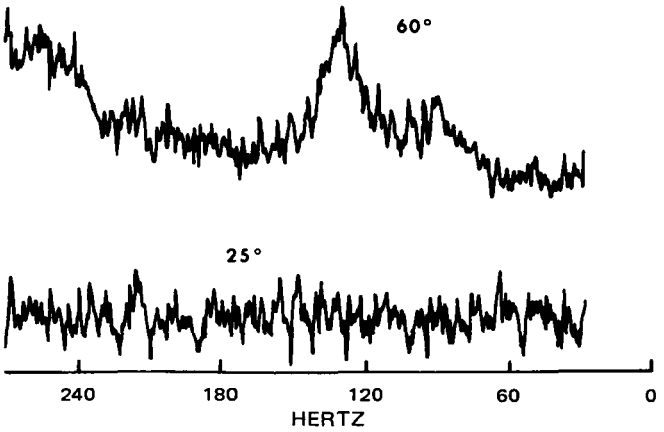

Figure 8—*NMR spectra for I (0.15% in 0.02% sodium sulfate solution) at 25 and 60°.*

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Solvolysis of a Substituted Imidazoline, Mazindol

H. V. MAULDING[x], P. TURI, D. GROSSMAN*, and J. P. NAZARENO

Abstract □ Hydrolysis of mazindol to form 2-(2-aminoethyl)-3-(*p*-chlorophenyl)-3-hydroxyphthalimidine was followed spectrophotometrically in aqueous solutions at temperatures between 37 and 70°, pH values up to 7.6, and an ionic strength of 0.2. The effects of acetate, formate, and phosphate buffers as well as ionic strength on the observed rate constants were investigated. An interesting nonlinear dependency of the k_{obs} with buffer concentration was noted. The velocity constants declined with increasing hydrogen-ion concentration; the log k–pH profile and rate law are given along with other relevant data.

Keyphrases □ Solvolysis—mazindol, a substituted imidazoline, pH values up to 7.6 □ Mazindol—solvolysis in aqueous solutions, pH up to 7.6

The chemical stability of organic medicinal agents from the viewpoint of the finished dosage forms and the kinetic aspects of the pertinent drugs are of great interest.

The transformation of the nonphenethylamine anorexigenic, mazindol[1] [5-(*p*-chlorophenyl)-2,5-dihydro-3*H*-imidazo[2,1-*a*]isoindol-5-ol] (I), to its hydrolysis product, 2-(2-aminoethyl)-3-(*p*-cholorophenyl)-3-hydroxyphthalimidine (II), was practically quantitative under the conditions employed as monitored by spectrophotometric and TLC methods. The process is somewhat analogous to the hydrolysis of 1,3-diphenyl-2-imidazolinium chloride recently reported (1).

EXPERIMENTAL

Kinetic Studies—A stock solution was prepared by dissolving 90 mg (3.16×10^{-4} mole) of I in 5 ml of 0.1 *N* HCl, and water was added to 50 ml. Two-milliliter aliquots were added to 200-ml volumetric flasks, equilibrated at various temperatures, containing 198 ml of the acidic or buffer solution. Ten-milliliter samples were withdrawn periodically and scanned[2] from 210 to 350 nm. The absorbance readings at 272 ± 1 nm were used to evaluate the rate of conversion of I to II.

A stock solution of II containing 95.5 mg (3.16×10^{-4} mole) in 50 ml of 0.01 *N* HCl was prepared. Two-milliliter aliquots were added to 198 ml of acidic or buffer solution previously equilibrated at various temperatures. Samples (10 ml) were taken periodically, and their UV spectra were scanned from 210 to 350 nm to monitor possible degradation of II.

Preparation of II from I—Two grams of I was refluxed in 100 ml of 48% aqueous ethanol for 2 hr until the solution became almost clear. The solvent was removed *in vacuo*. The resultant white solid dissolved in 50 ml of benzene with heating. Petroleum ether was added until the point of slight turbidity, and the compound was allowed to crystallize overnight at room temperature. The yield after two recrystallizations was 1.5 g (71%) of II, mp 170–171°.

Determination of pKa Values—A stock solution of 6.3×10^{-3} *M* I was prepared and added to 0.01 *M* tromethamine buffers (pH 7.8, 8.0, 8.2, and 8.4) along with 0.1 *N* HCl and 0.09 *N* NaOH to give a final concentration of 6.3×10^{-5} *M*. The samples were quickly read[2] at 272 nm. The pKa was calculated to be 8.55 ± 0.05 by the procedure of Albert and Sergeant (2).

Compound II (151.4 mg, 0.005 *M*) was dissolved in 95 ml of boiled water with the aid of 5 ml of 0.1 *N* HCl. The solution was titrated with 0.5-ml increments of 0.1 *N* KOH, giving a pKa value of 8.53 ± 0.05 at 25° (2).

[1] Sandoz Pharmaceuticals, East Hanover, N.J.

[2] Cary model 14 recording spectrophotometer.

