Acid Hydrolysis of 1,6-Dihydro-4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (1,6-Dihydrometamitron)

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Metamitron (**1**) does not undergo hydrolysis at pH 1−8 and up to 5 M H_2SO_4. The product of its two-electron reduction, 1,6-dihydrometamitron (**2**), on the other hand, undergoes at pH <3 relatively fast hydrolysis. The dependence of the measured rate constant on acidity indicates that the completely protonated form (AH_2^{2+}) predominating in strongly acidic media undergoes hydrolysis slower than the species bearing one less proton (AH^+). The latter most reactive species is present in highest concentration in solutions of pH between 0 and 2. This species is protonated on the 2,3-azomethine bond and yields as final products 2-hydrazino-2-phenylacetic acid (**4**) and acethydrazide (**5**). Kinetic, polarographic, and spectrophotometric measurements indicated for the first dissociation an average value $pK_a = -0.8$, for the second $pK_a = 0.95$. These observations together with the easy reduction of the 1,6-bond in metamitron (**1**) indicate that in nature the cleavage of metamitron may be preceded by its reduction to 1,6-dihydrometamitron (**2**), which is then hydrolyzed. Thus, anaerobic, reductive conditions are likely preferable for the total microbial degradation of metamitron.

Keywords: *Metamitron; 1,6-dihydrometamitron; as-triazine; acid hydrolysis; pesticide; herbicide cleavage; polarography; UV spectra*

INTRODUCTION

Biodegradation of numerous pesticides involves hydrolysis, which can be enzymatically catalyzed. Hydrolysis is undoubtedly directly involved in the cleavage of pesticides containing ester groups or groupings which resemble esters (Ecobichon, 1979). Hydrolysis (especially in acidic pH) as a degradation path has also been assumed to play a prominent role in the degradation of various pesticides containing a 1,2,4-triazine ring (Savage, 1977; Hance and Haynes, 1981; Pettygrove and Naylor, 1985; Allen and Walker, 1987; Kozak and Pavel, 1990; Locke and Harper, 1991a,b; Locke et al., 1994; Conn et al., 1996; Koskinen et al., 1996). Among these pesticides there are two related compounds, metamitron (**1**) [4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] and metribuzin [4-amino-3-methylthio-6-(1,1-dimethylethyl)-1,2,4-triazin-5(4*H*)-one]. The first has been used since 1973 (Coble and Schrader, 1973) in the U.S. and is still widely used in Europe (Fuehr and Mittelstaedt, 1979; Allen and Walker, 1987; Scholz et al., 1988; Parekh et al., 1994) to control broad leaf weeds in tomato and potato crops. The fate of metamitron in soil has been established mostly by following the decrease of the pesticide concentration with time, using radiolabeled herbicides (Scholz et al., 1988) or measuring the evolved CO_2 (Jarczyk, 1976; Fuehr and Mittelstaedt, 1979). The latter process suggested cleavage involving ring opening. Nevertheless, no evidence concerning the reaction pathway, intermediates, and products has been reported (Roberts, 1998), and the importance of an oxidation−reduction process in the course of degradation of such pesticides has not been—to our best knowledge—so far considered.

In the course of investigation of the electroreduction of metamitron (Ludvík et al., 1998a), we observed that compound **1** is in acidic media (up to 5 M H_2SO_4) stable over a period at least of several hours. Nevertheless, the azomethine bonds present in the triazine ring in positions 1,6 and 2,3 are relatively easily reduced. Electrochemically the reduction of the 1,6-C=N bond is more easy and occurs at potentials by about 0.6 V more positive than that of the 2,3-C=N bond (Ludvík et al., 1998a). On the other hand, a selective reduction of the 2,3-C=N bond of **1** can be achieved chemically using sodium borohydride (Draber et al., 1976; Ludvík et al., 1998a) (Scheme 1). Both reduction products, namely 1,6-dihydrometamitron 1,6-dihydro-4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (**2**) and 2,3-dihydrometamitron 2,3-dihydro-4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (**3**), undergo acid hydrolysis as has been demonstrated by the variation of polarographic currents with time (Ludvík et al., 1998a). It seems plausible that in soil reductases also attack the more easily reducible site yelding the 1,6-dihydro product. Hence, the investigation of the acid-catalyzed hydrolysis of 1,6-dihydrometamitron (**2**), using polarographic and spectrophotometric techniques, is reported in this study.

EXPERIMENTAL SECTION

Apparatus. DC-polarographic curves were recorded with an IBM Voltammetric Analyzer EC/225 using a dropping mercury electrode ($h = 74$ cm, $t = 2.4$ s) in a Kalousek cell with a liquid junction connection to SCE.

