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Synthesis and kinetic stability studies of progesterone derivatives

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Summary

The 3-potassium salt of carbomethoxyl amine derivative along with the betaine and pyridinium hydrazide chloride derivatives of progesterone were synthesized and their kinetics of decomposition in aqueous solution was studied. The rates of hydrolysis yielding progesterone was determined at 25 ° C over the pH range of 1–13 at an ionic strength of 0.5. The pH–rate profiles were accounted for by the specific acid-, base- and water-catalyzed spontaneous decomposition of these prodrugs. Maximum stability occurred at pH 6–7 in which region water was the principal catalyst in the decomposition pathway. Various buffer and temperature effects were also evaluated. In the slightly alkaline to basic medium the principal mode of prodrug degradation may be the cleavage of the carbon nitrogen bond by direct neucleophilic attack of the water molecule or the hydroxide ion at the 3-carbon of the A ring.

Introduction

Progesterone (4-pregnene-3,20 dione) I is an extremely hydrophobic steroid used exclusively for adjunctive therapy in menopause, endometrial hyperplasia, dysfunctional uterine bleeding, amenorrhea and premenstrual syndrome (Whitehead et al., 1980). Unfortunately the oral route of administration of progesterone has resulted in poor gastrointestinal absorption (Kinel et al., 1978; Wentz, 1981) and short biological half life (Sandberg and Slaunwhite; 1958). Poor aqueous solubility and high metabolic clearance rate (Little et al., 1966) are the major causes of its poor bioavailability. A potentially useful approach of increasing its bioavailability is the development of highly water soluble transient derivatives (prodrugs) with improved systemic absorption and reduced first-pass hepatic metabolism. Though some synthetic derivatives (e.g. medroxyprogesterone acetate, norgestrel, lynestreol ethylenediol diacetate) have been used orally, they suffer from a number of side effects which include depression, masculinization of the patient, headaches etc. (Morville et al., 1982; Huff, 1988).

In an effort to decrease the metabolism of progesterone, Bodor and Sloan (1982) synthesized a number of dithiazolidine prodrugs of progesterone derivatized at the 3 and 20 keto positions. However, all of the derivatives were found to be inactive or less active then their parent steroid in an animal model. This lack of activity

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has been explained by the rapid distribution of the thiazolidines out of blood into the tissues followed by a slow release of the parent drug such that the biological concentration of the steroid is always too low to be biologically effective.

Hydrazones of other keto steroids with betaine and pyridinium hydrazide chlorides did not produce any noticeable signs of toxicity in animals (Ward, 1953; Beckmuhl et al., 1945). This study has been undertaken in an effort to synthesize progesterone hydrazone prodrugs with improved aqueous solubility and reduced first pass metabolism and to study their stability with respect to pH, buffers and temperature.

Materials and Methods

Materials

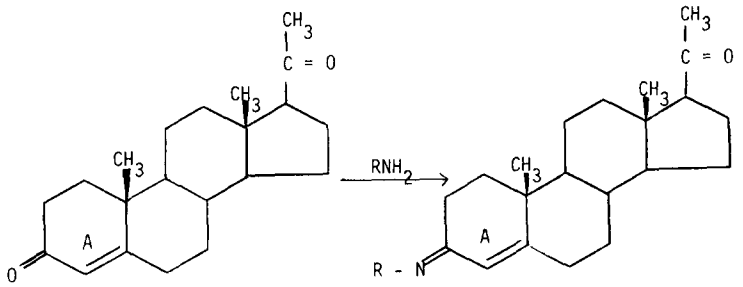
Progesterone, Girard reagents T and P and carboxymethoxylamine hemihydrochloride were supplied by Aldrich Chemicals, Milwaukee, WI. Buffer substances and all other chemicals were of reagent grade. Distilled, deionized water was used for the preparation of buffer solutions.