Table I—Observed First-Order Rate Constants, k_{obs} in hours^{-1}, for Decomposition of Mazindol in Aqueous Solution (6.32×10^{-5} *M*)[a]

Buffer	Buffer pH	Observed Rate Constants[b,c]			
		37°	45°	55°	70°
Phosphate buffer[d]	7.6	1.07	1.87	—	—
Phosphate buffer	7.15	0.41	0.88	2.03	—
Phosphate buffer	6.7	0.134	0.31	1.29	—
Phosphate buffer	6.35	—	0.20	0.48	—
Phosphate buffer	6.0	0.055	0.097	0.23	0.90
Phosphate buffer	5.7	0.023	0.056	0.14	0.56
Acetate buffer[e]	5.4	—	0.038	0.092	0.46
Acetate buffer	5.0	0.0136	0.033	0.073	0.25
Acetate buffer	4.5	0.0122	0.027	0.057	0.21
Acetate buffer	4.0	0.0103	0.023	0.051	0.17
Formate buffer[e]	3.75	0.0073	0.0156	0.038	0.134
Formate buffer	3.35	0.0054	0.0128	0.031	—
Formate buffer	3.00	0.0043	0.0119	0.024	0.088
Formate buffer	2.75	0.0032	0.0070	0.0177	0.065

[a] The reaction was followed by loss of absorbance at 272 nm and studied for at least two half-lives. Rate constants were reproducible within 15%. The pH values were measured at the specified temperatures (ionic strength 0.2). [b] Supplementary values for rate constants listed are as follows [pH and k (hr^{-1})]: At 37°: 7.62, 0.75; 7.1, 0.33; 6.65, 0.124; and 6.0, 0.051. At 55°: 6.67, 0.88; 6.64, 0.59; 6.5, 0.35; 6.0, 0.22; 5.85, 0.150; 6.67, 0.80; 6.61, 0.77; 6.5, 0.68; and 6.4, 0.51. At 60.5°: 6.8, 1.66. At 70° (run at formate-ion concentration of 0.08 *M* and ionic strength 0.2): 5.0, 0.22; 4.50, 0.15, 0.16; and 4.0, 0.14. [c] Some k_{obs} values deviate somewhat from those listed in Fig. 3. This is likely a consequence of slight pH deviations in the pH 6–7.2 region, which result in large changes in the k_{obs} at these pH values where the slope of the log k_{obs}–pH profile is +1. These experiments were run by different operators in different months, *etc.*, so other factors may also come into play. [d] Phosphate buffers were prepared according to Ref. 8, except the pH 6.00 buffer which was made from 0.115 *M* NaH_2PO_4 and 0.028 *M* Na_2HPO_4 and the pH 6.35 buffer which was adjusted with sodium hydroxide. [e] Acetate and formate buffers were produced from 8 g of sodium hydroxide, sufficient acid, and dilution with water to give the appropriate pH in 1 liter of solution.

All pH measurements were carried out on a pH meter[3] standardized with phthalate and borate buffers at the specified temperatures (2).

TLC—The solvent system utilized was chloroform–ethanol–ammonia (80:20:1). Silica gel GF plates were spotted with 20 μl of solution containing 25–50 μg of I and/or II. The walls of the tank were lined with filter paper saturated with solvent. The product (II) and reactant (I) showed R_f values of 0.8 and 0.9, respectively, by this method when the plates were dried and visualized under shortwave UV light.

RESULTS AND DISCUSSION

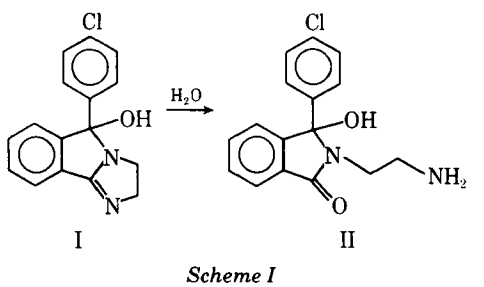
The solvolysis of I was studied between pH 0.3 and 7.2. The phthalimidine reaction product (II) was preparatively made from I (Scheme I) and was compared with an authentic sample of II[4]; they were found to be identical by NMR, mass, IR, and UV spectra, microanalysis, and melting-point determination.

Compound II was produced in almost quantitative yields from I by a first-order process. At higher acidities (below pH 2.6), the reaction was followed for at least 300 hr and exhibited pseudo-first-order kinetics for that time. Only I and II were discernible by TLC and UV spectrophotometry under the conditions examined.

The observed first-order rate constants were calculated from the expression:

$$\log (A_t - A_\infty) = \log (A_0 - A_\infty) - k_{obs}t/2.303 \qquad \text{(Eq. 1)}$$

where A_t, A_0, and A_∞ are absorbances at times t, zero, and infinity, respectively; k_{obs} is the observed or pseudo-first-order rate constant in hours^{-1}; and t is time in hours. Absorbance disappearance was followed at 272 ± 1 nm, with the hydrolysis product (II) and reactant (I) possessing molar absorptivities of 2.37×10^3 and 14.00×10^3 liters/mole cm, respectively. The absorbances for a given concentration of I and II were practically invariant over the pH scale studied (up to about pH 7.6).