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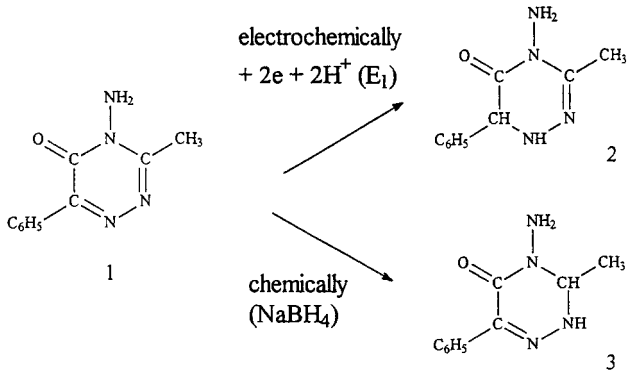
Scheme 1



UV−vis spectrophotometric data were obtained using the spectrometer Perkin-Elmer Lambda 19 in a 10 mm cell.

Chemicals, Reagents, and Solutions. *Metamitron* (**1**) [4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] was isolated by extraction from a sample of commercially available pesticide GOLTIX WP 70 and four times recrystallized from ethanol, mp 168.5−169 °C [Draber et al. (1976): 169 °C]. The identity was verified by comparison with ^{1}H and ^{13}C NMR spectra reported by Draber et al. (1976), and purity was checked by HPLC.

*1,6-Dihydrometamitron (**2**) [4-Amino-3-methyl-6-phenyl-1,6-dihydro-1,2,4-triazin-5(1H)-one].* To a solution of 1 g (0.005 mol) of metamitron(I) in 50 mL of acetonitrile was added 200 mL of an aqueous solution of 0.1 molar acetate buffer, pH 4.7. Electrolysis at −1.1 V was carried out in an argon atmosphere until 953 Coulombs passed (theoretical consumption, 946 C). The white precipitate formed (550 mg, 55%) had a mp 193.5 °C [recrystallized from methanol, in agreement with Riedl et al. (1996)]. ^{1}H NMR (DMSO-d_6): δ 2.04 (s, 3H, CH_3), 4.64 (s, 1 H, H_6), 5.00 (s, 2 H, NH_2), 6.91 (s, 1H, NH), 7.30−7.33 (m, 5 H, C_6H_5). ^{13}C NMR (DMSO-d_6): δ 17.1 (CH_3), 60.6 (C_6), 127.7 (C'_4), 127.8 ($C_{3,5}$), 127.8 ($C_{2,6}$), 136.5 (C_1), 146.6 (C_3), 165.3 (C_5). [These spectra are more detailed than those reported by Olmedo et al. (1994) and fully consistent with them.]

Anal. Calcd for $C_{10}H_{12}N_4O$: C, 58.29; H, 5.87; N, 27.19. Found: C, 58.23; H, 5.87; N, 27.61.

All acidic solutions were prepared by dilution of H_2SO_4 (Baker analyzed 96.5%).

Procedure. *Polarography.* Using a dropping mercury electrode in an electrolytic cell containing a reference calomel electrode, the voltage applied to these two electrodes was gradually increased. Current flowing between these two electrodes is recorded as a funcion of applied voltage. When resulting current−voltage curves are recorded, in the presence of reducible substances, so-called polarographic waves are observed. The limiting current and the potentials at which the current reaches one-half of its limiting value (the half-wave potentials) are measured at varying pH values. From their changes with pH, it was possible to deduce that 1,6-dihydrometamitron (**2**) undergoes in acidic media polarographic reduction in a single wave at −0.86 V, the half-wave potential of which at pH <1 is practically pH independent. This wave corresponds to the reduction of the protonated form of the azomethine bond in position 2,3 (Ludvík et al., 1998a).

For kinetic experiments, 10 mL of the solution of sulfuric acid was transferred to an electrolytic cell thermostated at 22 °C. After purging the supporting electrolyte by nitrogen for 3 min, 30 μL of 0.01 M freshly prepared stock solution of the triazine was added using a Hamilton or an Eppendorf pipet to a final concentration of 3×10^{-5} M. The mixture was purged for further 30 s and the current−voltage curve was recorded with a scan rate of 5 mV/s. In preliminary experiments, complete current−voltage curves were recorded, but as the half-times of studied reactions were between 10 and 100 min, only the current at −1.1 V was later measured.

Spectrophotometry. Preparation of reaction mixtures followed the same pattern as for polarography, but oxygen was not removed. Spectra were recorded from 600 to 200 nm within 1 min at 24 °C.

