plots of log $(A_t - A_\infty)$ versus time, where A_t and A_∞ are the absorbance readings at any time t and infinity, respectively.

An extensive acetate buffer dependency study on the rates of hydrolysis of 2a, followed at 322 nm (λ_{max} of 2a) and at pH values 4.4, 4.6, and 4.8, was carried out as described above except that 13–15 different buffer concentrations in the range 0.001–0.2 M were used.

Product Analysis—For all the enaminones, a number of solutions at the end of kinetic runs were analyzed by TLC. Precoated silica plates (Silica gel $60F_{254}$, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used. Diethylether was used as an eluent in most of the cases. In some cases, the UV scans for the kinetic solutions were compared with those for the expected products.

Enzymatic Hydrolysis Studies—The hydrolysis of enaminones 2d, 2g, and 2a (16 μM) at 25 °C in isotonic Sorensen phosphate buffer (pH 7.4) was studied at their λ_{max} in the presence and absence of various enzymes and tissues (porcine liver esterase, Sigma #E-3128, Type I, ~60 × 10^{-4} units/mL; carbonic anhydrase; rat and human plasma; and rat liver and intestinal homogenates). The spectrophotometric method described earlier was found to be sufficiently sensitive for the enzyme studies, while an HPLC method was needed for the rat liver and intestinal homogenate studies. The HPLC method utilized a LiChrosorb C18, 10-μm particle size, 250-mm Chromopak column. The mobile phase consisted of phosphate buffer (0.0925 M, pH 6.0):methanol (40:60). This adequately separated analine from all the enaminones (2d, 2g, and 2a). Only porcine liver esterase was extensively studied, as neither carbonic anhydrase nor the tissues showed any catalytic effects on the hydrolysis of the enaminones.

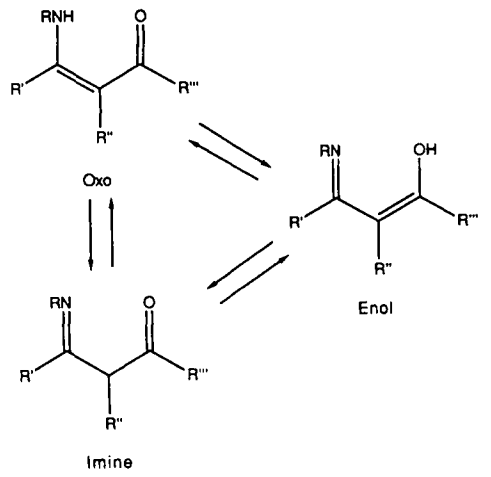
Dissociation Constants—The dissociation constants of a number of the dicarbonyl compounds were determined spectrophotometrically. Buffered aqueous solutions of the dicarbonyl compounds at various pH values were scanned (PE-555, Perkin-Elmer Coleman, Instrument Division, Oakbrook, IL) from 200 to 350 nm. Buffers used were HCl (pH 1–3), acetate (pH 3–6), phosphate (pH 6–9), and sodium hydroxide (pH 9–14). The buffer capacity of the solutions was not a problem. Nevertheless, pH values of the solutions were determined before and after scanning for those solutions with anticipated poor buffer capacity. The ionic strength of the solutions was maintained at 0.5 M with KCl and all measurements were carried out at 25 ± 0.05 °C. Stock solutions of the dicarbonyl compound were prepared in acetonitrile and 4–15 μL of the stock solutions were accurately added via Hamilton syringe to 3 mL of appropriate buffer to give concentrations in the range 20–100 μM, depending on the compound solubility and extinction coefficient. For any given compound, the concentration was maintained constant at all pH values. For each solution, the absorbance was measured against the corresponding buffer blank at 8–10 different pH values in the pH range of maximal spectral change. The dissociation constants were calculated via standard procedures described elsewhere[22] and are discussed in the *Results and Discussion* section.

Results and Discussion

Synthesis of Enaminones and Related Intermediates—All enaminones and related intermediates were synthesized using standard literature procedures with minor modifications. The coupling of amines with the dicarbonyl compounds was carried out using either the method of Stork et al.[15] or the milder, molecular sieve method.

The dicarbonyl compounds 3n and 3o required for enaminones 2n and 2o were synthesized by a method reported by Izawa and Mukaiyama[19] which is a general method for C-6 substituted 5,6-dihydro-4-hydroxy-2-pyrone. When this reaction was attempted using formaldehyde in the place of other aldehydes, in an attempt to synthesize the unsubstituted compound, multiple products of unknown structures were formed. In the present work, no attempts were made to optimize the reaction conditions for synthesis of the enaminones and the related intermediates, and most of the compounds reported here can be synthesized by alternative methods.