Scheme I

Compound II did not degrade further under the conditions employed except after 500 hr in 0.1 *N* HCl at 70°; a slight change in UV spectra was seen along with a third trace spot on the TLC plates. Figure 1 illustrates data obtained for the decomposition of I at various pH values at 55°. Table I shows typical results from which the rate–pH profile was constructed (Fig. 2).

Hydroxide-Ion Catalysis—The right-hand portion of the rate–pH profile in Fig. 2, above pH 6, has a slope of about 1. This result is partially the consequence of hydroxide-ion attack on the imidaz-

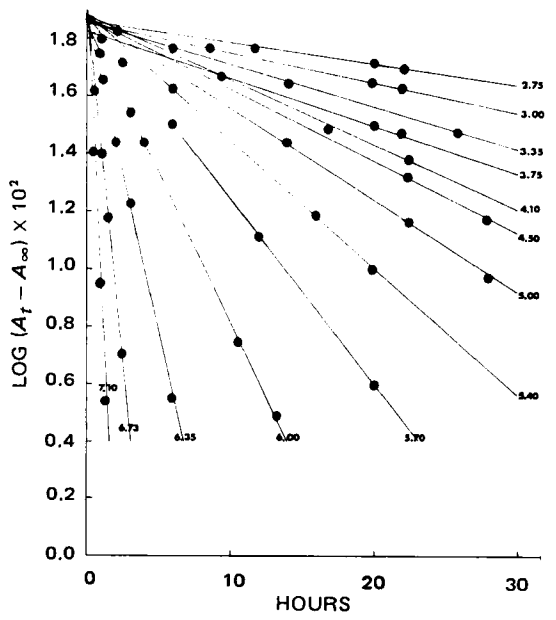


Figure 1—*Typical observed first-order plots for hydrolysis of mazindol at various pH values and 55°. The reaction was followed by loss of absorbance at 272 nm, with final attainment of residual absorbance, A_∞, characteristic of the reaction product (II).*

[3] Metrohm model E-300.

[4] Obtained from Dr. W. Houlihan, Sandoz Pharmaceuticals, East Hanover, N.J.

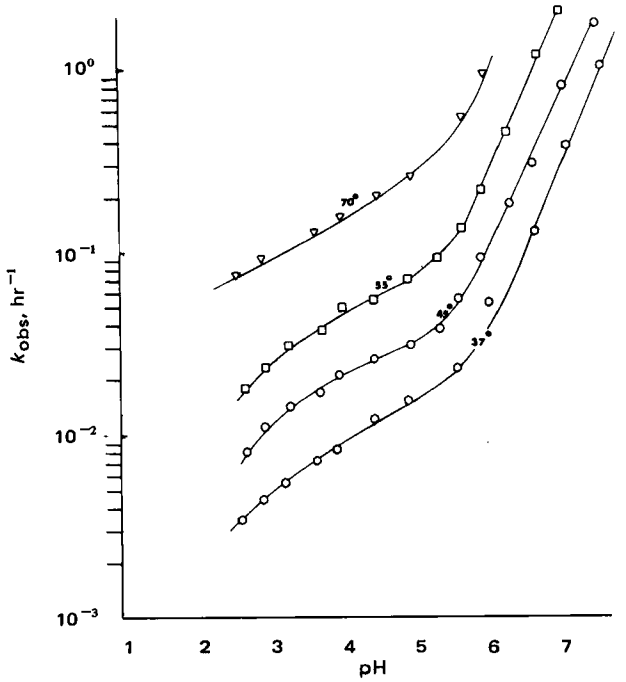


Figure 2—*Rate–pH profiles for the solvolysis of mazindol producing II at various temperatures. The reaction was monitored by absorbance loss at 272 nm (ionic strength 0.2).*

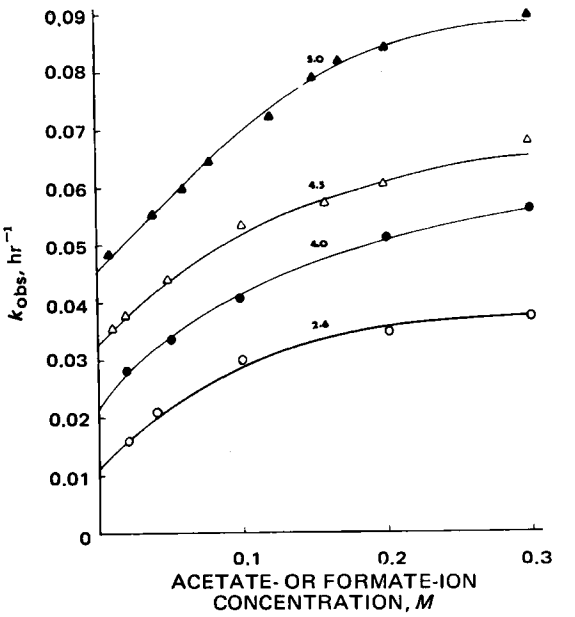


Figure 4—*Plot of observed first-order rate constants for hydrolysis of mazindol* versus *acetate- and formate-ion concentration at 55° (ionic strength constant) at various pH values. The pH 2.6 is formate buffer and the remainder are acetate systems.*