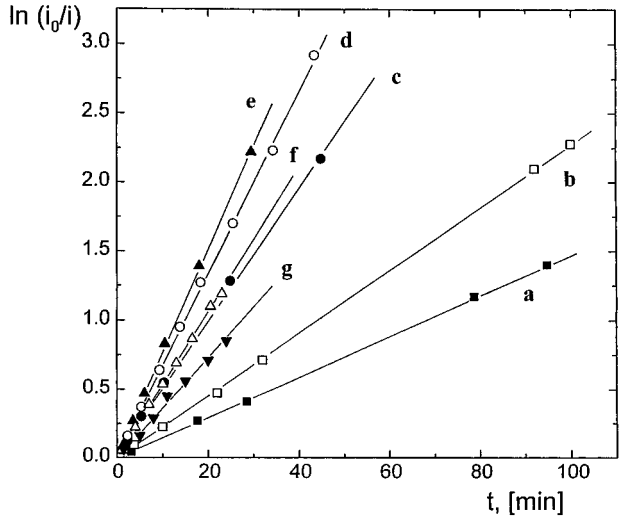


Figure 1. Hydrolysis of 2×10^{-4} M 1,6-dihydrometamitron (**2**) followed by polarography at 22 °C. Dependence of reduction rate on acidity. Plots of ln $i = f(t)$ in the following concentrations of sulfuric acid: (a) 0.01 M; (b) 0.03 M; (c) 0.1 M; (d) 0.3 M; (e) 1 M; (f) 2 M; (g) 4 M.

Identification of Products. For preparative hydrolysis, 18 mg of 1,6-dihydrometamitron (**2**) was dissolved in a small volume of ethanol added to 10 mL of 1.00 M H_2SO_4. The reaction mixture was after 1 h neutralized by sodium hydrogen carbonate to pH 6.8. After evaporation, the residue was twice extracted by a small volume of ethanol and dried (after filtration). The residue was dissolved in 0.5 mL of $CDCl_3$, to which 0.1 mL of d_6-DMSO was added. ^{1}H and ^{13}C NMR spectra were recorded using Varian Unity 200 spectrometer (200.057 MHz for ^{1}H and 50.306 MHz for ^{13}C). NMR analysis of the mixture (which was not separated due to the small amount available) indicated that the product contained only two chemical individuals (as proved by TLC): acethydrazide (proved by identity of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample) and 2-hydrazino-2-phenylacetic acid identified by its ^{1}H and ^{13}C NMR spectrum below.

*2-Hydrazino-2-phenylacetic Acid (**4**).* ^{1}H NMR: δ 4.59 (s, 1H, CH); 7.31−7.45 (m, 7H, 5 ArH, 2 NH_2); 9.05 (bs, 2H, COOH, NH). ^{13}C NMR: δ 67.5 (d, CH); 127.5 (d) and 128.0 (d, 5 x ArCH); 136.0 (s, ArC); 170.3 (s, CO).

*Acethydrazide (**5**).* ^{1}H NMR: δ 1.90 (s, 3H, CH_3); 4.01 (s, 2H, NH_2); 4.78 (s, 1H, NH). ^{13}C NMR: δ 20.3 (q, CH_3); 169.5 (s, CO).

Evaluation of Data. Both for polarography and spectrophotometry, the values of experimental rate constants k' (s^{-1}) were obtained from slopes of semilogarithmic plots. To evaluate the changes of absorbances with the time, a software MICROCAL ORIGIN, Version 4.10 (Microcal), was used enabling recording of plots of $A = f(t)$ at various wavelengths as well as $A_0 = f$(pH). Linearity of the ln $A = f(t)$ plots has been observed at all wavelengths, but the highest accuracy was obtained by using absorbance at 210, 220, and 240 nm for computations of the rate constant. The mentioned software was also used for nonlinear curve fitting of equilibrium data to obtain pK_a and for evaluation of rate and equilibrium constants from the dependence of measured rate constants (k'_h) on acidity.

RESULTS AND DISCUSSION

The cathodic polarographic wave of 1,6-dihydrometamitron (**2**) decreases in acidic media with time. The decrease which follows first-order kinetics (Figure 1) is not accompanied by an increase either of a cathodic nor of an anodic wave in the acidic medium used. The strictly first-order kinetics was verified by the indepen-