Structure of Enaminones—The tautomeric equilibria for the enaminones are shown in Scheme I. The enaminones can exist in oxo, imine, or enol forms. Table I gives the λ_{max} and ϵ_{max} values for some of the enaminones. The large values of λ_{max} and ϵ_{max} suggest that the enaminones probably do not exist in the imine form which would have a rather short λ_{max} because of the lack of conjugation. The absence of the methylene proton signal and the presence of vinylic proton signal in nonaqueous NMR solvents also confirms that imines are not present. However, both these techniques cannot distinguish between the oxo and the enol forms of the enaminones. Both the forms will be expected to have similar UV spectral properties, though the λ_{max} values are in good agreement with the estimated values for enaminones by application of Woodward's rule as described by Ostercamp.[23] The δ_{ppm} values for the NH and OH protons would also be



Scheme I

Table I—Wavelength for Maximum Absorbance (λ_{max})[a] and Molar Absorptivities (ϵ_{max})[a] Values for Some Enaminones

Enaminone	λ_{max}, nm	ϵ_{max} ± SD, M^{-1}
2a	322.0	21 200 ± 0500
2b	312.0	18 800 ± 0300
2c	313.0	19 900 ± 0100
2d	298.5	17 000 ± 0250
2f	311.5	22 400 ± 0300
2g	315.5	14 600 ± 4000
2h	313.0	9300 ± 1600
2i	295.5	17 000 ± 0100
2j	306.0	24 000 ± 0200
2k	309.0	23 800 ± 0100
2l	285.5	27 200 ± 0800
2m	283.0	19 800 ± 0100
2n	296.0	19 400 ± 0050
2o	296.0	19 400 ± 0050

[a] In aqueous buffer solutions at pH >7.

similar. From the splitting pattern of protons on the carbon adjacent to the enaminone nitrogen it has been shown that the enaminones exist in the oxo form, at least in nonaqueous solvents.[24] In the present work, a similar conclusion can be drawn for the enaminones **2b** and **2c**, both of which show multiplets for the protons adjacent to the nitrogen. For aniline enaminones this could not be observed because of the absence of such protons.

Greenhill,[25] from the measurements of pK_a values of some enaminones and of their enol ethers, has shown that the oxo:enol forms ratio for these enaminones is >10. Thus it appears that the enaminones primarily exist in the oxo form in aqueous solutions.

Hydrolysis Kinetics—The rates of hydrolysis of the enaminones in dilute, aqueous buffer solutions of constant pH, ionic strength (μ = 0.5 M, KCl), and temperature (25 ± 0.05 °C) were followed spectrophotometrically by monitoring the change in absorbance at the λ_{max} for each enaminone. Although the reaction of an amine with a carbonyl compound to form an enaminone is an equilibrium reaction, the reverse reaction, hydrolysis of the enaminone, may be treated as a unidirectional process in dilute aqueous solutions of the enaminones. This is because the magnitude of the equilibrium constant[26] and the concentrations of the amine and dicarbonyl compound formed after complete hydrolysis are such that complete dissociation of the enaminones is favored and the contribution from the reverse reaction (formation of enaminones) towards the observed rate constants is insignificant. This was further assured by the magnitude of A_∞, the absorbance reading at infinity, which was practically zero in all cases.

In order to confirm that simple first-order kinetics resulting in the formation of the corresponding dicarbonyl compound and amine were operative, some kinetic experiments were carried out by scanning over a wavelength range as a function of time. One such repetitive scan, Figure 1, shows a single isosbestic point indicating that the reaction is not complicated by multiple pathways or consecutive reactions. The products of the hydrolysis were confirmed as the corresponding amine and dicarbonyl compound by TLC and by comparison of UV spectra with those of standard solutions of amine and dicarbonyl compound.

The pseudo first-order rate constants for hydrolysis of enaminones were determined from semilogarithmic plots of $(A_t - A_\infty)$ versus time where A_t and A_∞ are as defined above. Figure 2 shows some examples of the semilogarithmic plots for the hydrolysis of **2a**.

The rate constants for hydrolysis of enaminones at zero buffer concentration, for acetate and phosphate buffers, were determined by extrapolation of the plots of the observed rate constants, k_{obs}, versus buffer concentrations to zero buffer concentration. The rate constants at zero buffer concentration, k_{obs}, were used to construct the pH–rate profiles for hydrolysis of the enaminones. The pH–rate profiles for hydrolysis of some of the enaminones are presented in Figures 3 and 4.