oline (I) in solution and may be written:

$$-d(I)_{tot}/dt = k_{obs}I_{tot} = k'_{OH^-}[OH^-][IH^+] + k_{OH^-}[OH^-][I] \quad \text{(Eq. 2)}$$

or:

$$k_{obs} = k'_{OH^-}[OH^-]f_{IH^+} + k_{OH^-}[OH^-]f_I \quad \text{(Eq. 3)}$$

where I plus IH^+ is the total compound in solution as neutral and protonated species, and k'_{OH^-} and k_{OH^-} are bimolecular rate constants for attack of hydroxide ion on the protonated fraction, f_{IH^+}, and the neutral fraction, f_I, respectively. The expression may be given as:

$$k_{obs} = k'_{OH^-}[OH^-]\frac{[H^+]}{[H^+] + K_{a_1}} + k_{OH^-}[OH^-]\frac{K_{a_1}}{K_a + [H^+]} \quad \text{(Eq. 4)}$$

At a pH above 7.6, the neutral molecule precipitated from aqueous solution. This precipitation, as well as a high reaction velocity, precluded study in these ranges. In the absence of other catalytic agents, *i.e.*, buffers, the k_{obs} of Eqs. 1–6 is actually the k_0 in Eq. 7.

The second term in the right-hand side of Eq. 4 may be omitted when $[H^+] \gg K_a$; $K_a/(K_a + [H^+]) \sim 0$. The resultant equation is written in the logarithmic form when $[H^+] \gg K_a$ in the usual manner:

$$\log k_{obs} = \log k'_{OH^-} + \log K_w - \log [H^+] \quad \text{(Eq. 5)}$$

or:

$$\log k_{obs} = \log k'_{OH^-} - pKw + pH \quad \text{(Eq. 6)}$$

In the range below 7.25 (about 1.30 units below the pKa), a plot of the log of the observed rate constant *versus* pH should give a straight line with a slope of 1 and an intercept of $\log k'_{OH^-} - pKw$.

Buffer Effects—Equations 4–6 hold in the absence of other catalytic species; however, a specific increment of the observed rate constant may be assigned to phosphate buffer catalysis at and above pH 5.7 (Fig. 2). The catalytic constants for $H_2PO_4^-$ and HPO_4^{-2} may be defined in the following manner, as mentioned by Notari and Caiola (3):

$$k_{obs} = k'_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{-2}}[HPO_4^{-2}] + k_0 \quad \text{(Eq. 7)}$$

The k_0 is composed primarily of $k'_{OH^-}[OH^-]$ as stated in Eq. 4. Equation 7 may be written as:

$$k_{obs} = \{k_{H_2PO_4^-}(R) + k_{HPO_4^{-2}}[HPO_4^{-2}] + k_0 \quad \text{(Eq. 8)}$$

where $R = [H_2PO_4^-]/[HPO_4^{-2}]$ and is invariant at a given pH. Thus, a plot of k_{obs} *versus* $[HPO_4^{-2}]$ with the pH constant gives:

$$\text{slope} = k_{H_2PO_4^-}(R) + k_{HPO_4^{-2}} \quad \text{(Eq. 9)}$$

A plot of the slopes of Eq. 8 at three or more pH values against the R values should yield a straight line with slope $k_{H_2PO_4^-}$ and a y intercept, $k_{HPO_4^{-2}}$, as expressed in Eq. 9.

The value for $k_{HPO_4^{-2}}$ at 55° was calculated using Eqs. 8 and 9 and found to be 6.8 liters/mole hr, with $k_{H_2PO_4^-}$ being negligible. The constants for $k_{HPO_4^{-2}}$ catalysis (liters per mole hour) at other

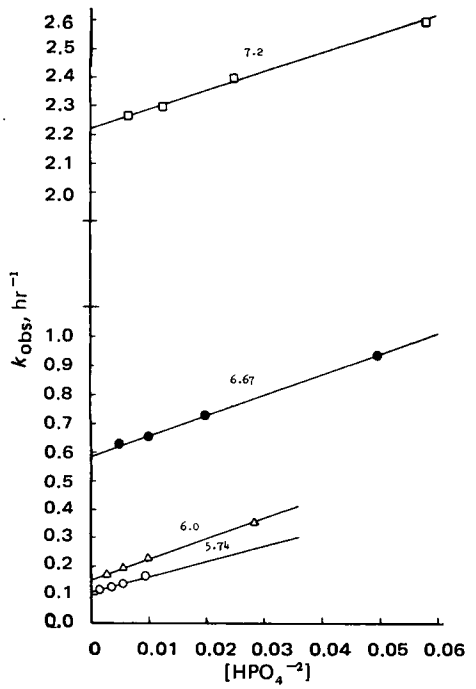


Figure 3—*Observed first-order rate constants for hydrolysis of mazindol at 55° and ionic strength 0.2, as a function of monohydrogen phosphate concentration at various pH values.*