Apparatus

^{1}H-NMR spectra were run on a Varian XL-200 spectrophotometer using tetramethyl silane as internal reference. Melting points were taken on a Thomas Hoover capillary melting point apparatus and are uncorrected. Rate of degradation of derivatives were monitored by Beckman Du-7 spectrophotometer. The pH measurements were made at the temperature of the study using a Fisher Accumet Model 825 MP pH meter.

Preparation of progesterone betaine hydrazide chloride (II)

Progesterone (**I**, Scheme 1; 500 mg, 0.001 mol) was refluxed with betaine hydrazide chloride (290 mg, 0.001 mol) commonly called Girard reagent T (Girard and Sandulesco, 1936) in methanol, for about 1 h, the apparent pH of the solution being adjusted to 5 with acetic acid. For completion of the reaction it was left at room temperature overnight. The solution was neutralized with aqueous sodium hydroxide to pH 7 before the work up because the Girard products are known to be stable at pH 7 but are readily hydrolyzed in acid medium. The neutralized solution was diluted with water and extracted several times with diethyl ether to remove the unreacted progesterone. It has been found that the use of 1 : 1 mole ratio of the Girard reagent to drug generates a 3-mono hydrazide derivative while an excess of the reagent produces a mixture of the 3,20-mono- and di-hydrazide derivatives. Lyophilizing the aqueous solu-



I

II R = $(CH_3)_3N^+CH_2CONH-$ Cl^-

III R = $C_5H_5N^+CH_2CONH-$ Cl^-

IV R = $K^{+-}OOCCH_2O-$

Scheme 1.

tion to dryness, however, yielded progesterone betaine hydrazide chloride in 85% yield. Recrystallization from the methanol–benzene mixture yielded a solid with m.p. 120 °C. Anal. calcd. for $C_{26}H_{40}O_2N_3Cl$: C, 67.52; H, 8.72; N 9.09; Cl, 7.67. Found: C, 67.48; H, 8.75; N, 9.08; Cl, 7.60. Molar absorptivity at λ_{max} (281 nm) 3157.

Preparation of progesterone pyridinium acetohydrazide chloride (III)

Progesterone (500 mg, 0.001 mol) was refluxed with pyridinium acetohydrazide chloride (187 mg, 0.001 mol) commonly known as Girard reagent P, in 50 ml of methanol at pH 5. The reaction was carried out in the same manner as described for compound **II**. The usual workup however yielded 80% of progesterone pyridinium acetohydrazide chloride (**III**). Recrystallization from methanol–benzene mixture yielded a solid with melting point 75 °C. Anal. calcd. for $C_{28}H_{36}O_2N_3Cl$: C, 70.05; H, 7.55; N, 8.75; Cl, 7.38. Found: C, 70.01; H, 7.60; N, 8.70; Cl, 7.32. Molar absorptivity at λ_{max} (281 nm) 3947.

Preparation of potassium salt of carboxymethoxyl amine of progesterone (IV)

A solution (500 mg, 0.001 mol) of progesterone and (0.416 g, 1.2 mol) of *O*-carboxymethoxylamine hemihydrochloride in 50 ml of ethanol containing 3 ml of 2 N KOH was refluxed for 3 h (Erlanger et al., 1959). The reaction mixture was reduced to a small volume under vacuum and water was added. The pH was adjusted to 10–10.5 with 2 N KOH. After being extracted twice with ethyl acetate to remove the unreacted progesterone the aqueous phase was lyophilized to obtain in 85% yield, the potassium salt of the carboxymethoxyl amine of progesterone. Recrystallization from methanol–chloroform mixture yielded a solid with melting point 250 °C. Anal. calcd. for $C_{23}H_{32}O_4NK$: C, 64.90; H, 6.71; N, 2.91; K, 8.14. Found: C, 64.89; H, 6.75; N, 2.90; K, 8.16. Molar absorptivity at λ_{max} (266 nm) 1578.