A general mechanism for the hydrolysis of enaminones is shown in Scheme II. It has been proposed that protonation of the vinyl carbon (step 2) is the rate-determining step for the hydrolysis of enaminones and that all subsequent steps in the hydrolysis are relatively fast.[9,10,27,28] The pH–rate profiles for all the enaminones, except those for **2b** and **2c**, can be explained by this mechanism. The Scheme is consistent with that reported for the hydrolysis of enamines in general. The pH–rate profiles for **2b** and **2c** will be discussed separately. For the other enaminones, the rate constant for the hydrolysis can be expressed by eqs 1 and 2:

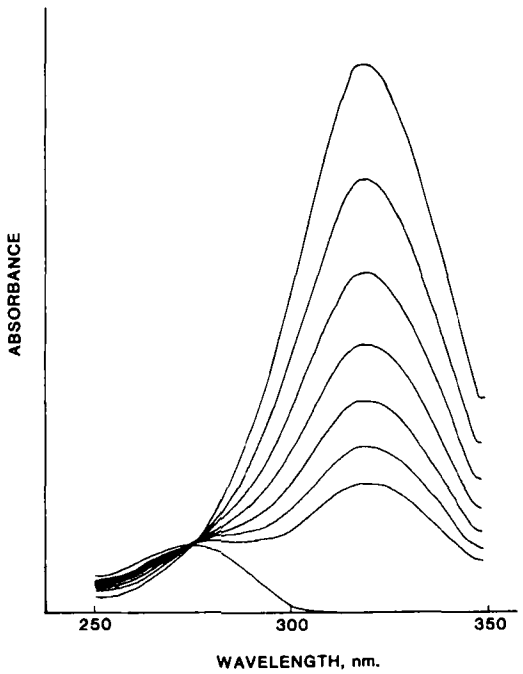


Figure 1—Repetitive UV scans (time interval, 2 min) for hydrolysis of 4-(*N*-phenylamino)-3-pentene-2-one (**2a**) at pH 5.0 (25 °C; μ = 0.5 M).

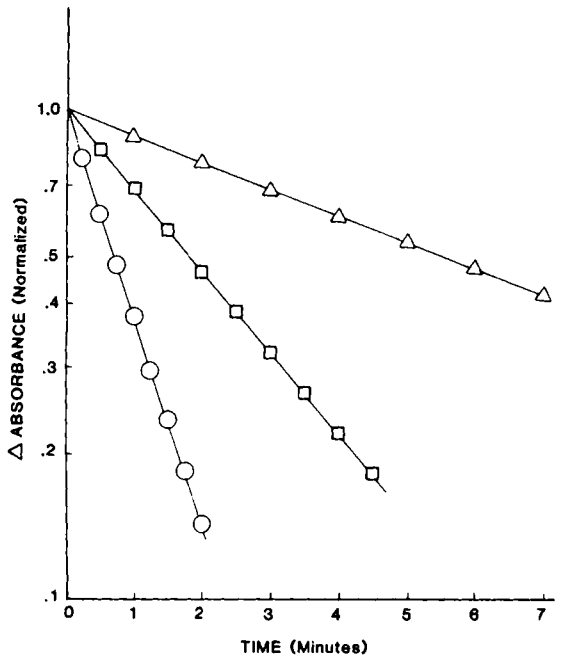


Figure 2—Semilogarithmic plots for the hydrolysis of 4-(*N*-phenylamino)-3-pentene-2-one (**2a**) at 25 °C in 0.05 M acetate buffer (μ = 0.5 M). Key: (○) pH 4.0; (□) pH 4.6; (△) pH 5.0.

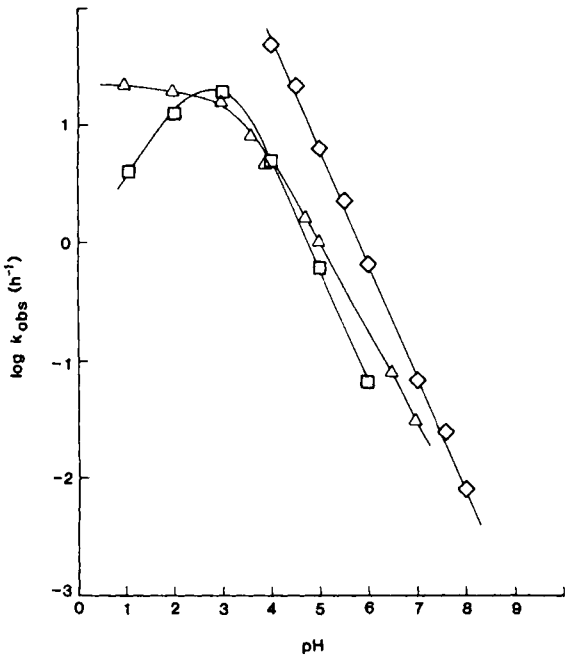


Figure 3—Log k_{obs} versus pH profiles for the hydrolysis of pentane-2,4-dione enamines of aniline (**2a**, ◇), phenethylamine (**2b**, □), and D,L-valine (**2c**, △) at 25 °C and μ = 0.5 M.

$$k_{obs} = k_H f_E \quad (1)$$

where k_H is the specific acid catalysis rate constant for the hydrolysis of the free base form of the enaminone (E) and f_E is the fraction of the enaminone in the free base form; and

$$k_{obs} = k_H[H^+]K_a/([H^+] + K_a) \quad (2)$$

where K_a is the acid dissociation constant of the protonated enaminone (EH), and $[H^+]$ is hydrogen ion concentration.