Table II—Catalytic Constants[a] for Solvolysis of Mazindol in Aqueous Solution at Various Temperatures[b]

Constant	70°	55°	45°	37°
k'_{OH^-}	4.6×10^6	2.1×10^6	1.1×10^6	8.1×10^5
k''_{OH^-}	9×10^8	4.2×10^8	$(2.45 \times 10^8)^c$	$(1.55 \times 10^8)^c$
k''_{H_2O}	1.5×10^{-4}	6.5×10^{-5}	$(3.6 \times 10^{-5})^d$	$(2.2 \times 10^{-5})^d$
$k_{HPO_4^{-2}}$	18.0	6.8	2.6	1.5
$k_{CH_3COO^-}[CH_3COO^-]$	—	0.03	—	—

[a] Constants were calculated in liters per mole hour, except $k_{CH_3COO^-}[CH_3COO^-]$ which was estimated from data in Fig. 4 and k''_{H_2O} which was estimated in hours^{-1}. [b] Other pertinent values are: pKa_1, 8.55 (spectrometric determination at 25°), and pKa'_2, 2.9 (kinetic determination at 55°). [c] Determined by extrapolation of the Arrhenius equation. [d] Estimated from Eq. 12.

temperatures were: 37°, 1.5; 45°, 2.6; and 70°, 18.0 (Table II). Figure 3 shows an example of the utilization of Eq. 8 where the observed first-order constant is graphed on the ordinate and the $[HPO_4^{-2}]$ is graphed on the abscissa.

It is evident that the log k–pH profile, where $f_{IH^+} = 1$, in the phosphate buffer region may be adequately described in the following terms:

$$k_{obs} = k'_{OH^-}[OH^-] + k_{HPO_4^{-2}}[HPO_4^{-2}] \qquad \text{(Eq. 10)}$$

The k'_{OH^-} may be determined from Eq. 10 and/or the y intercepts in Fig. 3 by division of that value by the hydroxide-ion concentration at that temperature. The k'_{OH^-} values (liters per mole hour) were: 70°, 4.6×10^6; 55°, 2.1×10^6; 45°, 1.1×10^6; and 37°, 8.1×10^5 (Table II).

In the acetate and formate buffer range (pH 2.7–5.4) a nonlinear dependency of rate constants on conjugate base existed contrary to the expected straight lines (Fig. 4). This phenomenon was encountered by Martin *et al.* (4) in the *S*- to *N*-acetyl transfer of *S*-acetyl-β-mercaptoethylamine and was further investigated by Barnett and Jencks (5). These investigators predicted a sequence containing a catalyzed rate-determining step at low acetate and formate concentrations, followed by an uncatalyzed rate-determining step at higher buffer concentrations. Notari *et al.* (6) observed the same occurrence in the general-acid-catalyzed deamination of arabinosylcytosine.

With the imidazoline (I), the increments attributable to acetate and formate catalysis exhibited this nonlinear dependency. No attempt was made to dissect the function into constants. The amount contributed by hydroxide ion in the region, pH 2.6–5.0, may be estimated by extrapolation of acetate- and formate-ion concentrations to zero. These amounts at 55° (Fig. 4) were 0.012, 0.022, 0.033, and 0.046 hr^{-1} for pH values 2.6, 4.0, 4.5, and 5.0, respectively, and were the result of specific base (hydroxide-ion) catalysis. These values should be composed of two components: (*a*) k'_{OH^-}, and (*b*) k''_{OH^-}, which is indicative of hydroxide-ion reaction with a diprotonated species.

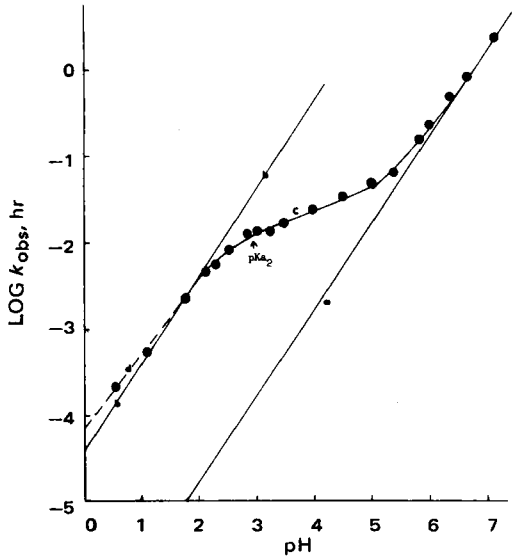


Figure 5—*Synthesis of the log* k–*pH profile for the solvolysis of mazindol at 55° (ionic strength 0.2). Line a represents hydroxide-ion attack on monoprotonated mazindol,* $k'_{OH^-}[OH^-]$, *while line b describes hydroxide-ion reaction with diprotonated mazindol,* $k''_{OH^-}[OH^-]$. *Line c is a summation of the two functions, where* $K_{a2} = 1.26 \times 10^{-3}$ M. *Line d is a summation of line b plus the increment added by water hydrolysis of the substrate as given in Eq. 11. The solid dots represent the experimental data points extrapolated to zero buffer concentration.*

Log *k*–pH Profile—Table III lists rate constants and half-life times for hydrolysis of I under acidic conditions.