Solubility determinations

A quantity of each derivative exceeding the amount required to produce a saturated solution was shaken for about 2 days in 4 ml vials containing 2 ml of distilled deionized water at 25 ± 0.5 °C. Each sample was then filtered through a 0.45 μm cellulose acetate Millipore filter. The filtrate was collected in fractions for analysis to ensure that there was no misleading solubility measurements resulting from adsorption onto the filter. Filter adsorption was assumed to be negligible when the concentrations of successive fractions agree within ±5%. Table 1 gives the solubilities of different prodrugs at pH 7.4.

TABLE 1

Aqueous solubility of progesterone and its prodrugs at pH 7.4

Compound	Molar solubility (mean ± S.D.) × 10^2
I	0.0006 ± 0.0002
II	4.97 ± 0.05
III	5.83 ± 0.10
IV	9.44 ± 0.08

$n = 3$.

Kinetic studies

All the kinetic studies were performed in aqueous buffer solutions at 25 °C. The degradation of the prodrugs was monitored by ultraviolet spectrophotometric method. The following buffers were used: at pH < 3, hydrochloric acid; at pH 3–5, citrate; at pH 5–6, acetate; at pH 7–8 phosphate; at pH 9–10, borate and at pH > 10 sodium hydroxide. Citrate buffer at pH 5 was only used for the buffer catalysis studies. A constant ionic strength of 0.5 M was maintained for each buffer by adding an appropriate amount of sodium chloride. The compounds **II** and **III** were measured at 281 nm whereas 266 nm was the wavelength of choice for compound **IV**. Studies within the pH range of 1–3 and 10–13 were done directly in the cuvettes of the UV spectrophotometer because the reactions were very fast and sampling was difficult. Absorbance was continuously monitored against time in the thermostatically controlled sample compartment at the analytical wavelength. The initial reactant concentration was 38 mM. For fast reactions, results were obtained for 4–5 half-lives, for other relatively slower reactions (pH 6 and 7) data were obtained for at least 3 half-lives.

Linearity of the logarithm of reactant concentration vs time plots was evident over the time duration of sampling. Buffers in the concentration range of 10–90 mM were employed and the buffer-independent rate constants were used for the construction of pH-rate profiles. Standard curves constructed by plotting concentration against absorbance were linear with correlation coefficient of 0.997–0.999 in all cases.

Results and Discussion

Table 1 indicates that the compounds **II**, **III**, and **IV** have solubilities in the increasing order and all of them are more than 3 orders of magnitude more soluble in aqueous phase than **I** (progesterone).

Kinetic studies

The kinetic studies were performed by following the decrease in absorbance at 281 nm for the compounds **II** and **III** and at 266 nm for compound **IV**. The choices of the wavelengths were made such that interference due to the absorbance of progesterone at that wavelength could be eliminated totally as molar equivalent amounts of progesterone is produced during the course of degradation.

The release of progesterone from the products were confirmed by an independent confirmatory test. After the complete decomposition of the products the solution was extracted with ether which on subsequent drying and purification by column chromatography gave a white solid m.p. 128 °C (Lit. 129–130 °C) identified as progesterone by TLC ($R_f = 0.7$) in (90 : 5) $CHCl_3$–acetone, m.p. and co-TLC with a standard compound. More over the ^{1}H-NMR showed the distinct shifts at δ9.28 (CH_3-18) and δ8.85 (CH_3-19) protons. The mass spectra gave the molecular ion at $-315(M^+)$, 300 (M-CH_3), 295 (M-H_2O), 281 (M-($H_2O + CH_3$), 271 (M-$COCH_3$) and 229 (M-D ring + H) corresponding to progesterone.

The observed pseudo-first-order rate constants (k_{obs}) for the overall degradation of **II**, **III** and **IV** were calculated from the slopes of the linear regression lines obtained by plotting the logarithm of the residual amount of each prodrug against time.