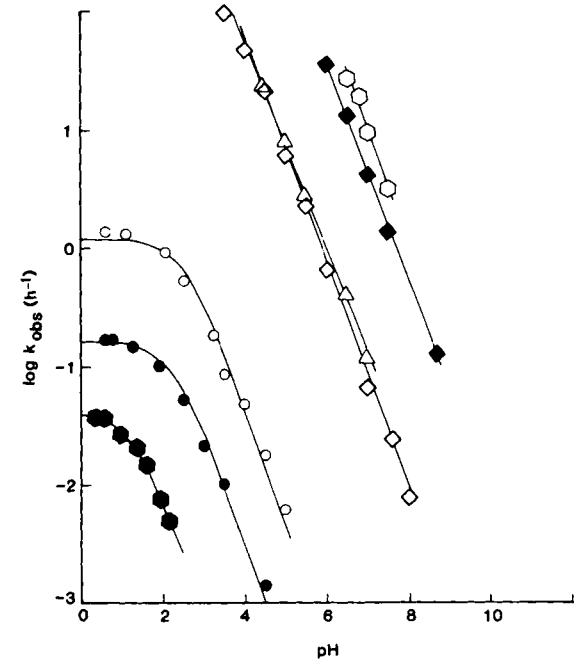
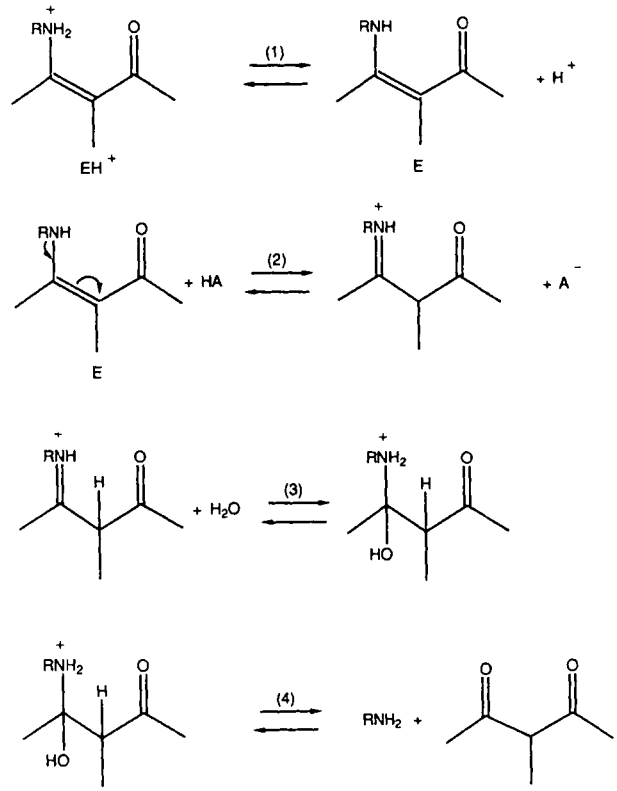


Figure 4—Log k_{obs} versus pH profiles for the hydrolysis of **2a** (◇), **2d** (◆), **2g** (△), **2h** (○) **2i** (●), **2j** (○), and **2k** (●) at 25 °C and μ = 0.5 M.



Scheme II

For reaction conditions where $[H^+] >> K_a$, eq 2 collapses to eq 3:

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

Therefore, a plot of log (k_{obs}) versus pH should be independent of pH under these conditions. For reaction conditions where $[H^+] << K_a$, eq 2 reduces to eq 4:

$$k'_{obs} = k_H[H^+] \quad (4)$$

Therefore, a plot of log (k_{obs}) versus pH under these conditions will be described by eq 5:

$$\log k_{obs} = \log k_H - pH \quad (5)$$

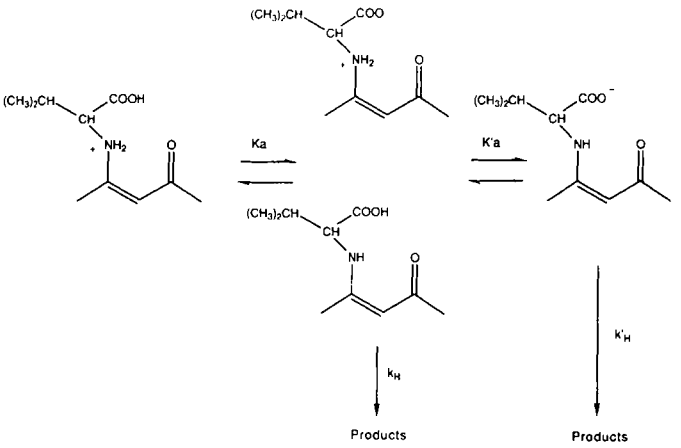
Thus, for pH values below the pK_a of the enaminone, log k_{obs} will be constant and independent of pH, while for pH values above the pK_a of the enaminone, log k_{obs} will vary linearly with the pH with an expected slope of −1, indicating apparent hydronium ion catalysis.