The curvature of the real data points in Fig. 5 indicate the likelihood of a second pKa. The predisposition of the velocity constants to increase as the pH rises point toward specific hydroxide-ion catalysis of both mono- and diprotonated species. Due to the prevalence of buffer catalysis in the acidic region, reaction with water might be considered since the two frequently occur concomitantly.

At low pH values, pH < 2, one may write an equation of the type:

$$k_{obs} \approx k_0 = (k''_{OH^-}[OH^-] + k''_{H_2O})f_{IH_2^{+2}} \qquad \text{(Eq. 11)}$$

which simplifies when $f_{IH_2^{+2}} \approx 1$ to:

$$k_{obs} = k''_{OH^-}[OH^-] + k''_{H_2O} \qquad \text{(Eq. 12)}$$

explaining the results of Fig. 5. Thus, a plot of k_{obs} *versus* $[OH^-]$ at low pH values, *i.e.*, between 0 and 2, should yield a straight line of slope k''_{OH^-} and intercept k''_{H_2O}. The k''_{OH^-} values are listed in Table II along with results at 37 and 45° obtained by extrapolation of the Arrhenius plot.

Line b of Fig. 5 was derived from the function $k''_{OH^-}[OH^-]$, while line d is a combination of the function plus the increment attributable to k''_{H_2O}. The k''_{H_2O} values of 1.5×10^{-4} (70°) and 6.5×10^{-5} (55°) in hours^{-1} (Table II) were calculated from Eq. 12.

These findings support the thought that two principal kinetic

Table III—Observed First-Order Rate Constants for Hydrolysis of Mazindol in Acidic Solution (6.32×10^{-5} *M*)[a]

	Observed Rate Constants, hr^{-1}		$t_{1/2}$, hr	
HCl	55°	70°	55°	70°
0.005 *N*	0.0058	—	120.0	—
0.010 *N*	0.0047	—	143.8	—
0.02 *N*	0.0020	0.0069	346.7	100.3
0.10 *N*	0.0006	0.0025	1045.5	278.2
0.20 *N*	—	0.0017	—	401.2
0.50 *N*	0.00018	0.0011	3850.0	605.3
0.75 *N*	—	0.00086	—	802.3
1.00 *N*	—	0.00076	—	903.1
H_2PO_4–NaH_2PO_4[b]				
pH 2.50	0.0086	—	79.9	—
pH 2.75	0.0115	—	70.0	—
pH 3.00	0.0144	—	47.9	—
pH 3.25	0.0153	—	44.9	—

[a] Reactions were followed by loss of absorbance at 272 nm and monitored for at least 300 hr. Ionic strength was made to 0.2 with sodium chloride where possible. [b] Solutions were made from 0.05 *M* $NaH_2PO_4 \cdot H_2O$ with phosphoric acid added to give the proper pH. No evidence of phosphoric acid or phosphorus acid catalysis was noted.

Table IV—Effect of Ionic Strength (μ) on Observed First-Order Velocity Constants for Hydrolysis of Mazindol in Aqueous Solution (6.32×10^{-5} *M*) in Acetate and Phosphate Buffers at 55°

Buffer[a]	pH[b]	$\mu^{1/2}$	μ	k, hr^{-1}
Phosphate	6.67	0.10	0.01	0.862
Phosphate	6.67	0.224	0.05	0.802
Phosphate	6.67	0.447	0.20	0.715
Phosphate	6.67	0.707	0.50	0.675
Phosphate	6.67	1.0	1.0	0.595
Acetate	4.5	0.10	0.01	0.045
Acetate	4.5	0.224	0.05	0.040
Acetate	4.5	0.447	0.20	0.036
Acetate	4.5	0.707	0.50	0.029
Acetate	4.5	1.0	1.0	0.024

[a] Reaction solutions (200 ml) were prepared by taking 10 ml of acetate or phosphate buffer (μ = 0.2) with addition of sodium chloride to bring the solutions up to the stated ionic strengths. Phosphate buffers were prepared according to the specifications of Ref. 8. Acetate buffers were made by adding acetic acid to 8 g of sodium hydroxide to give the proper pH and dilution to 1 liter with water. [b] Due to the extreme effect of pH on rate constants of pH 6.67 phosphate buffer, a small amount of 1 *N* NaOH had to be added to the solutions containing larger amounts of sodium chloride. The most added was 0.2 ml of 1.0 *N* NaOH to adjust pH to 6.67.

factors work at pH < 2: (*a*) spontaneous decomposition, and (*b*) hydroxide-ion attack on some polyprotonated form of I.