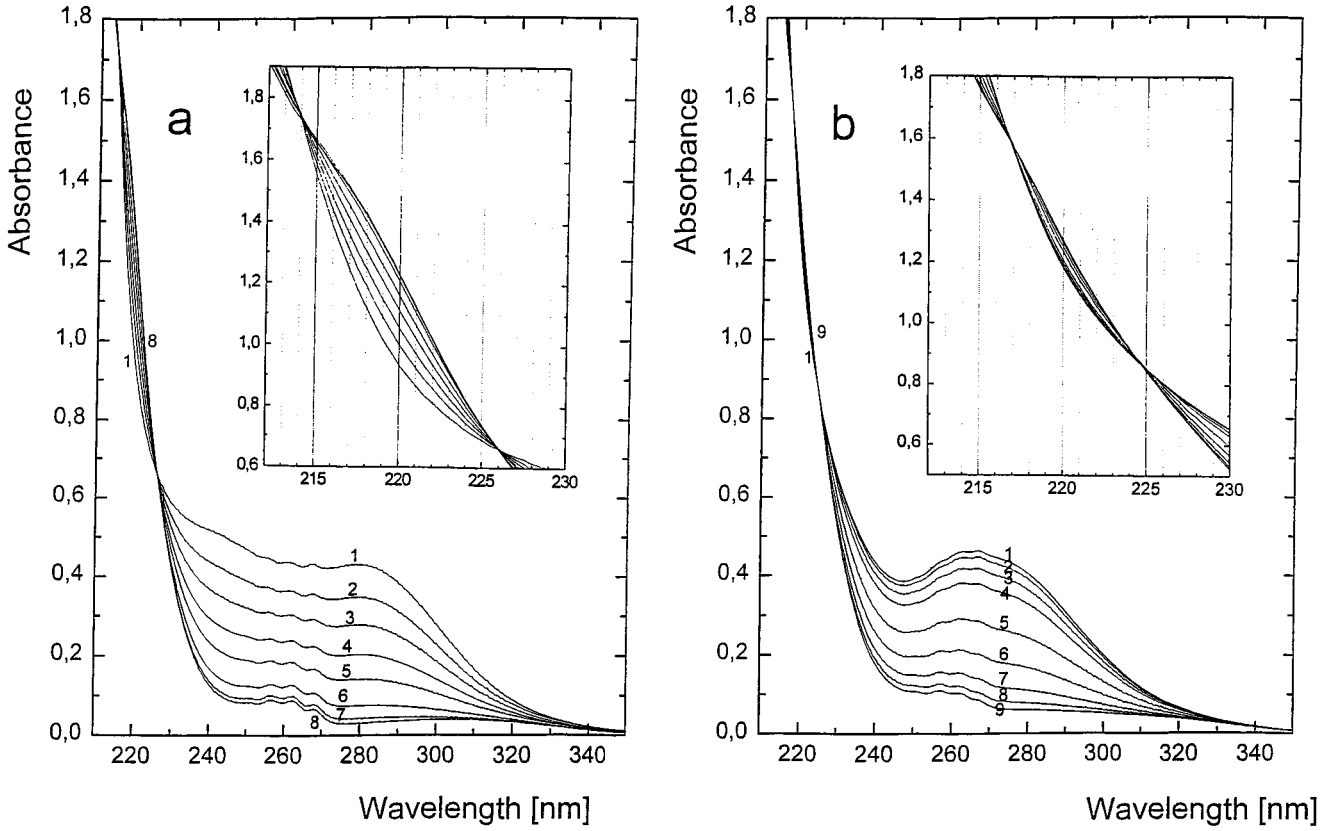


Figure 2. Hydrolysis of 2×10^{-4} M 1,6-dihydrometamitron (**2**) followed by spectrophotometry. (a) Time-dependence of spectra in 1 M H_2SO_4 at reaction times: (1) 90 s; (2) 240 s; (3) 420 s; (4) 660 s; (5) 960 s; (6) 1560 s; (7) 2460 s; (8) 5460 s. (Inset) Detail of spectra in the 212−230 nm range. (b) Time dependence of spectra in 0.03 M H_2SO_4 at reaction times: (1) 40 s; (2) 200 s; (3) 500 s; (4) 920 s; (5) 2120 s; (6) 3620 s; (7) 5420 s; (8) 7220 s; (9) 9020 s. Insert: Detail of spectra in the 212−230 nm range.

Table 1. Rate Constant of Hydrolysis of 1,6-Dihydrometamitron (2) at Varying Initial Concentrations of the Reactant (0.3 M H_2SO_4, 22 °C)

1,6-dihydrometamitron concentrated (mol $L^{-1} \times 10^{-5}$)	k'_h ($s^{-1} \times 10^3$) polarography
1.0	1.00
3.0	1.04
7.5	1.06
20	1.00

dence of the value of the rate constant of the initial concentration of the substrate (Table 1).

In the course of hydrolysis, absorption spectra of 1,6-dihydrometamitron (**2**) show a decrease of absorbance between 230 and 320 nm and an increase of absorbance at about 220 nm with time. Presence of isosbestic points (i.e., points where the absorbance remains time-independent) at 226 and 214 nm (at sulfuric acid solutions more concentrated than about 1.0 mol L^{-1}) or 225 and 217 nm (in less acidic solutions) (Figure 2) indicates simple kinetics (as for competitive or consecutive reactions isosbestic points are usually not observed). As mentioned above, absorbance at 236, 265, and 325 nm were used for obtaining of the first-order rate constant (k'_h).