The pH–rate profiles for enaminones **2i**, **2j**, and **2k** are shown in Figure 4. Both the pH-independent region and the hydronium ion-catalyzed region were observed for these enaminones. The data for **2i**, **2j**, and **2k** were fit to eq 2 using a computer program[29] and the values of k_H and K_a were estimated. The solid lines in Figure 4 were drawn using the estimated values of k_H and K_a. The values of k_H are given in Table II. The values of pK_a were estimated as 2.57 ± 0.09 for **2j**, 2.31 ± 0.07 for **2k**, and 1.34 ± 0.03 for **2i**.

For all the other enaminones studied, only the linear portions of the pH–rate profiles were observed within the pH range studied. The data for all these enaminones were fit to eq 4 by linear regression. The values of the apparent hydronium ion catalysis constants, k_H, obtained from this analysis are included in Table II. These k_H values were used to draw the lines for enaminones **2a**, **2d**, **2g**, and **2h** in Figure 4.

The pH–rate profiles for enaminones **2b** and **2c** need special comment. As indicated in Figure 3, though the pH–rate profile for **2b** shows apparent hydronium ion catalysis at pH values >4, the pH–rate profile goes through a maxima and the observed rate constants decrease with pH values <3. This may be explained by invoking a change in the rate-determining step for the hydrolysis reaction from protonation of vinyl carbon (step 2 in Scheme II) to the breakdown of the carbinolamine (step 4 in the Scheme II).

The amine component for the enaminone **2c** was D,L-valine, an α-amino acid. Thus, this enaminone has an ionizable carboxyl group in its structure, and the enaminone could exist in four states of ionization. Assuming the concentration of zwitterionic species in the solution to be negligible, hydrolysis of **2c** may be represented by Scheme III and the rate constant for the hydrolysis of the enaminone will be given by eq 6:[4]



Scheme III

$$k_{obs} = \frac{(k_H[H^+] + k'_H K'_a)k_a[H^+]}{([H^+]^2 + K_a[H^+] + K_a K'_a)} \quad (6)$$

where k'_H is the second-order rate constant for hydronium ion-catalyzed hydrolysis of the anionic form of the enaminone (E^-, the carboxylic group is in its carboxylate form) and K'_a is presumably the dissocation constant of the carboxylic group. All other symbols have the same meanings as in eq 1. The line drawn for enaminone **2c** in Figure 3 was generated by fitting the data to eq 6 using the computer program referred to earlier.[29] The estimated values of k_H, k'_H, K_a, *and* K'_a are $3.16 \times 10^4\ M^{-1}h^{-1}$, $3.02 \times 10^5\ M^{-1}h^{-1}$, 1.26×10^{-3} M (pK_a = 2.9), and $4.36 \times 10^{-6}\ M^{-1}h^{-1}$ (pK'_a = 5.36), respectively.

The pH–rate profiles for enaminones **2a**, **2b**, and **2c** are shown in Figure 3 for comparison. The data allows one to compare the effect of the pK_a of the amine on the stability of the enaminones. The three enaminones were synthesized using different amines, namely aniline (pK_a = 4.7), phenethylamine (pK_a = 9.8), and D,L-valine (pK_a = 9.7), respectively. The derivatizing dicarbonyl compound was pentane-2,4-dione (acetylacetone). Though the values of pK_a for the amines ranged from 4.7 to 9.8, for the most part, the pH–rate profiles for the hydrolysis of the enaminones were quite similar, both qualitatively and quantitatively. The k_H values differ by only an order of magnitude. This observation is very useful when considering enaminones as prodrugs of drugs containing a primary amino group. It allows one to develop a general strategy in designing enaminones as prodrugs regardless of the pK_a of the amine. Also, the information collected for a model amine can be utilized in predicting a priori the stability behavior of enaminones of other primary amine-containing compounds.

Figure 4 demonstrates the effect of the structure of the dicarbonyl compound on the stability of enaminones. The pH–rate profiles for hydrolysis of a number of the other enaminones dramatically demonstrate the effect of the structure of the dicarbonyl compound on the stability of the enaminones. Contrary to the suggestion of Larsen and Bundgaard,[1] intramolecular hydrogen bonding between the enaminone N-H group and the β-carbonyl group cannot account for the relative stability since enaminones formed with cyclic dicarbonyl compounds, where such interactions are not possible, were always more stable than their acyclic analogues. The model amine used in this case was aniline. It

Table II—Apparent Hydronium Ion-catalyzed Rate Constant (k_H) for the Hydrolysis of Some Enaminones and the pK_a of Some of the Corresponding Dicarbonyl Compounds Used to Form the Enaminone