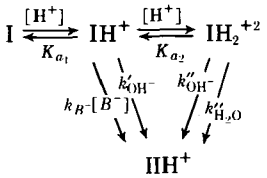
Figure 5 contains a second diagonal line of slope 1, line a, which signifies hydroxide-ion reaction with the monoprotonated molecule, $k'_{OH^-}[OH^-]$, from Eq. 4 (Table II). Once the bimolecular rate constants for attack of hydroxide ion on monoprotonated and diprotonated imidazoline were determined, the K_{a_2} could be estimated (Eq. 13) by means of data fitting to meet experimental results. The intermediate area between lines a and b, line c, is a combination of the two functions $k'_{OH^-}[OH^-]$ and $k''_{OH^-}[OH^-]$, with K_a 1.26×10^{-3}, pKa 2.9, being used for analysis of the data. A small contribution was made by the k''_{H_2O}, but it may be neglected above pH2.0.

The k''_{OH^-} values of 9×10^8 (70°) and 4.2×10^8 (55°) liters/mole hr were evaluated from the data in Table III by means of Eqs. 11 and 12. These values are more than two orders of magnitude above the k'_{OH^-} values for similar temperatures, which were 4.6×10^6 and 2.1×10^6 liters/mole hr, respectively. Results of this type may be expected for the reaction of hydroxide ion with IH_2^{+2}, because the constants should be considerably larger than those for the reaction of OH^- with IH^+.

Figure 5 illustrates the theoretical log k_0–pH profile, using the relationship:

$$k_0 = k'_{OH^-}[OH^-]\frac{[H^+]}{[H^+]+K_{a_1}} + (k''_{OH^-}[OH^-] + k''_{H_2O})\frac{[H^+]}{[H^+]+K_{a_2}} \quad \text{(Eq. 13)}$$

Ionic Strength Effects—The perturbation of the observed first-order velocity constants by addition of neutral salts at constant pH values are shown in Table IV. These results imply reaction of a negative and positive ion at pH 6.67 and 4.5. This finding



Scheme II—Schematic diagram for the solvolysis of mazindol and its protonated forms, IH^+ and IH_2^{+2}, to produce 2-(2-aminoethyl)-3-(p-chlorophenyl)-3-hydroxyphthalimidine (II) in its protonated form, IIH^+. (The possibility exists of diprotonated II, but it was not noted by the experimental methods utilized.) Both molecules primarily protonated (95+%) under the conditions investigated.

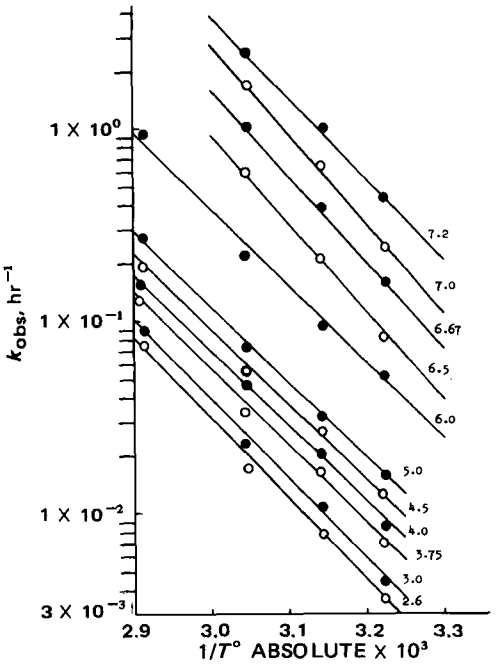


Figure 6—*Typical Arrhenius plots for hydrolysis of mazindol in aqueous media at the indicated pH values.*

cannot be considered conclusive since the Debye–Hückel theory and Brønsted–Bjerrum equation are valid for solutions below 0.01 *M* of 1:1 electrolytes (7).

Dependency of Reaction Rate on Temperature—The Arrhenius parameters for solvolysis of I were determined from the slopes and intercepts of plots of log k_{obs} *versus* the reciprocal of the absolute temperature in accordance with the equation:

$$\log k_{obs} = \log P - E_a/2.303RT \quad \text{(Eq. 14)}$$

with R being 1.987 cal deg^{-1} $mole^{-1}$. The results for several pH values are illustrated in Fig. 6. The apparent energies of activation between pH 6 and 7.2 were 20.9–22.3 kcal/mole, while those in the acetate and formate buffers varied between 17.9 and 21.1 kcal/mole. The k'_{OH^-} values give a E_a equal to 11.35 kcal/mole; $k_{HPO_4^{-2}}$ was found to have 20.9 kcal/mole as the activation energy.