Variation of the value of the experimentally obtained rate constant k'_h with acidity in solutions of sulfuric acid with acidity (Table 2, Figure 3) indicates that the rate-determining steps are preceded by two rapidly established acid−base equilibria. The form which predominates in the solution at pH >3 is denoted as *A*. In the hydrolysis, the monoprotonated form AH^+ and the diprotonated one AH_2^{2+} are involved. The monoprotonated form AH^+ undergoes hydrolysis faster with a rate constant k_2, the diprotonated form AH_2^{2+} slower with rate constant k_1, following the reaction scheme corresponding to eq 1:

$$\begin{array}{ccccc} AH_2^{2+} & \overset{-H^+}{\underset{K_1}{\rightleftharpoons}} & AH^+ & \overset{-H^+}{\underset{K_2}{\rightleftharpoons}} & A \\ \downarrow k_1 & & \downarrow k_2 & & \end{array} \qquad (1)$$

For such scheme the dependence of the measured rate constant k'_h on activity of hydrogen ions follows eq 2:

$$k'_h = \frac{k_1 a_{H+}{}^2 + k_2 K_1 a_{H+}}{K_1 K_2 + K_1 a_{H+} + a_{H+}{}^2} \qquad (2)$$

Good fit of experimental data for k'_h obtained by polarography at 22 °C (Table 2) was found for $k_1 = (4.55 \pm 0.29) \times 10^{-4}$ s^{-1}, $k_2 = (15.23 \pm 0.69) \times 10^{-4}$ s^{-1}, $K_1 = 5.89 \pm 1.29$ mol L^{-1}, and $K_2 = 0.084 \pm 0.010$ mol L^{-1} (Figure 3). For data obtained by spectrophotometry at 25 °C, the best fit of experimentally found k'_h (Table 2) has been obtained for $k_1 = (8.86 \pm 1.27) \times 10^{-4}$ s^{-1}, $k_2 = (19.53 \pm 2.98) \times 10^{-4}$ s^{-1}, $K_1 = 6.19 \pm 5.32$ mol L^{-1}, and $K_2 = 0.20 \pm 0.07$ mol L^{-1}. The data obtained by the two independent techniques are in very good agreement, considering the small difference in temperature.

To exclude participation of the hydrogen sulfate anion or sulfuric acid in the hydrolytic process, preliminary studies of hydrolysis of **2** were carried out in perchloric

Table 2. Dependence of Rate Constants of Hydrolysis of 1,6-Dihydrometamitron (k'_h) on Acidity, 22 °C

H_2SO_4 concentrated (mol L^{-1})	pH, $H°$	k'_h ($s^{-1} \times 10^4$) polarography	k'_h ($s^{-1} \times 10^4$) spectrophotometry
0.01	1.75	2.5	1.2
0.03	1.5	3.8	2.5
0.1	1.0	8.3; 8.6[a]	5.4
0.3	0.5	11.6	13.4
1.0	−0.2	12.1; 12.8[a]	14.7
2.0	−0.85	9.5; 9.0[a]	14.3
3.0	−1.35		10.9
4.0	−1.84	6.2	
5.0	−2.28	4.6	9.2
7.0	−3.37	4.2	

[a] Repeated measurements.

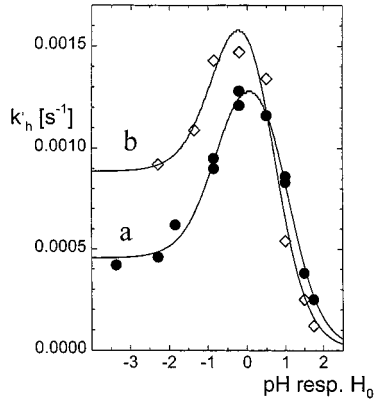


Figure 3. Dependence of measured first-order rate constants k'_h for the reaction of 2×10^{-4} M 1,6-dihydrometamitron on acidity at 22 °C, pH > 0, H_o < 0. (a) Reaction rate followed by polarography (experimental points). The curve was calculated using eq 2 with the following parameters: $k_1 = (4.55 \pm 0.29) \times 10^{-4}$ s^{-1}, $k_2 = (15.23 \pm 0.69) \times 10^{-4}$ s^{-1}, $K_1 = 5.89 \pm 1.29$ mol L^{-1}, and $K_2 = 0.084 \pm 0.010$ mol L^{-1}; (b) reaction rate followed by spectrophotometry (experimental points). The curve was calculated using eq 2 with the following parameters: $k_1 = (8.86 \pm 1.27) \times 10^{-4}$ s^{-1}, $k_2 = (19.53 \pm 2.98) \times 10^{-4}$ s^{-1}, $K_1 = 6.19 \pm 5.32$ mol L^{-1}, and $K_2 = 0.20 \pm 0.07$ mol L^{-1}.