Enaminone	k_H ± SD, $M^{-1}h^{-1}$	Dicarbonyl pK_a ± SD
2a	$5.10 \pm 0.28 \times 10^5$	8.72 ± 0.13
2b	$5.07 \pm 0.13 \times 10^4$	5.10 ± 0.12
2d	$3.71 \pm 0.13 \times 10^7$	10.61 ± 0.15
2e	$1.71 \pm 0.15 \times 10^7$	—
2f	$1.09 \pm 0.02 \times 10^6$	8.11 ± 0.20
2g	$4.24 \pm 0.18 \times 10^5$	10.15 ± 0.10
2h	$9.40 \pm 1.60 \times 10^7$	11.99 ± 0.05
2i	$8.90 \pm 0.40 \times 10^{-1}$	—
2j	$4.50 \pm 0.50 \times 10^2$	4.93 ± 0.22
2k	$3.30 \pm 0.40 \times 10^1$	4.50[a]
2l	$1.30 \pm 0.09 \times 10^1$	—
2m	$5.63 \pm 1.15 \times 10^{-1}$	—
2n	$1.82 \pm 0.02 \times 10^2$	3.76[b]
2o	$2.81 \pm 0.05 \times 10^2$	5.06 ± 0.08

[a] From W. D. Kumler, *J. Am. Chem. Soc.* **1938**, *60*, 859–865.
[b] From Boothe, J. H., et al. *J. Am. Chem. Soc.* **1953**, *75*, 1732–1733.

is quite clear from Figure 4 and the data in Table II that even subtle changes in the structure of the dicarbonyl compound have a significant and dramatic effect on the stability of enaminones. For example, compare the stability of **2g** with that of **2h**, compounds whose stability differ by a factor of 200. Though not all the aniline enaminones are included in Figure 4, for the sake of clarity, the values of k_H for all the aniline enaminones are given in Table II. The stability of enaminones of aniline varies by about eight orders of magnitude with change in the structure of the dicarbonyl compound. Also included in Table II are some of the pK_a values for the corresponding dicarbonyl compounds used to form the enaminones. Note that the range of dissociation constants is in the same order of magnitude as the k_H values. A plot of log k_H versus the pK_a values is shown in Figure 5. The strong correlation between these two parameters with a slope close to unity (0.9 ± 0.1) indicates that the transition state for enaminone hydrolysis is probably similar to that for the dissociation equilibrium for the corresponding dicarbonyl compound. This suggests that the rate-determining step for the acid-catalyzed degradation of the enaminones is proton addition to the vinyl carbon, as illustrated in Scheme IV. The similarity to the dissociation equilibria for the dicarbonyl compound can be seen in Scheme V. Note that the reported pK_a values are observed values uncorrected for the keto:enol ratio of the dicarbonyl compound. It can be speculated, based on Scheme V, that a stronger correlation might exist between

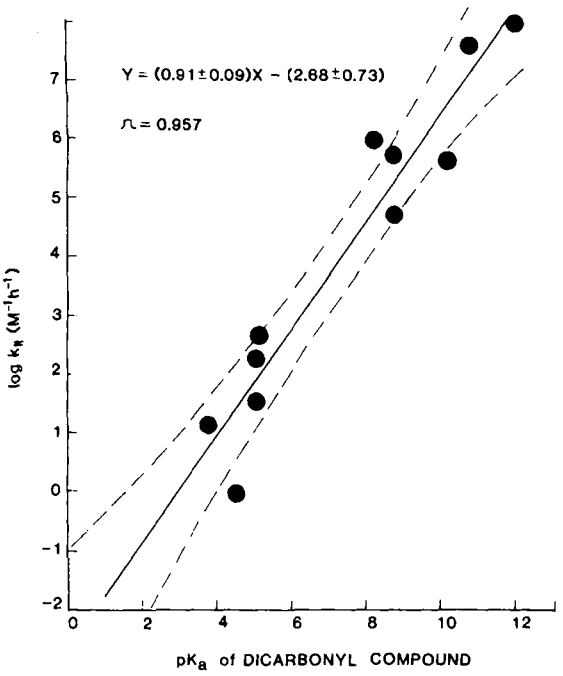
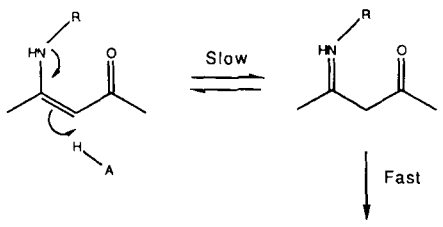
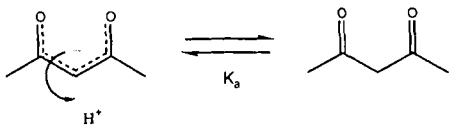


Figure 5—A plot of log k_H for the hydrolysis of the enaminones versus pK_a of the corresponding dicarbonyl compound used to form the enaminone. Broken lines represent the 95% confidence interval.