SUMMARY

Mazindol (I) hydrolyzes in aqueous solution to yield a phthalimidine product (II) in practically quantitative amounts.

The solvolysis possibly proceeds through a tetrahedral intermediate similar to that suggested by Robinson (1). The intermediate should show a diminution of absorbance if it has an appreciable lifetime. This phenomenon was not encountered under the conditions studied.

The process is a relatively facile transformation at moderate temperatures in neutral media, which leads to II as a simple hydrolytic product. This transformation is an instance where the velocity constants vary directly with pH, signifying hydroxide-ion catalysis over the entire range. Negligible acid catalysis was encountered, but both general-base and water catalysis were manifest. The observed or apparent rate constant may be reasonably well described between pH 7.6 and zero by the following relationship:

$$k_{obs} = k'_{OH^-}[OH^-]\frac{[H^+]}{[H^+]+K_{a_1}} + (k''_{OH^-}[OH^-] + k''_{H_2O})\frac{[H^+]}{[H^+]+K_{a_2}} + k_B[B^-][I_{tot}] \quad \text{(Eq. 15)}$$

with $k_B[B^-]$ indicative of species in HPO_4^{-2}, acetate, and formate. Acetate and formate must be estimated due to a nonlinear dependency of the rate constant on the concentration of conjugate base. At higher acidities, the reaction rate is mainly dependent upon hydroxide-ion concentration and water attack on the diprotonated molecule in solution.

Arrhenius parameters are given and may allow prediction of shelflife as well as stability in biological media at various temperatures and conditions. Salt effects are also discussed and point toward reaction between two oppositely charged ions. A diagram of the entire process is shown in Scheme II.

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Isolation and Identification of Three New Flavones from *Achillea millefolium* L.

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Abstract □ Column chromatography on silica gel of a petroleum ether extract of the flowering heads of *Achillea millefolium* L. allowed three flavones to be separated and identified. Spectral studies (PMR, mass spectrometry, and UV) and a comparison with data for compounds reported in the literature established the flavones as 5-hydroxy-3,6,7,4′-tetramethoxyflavone, artemetin, and casticin. These compounds have not been reported previously as constituents of *A. millefolium*.

Keyphrases □ Flavones—chromatographic separation and spectral identification from *Achillea millefolium* □ *Achillea millefolium*—chromatographic isolation and spectral identification of flavone components □ Medicinal plants—*Achillea millefolium*, isolation and identification of flavone components

Achillea millefolium L. (Compositae), commonly known as yarrow, grows abundantly throughout America and Europe (1). The plant has been a popular medicinal remedy, having been known to Dioscorides (2), and has been recommended for use as a tonic, a stomachic, a hemostatic, an antispasmodic, an antihemorrhoidal, an emmenagogue, an antiseptic, and an anthelmintic (3).

Chemical investigations of the plant have been numerous, extending back for more than 200 years (4, 5). One class of compounds that has received considerable attention is the flavonoids; they have been proposed as the cause of the spasmolytic activity of *A. millefolium*. Horhammer (6) examined a number of flavones and extracts of different plants for antispasmotic activity and showed *A. millefolium* to be the most active plant. He found that the plant contained apigenin and luteolin-7-glycosides. These flavones, as well as caffeic acid, were found also by Michaluk (7), and the presence of caffeic acid was more recently verified (8). In 1964, luteolin-7-glucoside was isolated from *A. millefolium* and synthesized (9). More recently, the presence of isorhamnetin was reported (10).

The present work yielded three polymethoxy flavones not previously reported as constituents of this plant.

EXPERIMENTAL

Plant Material—The flowering heads of *A. millefolium* were harvested in June 1970 at the University of Illinois Drug and Horticultural Experimental Station, Downers Grove, Ill. The seeds of wild growing *A. millefolium* were collected in the Bemis Woods, McHenry County, Ill., and were planted in a greenhouse at the Station. Shoots were transplanted from one generation to the next to assure that material of the same species was maintained. Only the inflorescence of the plant was used and was harvested at the time of opening of the flowers. The plant was identified as *A. millefolium* by chromosome count[1].

Extraction—One kilogram, 500 g at a time, of air-dried, coarsely ground plant material was extracted in a soxhlet extractor. Each 500-g portion was extracted for 24 hr with 3400 ml of petroleum ether, the extract was removed, 2500 ml of fresh solvent was added, and the extraction was continued for an additional 48 hr. All solvents were combined, and an insoluble precipitate, F1, was filtered off and set aside. The filtrate was condensed by evaporation to 1500 ml and again filtered, yielding another precipitate, F2.

The precipitate F1 was crystallized from ligroin, and the filtrate was combined with F2. The crystals were triturated with ether, and the filtrate again was added to F2. Finally, the crystals were recrystallized from chloroform–ether to yield 3.75 g of achillin, a sesquiterpene lactone previously isolated from this plant (11), and this filtrate was combined with F2.

[1] Performed by Dr. Edward D. Garbner, Department of Botany, University of Chicago.