Table 3. Hydrolysis of 1,6-Dihydrometamitron in Solutions of Perchloric Acid, 22 °C

$HClO_4$ concentrated (mol L^{-1})	pH, $H°$	k'_h ($s^{-1} \times 10^4$) polarography	k_{calc} ($s^{-1} \times 10^4$)[a]
0.1	1.0	5.3	5.0
0.4	0.0	7.15	7.7
2.0	−0.82	3.55	4.3

[a] Calculated from $k_{calc} = (k_1 a_{H+}^2 + k_2 K_1 a_{H+})/(K_1 K_2 + K_1 a_{H+} + a_{H+}^2)$ for $k_1 = 0.1 \times 10^{-4}$ s^{-1}; $k_2 = 10 \times 10^{-4}$ s^{-1}; $K_1 = 5.0$; $K_2 = 0.1$.

acid within acidity range from pH 1.0 to $H_o = -0.82$. [For strongly acidic and strongly alkaline solution, pH scale is replaced by acidity functions, for example, $H_o = -\log a_{H+}$. The values of such functions are obtained spectrophotometrically using indicators. They differ for solutions of individual strong acids and are tabulated (Boyd, 1969).] The change in concentration of 1,6-dihydrometamitron (**2**) in this medium also follows first-order kinetics and the dependence of measured values of rate constant k'_h on acidity (Table 3) is consistent with the eq 2. The smaller values of $k_1 = 1 \times 10^{-5}$ s^{-1}, $k_2 = 1 \times 10^{-3}$ s^{-1}, $K_1 = 5.0$ mol L^{-1}, and $K_2 = 0.1$ mol L^{-1} indicate difference in counterion effects, but may also be influenced by differences in used acidity functions which have to be considered only as a first approximation and may not strictly apply to such highly charged species.

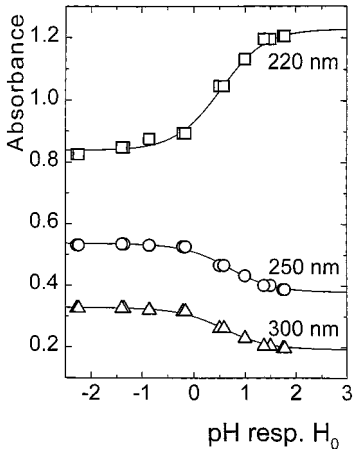


Figure 4. Dependence of absorbances (extrapolated to $t = 0$) on acidity (pH > 0, H_o < 0) in solutions of 3×10^{-5} M 1,6-dihydrometamitron: concentration of sulfuric acid: (1) 0 M, i.e., buffer pH 4.2; (2) 0.03 M; (3) 0.1 M; (4) 0.3 M; (5) 1 M; (6) 5 M. (Inset) Dependence of absorbance on pH and H_o at selected wavelengths.