Scheme IV



Scheme V

log k_H and $pK_{a,diketone}$.

Consistent with this proton addition mechanism is the observation of general acid catalysis for the degradation of the enaminones. Linear plots of k_{obs} versus phosphate buffer concentrations in the pH range 6.2–7.6 for **2a** hydrolysis were observed. When the slopes (k_{cat}) of the catalytic plots were plotted versus the fraction of phosphate in its monoanionic form, $H_2PO_4^-$ (see Figure 6), it was obvious that the reaction was subject to general acid and not general base catalysis. That is, no catalysis by HPO_4^{2-} was observed. In the pH range 4.4–4.8, plots of k_{obs} versus total acetate buffer concentration (see Figure 7) were nonlinear. This is consistent with previous observations,[27,28] including those of Fedor[14] who studied the hydrolysis of the acetylacetone adduct of cycloserine. Note that the initial slopes of the k_{obs} versus total acetate concentration (broken lines in Figure 7) increase with decreasing pH; this is consistent with general acid catalysis. Larsen and Bundgaard[1] did not observe a similar behavior in their study, probably because of the limited buffer concentration used. The observations of nonlinear buffer catalysis at low pH values in the presence of relatively strong general acid, acetic acid versus $H_2PO_4^-$, indicate a change in the rate-determining step from protonation of the vinyl carbon at low buffer concentration or in the presence of very weak general acids to either formation of the carbinolamine from its Schiff base or hydrolysis of the carbinolamine.[27]

The results of this study support one important reason for lack of interest in the development of enaminones as prodrugs, particularly for oral drug delivery. The nature of the

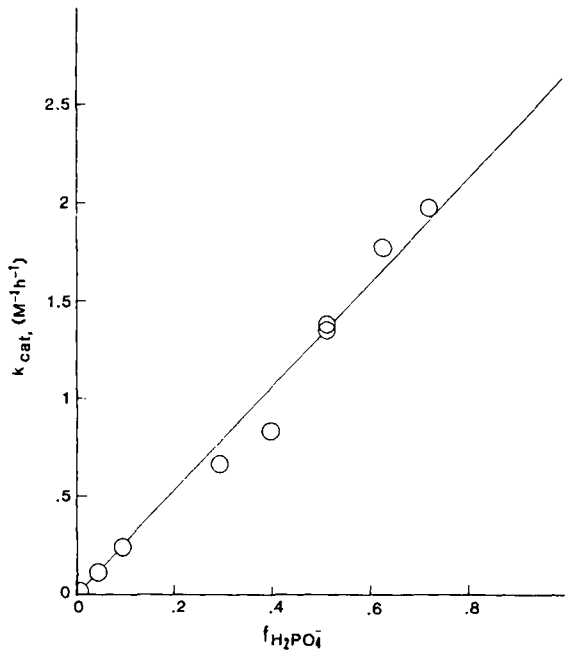


Figure 6—A plot of buffer catalysis constant, k_{cat}, versus the fraction of monobasic phosphate for the hydrolysis of 4-(*N*-phenylamino)-3-pentene-2-one, (**2a**) at 25 °C and μ = 0.5 M. The pK_{a2} value of 6.62 was used for the second dissociation constant of phosphoric acid at this ionic strength.

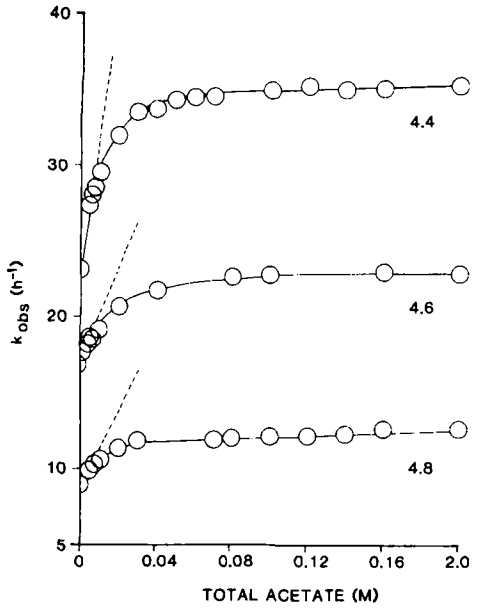
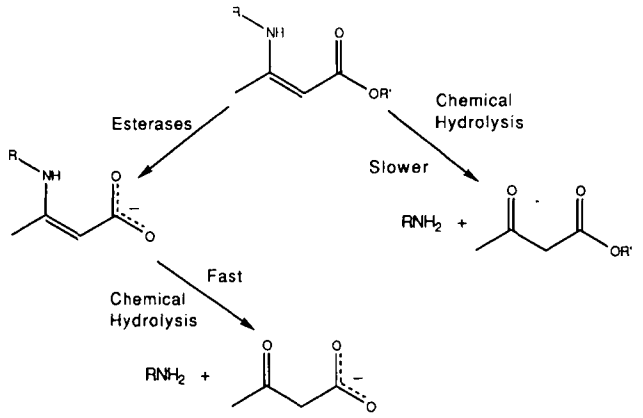