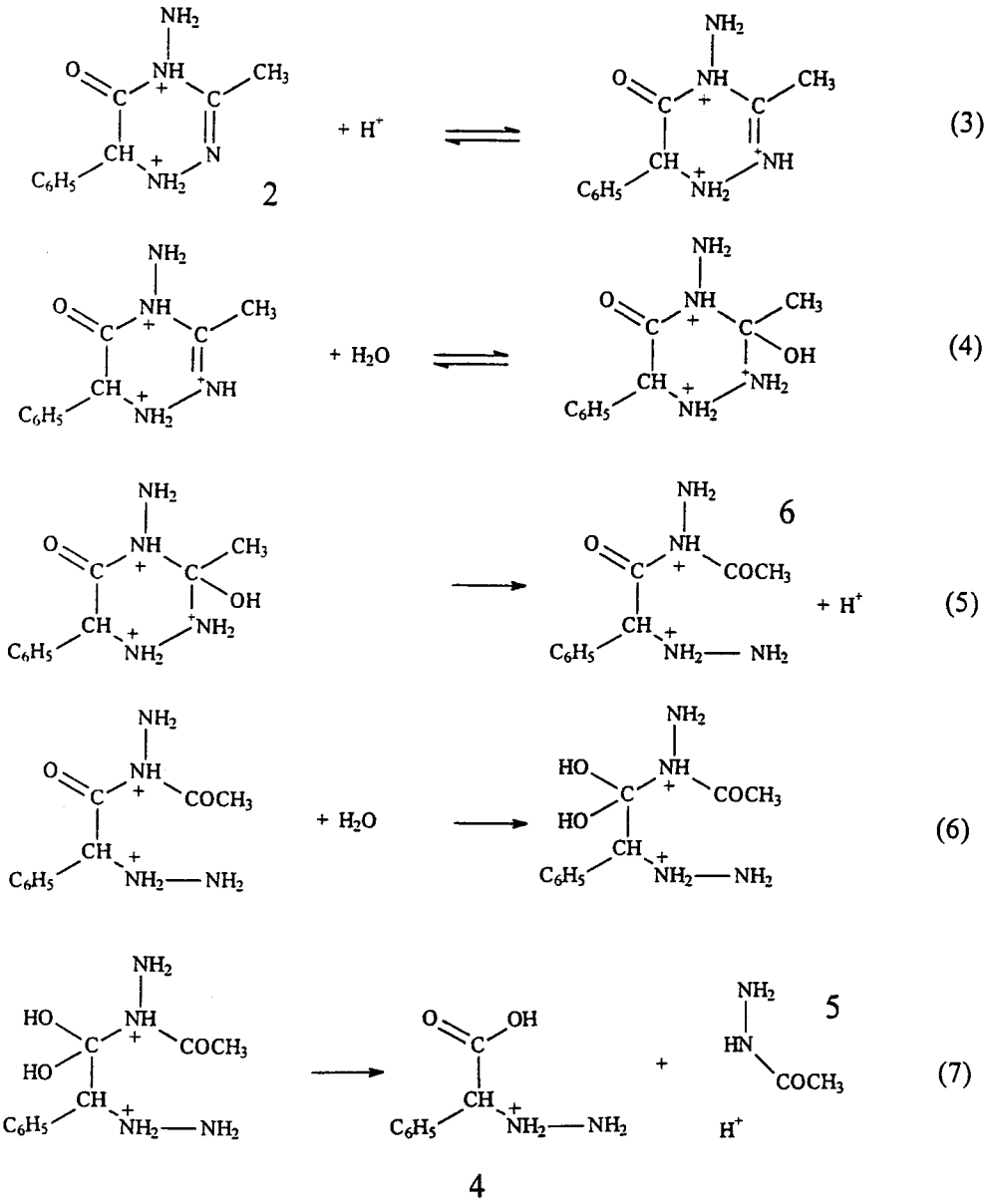
The value of $pK_2 = 1.07$ from kinetics followed by polarography and $pK_2 = 0.70$ from kinetic data obtained by spectrophotometry are in good agreement with $pK = 1.0$ obtained from the shifts of half-wave potentials of 1,6-dihydrometamitron (**2**) (Ludvík et al., 1998a) and $pK = 0.9$ obtained from the pH dependence of the absorbance at 325 nm extrapolated to $t = 0$ (Figure 4). This pK_2 is hence attributed to the protonation of the azomethine bond. The value $pK_1 = -0.77$ from kinetics followed by polarography and $pK_1 = -0.79$ from kinetics using spectrophotometry corresponds to the pK_a range in which the diprotonated forms of hydrazines dissociate (Perrin, 1965). Protonation of the amidic carbonyl cannot be, nevertheless, excluded.

At pH below 3, where this study has been carried out, both cyclic hydrazino nitrogens in position 1 and 4 exist in solution predominantly in the protonated form, as the pK_a values for $RN^+H_2NH_2 = RNHNH_2 + H^+$ are between 6 and 8 (Perrin, 1965). Arbitrarily for the hydrazino grouping in position 4, we assume that the protonation occurs preferably on the cyclic nitrogen rather than on the exocyclic one.

On the basis of the dependence of kinetics on acidity, the change of absorption bands in the UV spectra in the course of reaction and on identification of products **4** and **5** (see Experimental and later), it is possible to propose for the acid-catalyzed hydrolysis of 1,6-dihydrometamitron (**2**) the following reaction scheme in Scheme 2.

There is an alternative to protonation of the 2,3-azomethine group in eq 3, namely a protonation of the amidic carbonyl group in position 5, followed by a hydrolytic ring opening between C-5 and N-4. Following arguments can be presented against this alternative: (a) the azomethine group is generally more easily protonated than an amidic carbonyl (Perrin, 1965); (b) the shifts of half-wave potentials of the reduction of the 2,3-azomethine bond with pH indicate protonation of the azomethine group prior to the electron transfer (Ludvík et al., 1998a); (c) covalent addition of water to the 2,3-azomethine bond, assumed in reaction 4, has been proved (Ludvík et al., 1998a) by the decrease of the

Scheme 2



polarographic reduction current below a value corresponding to a two-electron diffusion controlled process; (d) pK_2 value obtained from kinetic data agrees well values obtained by polarography and spectrophotometry, which are attributed to protonation of the 2,3-C=N bond.

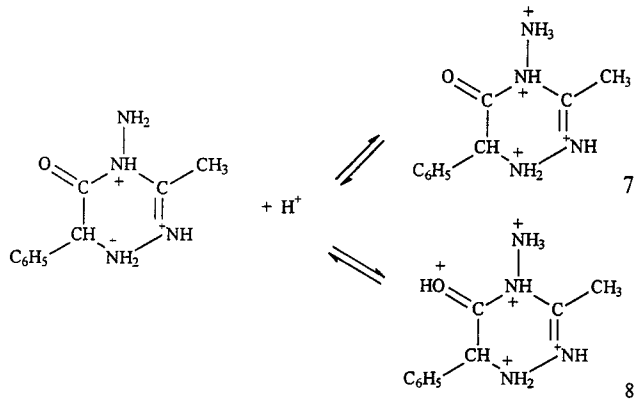
It is necessary to consider a second hydrolytic step (eqs 6 and 7), as 2-hydrazino-2-phenylacetic acid (**4**) has been, together with acethydrazide (**5**), identified as final products. Their formation is also in agreement with the retrosynthesis of **1**.