Figure 7—A plot of k_{obs} versus total acetate buffer concentration for the hydrolysis of 4-(*N*-phenylamino)-3-pentene-2-one (**2a**) at pH 4.4, 4.6, and 4.8 (25 °C, μ = 0.5 M).

pH–rate profiles is such that if an enaminone is designed to have sufficient chemical stability under the acidic conditions encountered in the stomach, the enaminone will be too stable to release the parent amine under physiological pH conditions. On the other hand, if an enaminone is designed to break down reasonably quickly under physiological pH conditions, it will be extremely unstable under gastric pH conditions.

It can be seen from Table II that cyclic enaminones are chemically more stable than acyclic enaminones (compare the five order of magnitude difference in the chemical stability of **2e** with that of **2o**). One possible prodrug strategy would be to design a chemically stable cyclic enaminone which is converted to a chemically unstable acyclic enaminone in vivo. Thus, the degradation of the enaminone in vivo will be triggered by ring opening. Similarly, enaminones are relatively more stable than simple enamines due to the extended conjugation of the vinylic double bond with the carbonyl group. This generalization holds whether the carbonyl group is from a ketone or an ester function. As indicated in Scheme VI, if the ester bond is cleaved by esterases, the keto carboxylate type enaminone formed should chemically hydrolyze faster than the parent enaminone because after ester hydrolysis, there is a significant reduction in the π-bond overlap and therefore the stabilization of the enaminone is lost.

This hypothesis was tested for two enaminones, **2d** and **2g**. The hydrolysis of **2d** and **2g** at 25 °C in isotonic Sorensen phosphate buffer (pH 7.4) was studied spectrophotometrically at 298.5 nm for **2d** and 315.5 nm for **2g** in the presence and absence of various enzyme systems. Porcine liver esterase (Sigma # E-3128, Type I) appeared to catalyze the hydrolysis of **2d** and **2g** relative to their chemical hydrolysis (e.g., at pH 7.4 and 25 °C, the apparent half-lives for chemical hydrolysis of **2d** and **2g** were 18 min and 15.5 h, respectively). In the presence of $\approx 60 \times 10^{-4}$ units/mL activity of porcine liver esterase and an initial substrate concentration of 16 μM, the apparent half-lives were reduced to 2 and 10 min for **2d** and **2g**, respectively. Apparent Michaelis–Menten kinetics were observed when substrate and enzyme levels were varied. No catalysis by porcine liver esterase was observed for the hydrolysis of **2a**, an enaminone structurally similar to **2d** but without the ester function (e.g., the half-life for hydrolysis of



Scheme VI

2a was the same, ≈34 h, in the presence and absence of porcine liver esterase). Hence it appears that the presence of the ester group was necessary for the catalytic effect by porcine liver esterase. Aniline, as expected, was observed to be a product of both the chemical- and enzyme-catalyzed hydrolysis. The corresponding ketoesters were the other products of the chemical hydrolysis. Although the second product of the enzyme-catalyzed hydrolysis could not be directly identified, it was not the corresponding ketoester as assessed by TLC. Thus it appears, as expected, that the ketoester is hydrolyzed prior to enaminone hydrolysis. Catalysis of the hydrolysis of **2d** and **2g** was not observed when studies were carried out in the presence of rat and human plasma, rat liver and intestinal homogenates, and carbonic anhydrase. Thus it appears that the ketoester type enaminones are enzymatically labile, but their enzymatic lability is limited to some specific enzyme(s) that do not appear to be ubiquitous.

This appears to be the first time that enzyme-aided hydrolysis of enaminones has been reported. Our results suggest that it may be possible to utilize chemically stable ketoester type enaminones as prodrugs of primary amino group-containing drugs by designing enaminones with the desired chemical stability characteristics but which are subject to enzyme-aided hydrolysis via the mechanism. The general utility of the concept, however, requires more detailed exploration.

In conclusion, enaminones of primary amines with 1,3-dicarbonyl compounds are relatively unstable under acidic pH conditions. The chemical stability of the enaminones is relatively insensitive to changes in the pK_a of the amine, while the chemical stability of the enaminones is extremely sensitive to the structure of the derivatizing dicarbonyl compound. Based on chemical stability considerations alone, these enaminones do not appear to be good candidates as prodrugs of primary amines. However, research on the design of enaminones destabilized by a triggering enzymatic event that results in the loss of conjugation (e.g., ester or lactone hydrolysis or an oxidation/reduction event) may prove worth pursuing.

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