The second hydrolytic step is either faster than the first one or takes place during the manipulation of the sample for NMR. This is indicated by simple first-order kinetics, with no indication of two consecutive processes of comparable rate. The fast consecutive second hydrolytic process is in agreement with a change in the UV spectra during hydrolysis. The decrease of the absorption band centered at about 280 nm with molar absorptivity ϵ of the order of 10^3 L mol^{-1} cm^{-1} indicates a decrease in concentration of **2** with a benzene ring conjugated with the heterocyclic ring. Formation of a weaker absorption band centered around 260 nm (with ϵ of the order of 10^2 L mol^{-1} cm^{-1} with fine structure) is characteristic for a formation of a compound in which the benzene ring is not conjugated with an exocyclic group. This absorption spectrum is attributed to the 2-hydrazinyl-2-phenylacetic acid (**4**), confirmed by NMR. As the decrease in concentration of **2** follows strictly first-order kinetics, the intermediate **6** is not stable.

Hydrolysis of 1,6-dihydrometamitron (**2**) is an irreversible process. This has been proved by a hydrolysis in 0.1 M H_2SO_4 for 1 h, followed by adjustment of pH to 6 and later to 11.9. Absence of a reduction wave at either pH confirmed the irreversibility of hydrolysis. Anodic wave at −0.29 V observed at pH 11.9 indicates the presence of a hydrazino grouping that can be formed in alkaline solutions by hydrolysis.

The hydrolysis of the bisprotonated species (AH_2^{2+}) **7** and **8** in strongly acidic media is slower than that of the monoprotonated species AH^+ formed in reaction 3 ($k_1 < k_2$). We have no evidence indicating whether the second protonation occurs on the exocyclic nitrogen yielding **7** or on the 5-amidic carbonyl forming **8**

Scheme 3



(Scheme 3). We can offer no evidence if in this hydrolytic process the 2,3 or 4,5 bond is cleaved first. This excludes the possibility to offer an explanation why the hydrolysis of the diprotonated species is slower than that of the monoprotonated form.

Ring opening has been observed also for 1,2,4-triazine-4-oxides (Neunhoeffer and Boehnisch, 1976), but the cleavage occurs between C-3 and N-4. Presence of N → O in position 4 or absence of C=O in position 5 may cause the difference in mechanism.

CONCLUSIONS

As metamitron (**1**) is resistant to an acid-catalyzed hydrolysis, but 1,6-dihydrometamitron (**2**) is relatively easily hydrolyzed, it is proposed that in nature the hydrolysis of the pesticide is preceded by a reduction of the 1,6-azomethine bond. This conclusion is supported by the fact that the reduction of the 1,6-azomethine bond in metamitron (**1**) occurs at rather positive potentials comparable to those at which the reduction of nitro compounds occurs. A potential range between −0.2 and −0.8 V (depending on pH) means that reduction occurs relatively easily. It seems plausible that reductases which act on organic nitro compounds and contribute to the conversion of organic compounds in the ecosystem could reduce the 1,6-C=N bond in metamitron as well.

Effective microbial metabolism of nitroaromatics (e.g., trinitrotoluene) and nitroheterocycles (hexahydro-1,3,5-trinitro-1,3,5-triazine) occurs reductively (Boopathy et al., 1993; McCormick et al., 1981; Harvey et al., 1990; Preuss et al., 1993). Therefore, it is expected that similar nonspecific (Preuss et al., 1993) or specific reductive microbial pathways (Preuss et al., 1993) could promote the effective hydrolysis of the metamitron molecule.

The only suggestion of accumulation of this chemical in the environment is that the chemical is found in some surface waters. However, this is normally associated with immediate runoff events following spraying, not due to lack of metabolism in surface soils (Lundberg et al., 1995). Since this chemical is not known to accumulate in agricultural soils, it is expected that microaerophilic or localized anaerobic microenvironments in the soil foster the microbial degradation of this compound.

It is generally known that hydrolyses which in vitro occur only under extreme conditions can be facilitated by hydrolases under physiological conditions. It is thus possible that the hydrolysis of the 1,6-dihydrometamitron, which occurs in vitro at pH <3, takes place enzymatically in soil under natural conditions. Thus, in nature, anaerobic, reductive conditions are preferred for the complete cleavage of metamitron.

Preliminary experiments indicated that structurally related metribuzin, more frequently used in the U.S., shows a pattern of reduction (Ludvík et al., 1998b) and hydrolysis similar to that of metamitron. More detailed investigation of its hydrolysis is in progress.

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