General acid–base catalysis

The hydrolysis of compounds **II** and **III** were found to be independent of the catalytic effects by most of the buffer species utilized in the present study except the citrate buffer. The buffer catalytic effects could be determined by measuring the rates of degradation at constant pH, ionic strength and temperature varying only the buffer concentration at a specific pH. The concentration of buffers studied were 1, 3, 6 and 9×10^{-2} M respectively at several pH values within the effective range of the buffers.

Typical plots exhibiting the catalytic effect of citrate buffer on compounds **II** and **III** at various pH values are shown in Figs. 1 and 2, respectively. There was however no catalytic effect of citrate buffer on compound **IV**. The graphs show linear relationships at constant pH in all cases. Extrapolation of such plots to zero buffer concentration provides as intercepts the pseudo-first-order rate constant k_{H^+}, corresponding to the non-buffer catalysed decomposition process. The observed rate constant k_{obs} may be mathematically expressed in the citrate buffer system as Eqn. 1:

$$k_{obs} = k_{H^+} + k_{H_3A}[H_3A] + k_{H_2A^-}[H_2A^-] + k_{HA^{2-}}[HA^{2-}] + k_{A^{3-}}[A^{3-}] \quad (1)$$

where k_{H_3A}, $k_{H_2A^-}$, $k_{HA^{2-}}$ and $k_{A^{3-}}$ are the second order catalytic rate constants associated with the undissociated mono-, di- and tri-ionized citrate acid species respectively. Since the graph shows that the catalytic effect was observed only between pH 3 and 6, the H_2A^- and HA^{2-} ions are the predominant catalytic species with the involved citrate pK_a of about 4.78. So Eqn. 1 can be rewritten in terms of total citrate concentration $[A]_T$, as shown by Eqn. 2:

$$k_{obs} = k_{H^+} + \left(k_{HA^{2-}} + \left(k_{H_2A^-} - k_{HA^{2-}}\right) f_{H_2A^-}\right) \cdot [A_T] \quad (2)$$

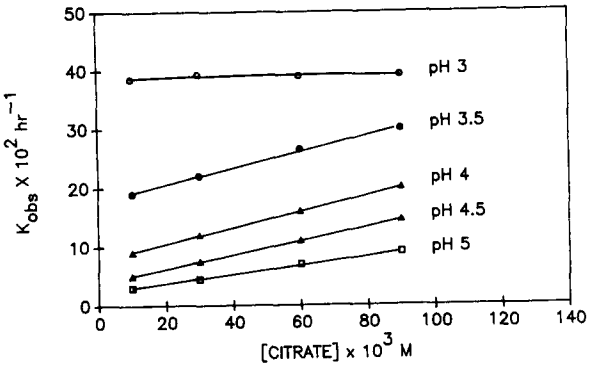


Fig. 1. The effect of citrate buffer concentration on the observed rate constants for the degradation of compound **II** at various pH values (25 °C; $\mu = 0.5$).

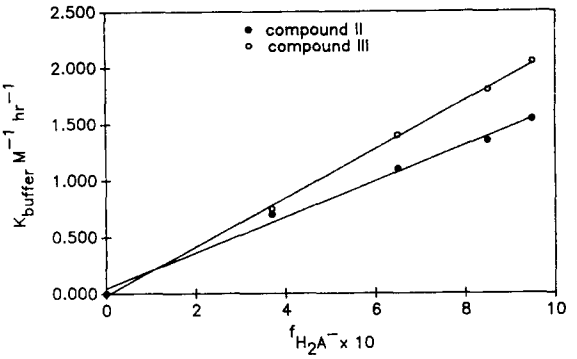


Fig. 3. Dependence of the apparent second-order rate constant for citrate buffer-catalyzed degradation of compounds **II** and **III** at 25 °C on the fraction of dihydrogen citrate buffer species in the buffers.

The quantity $f_{H_2A^-}$ refers to the fraction of the dihydrogen citrate buffer species in the solution. Slopes of the linear plots shown in Fig. 1 and Fig. 2 were calculated for each pH and plotted against the fraction of H_2A^- species at that pH. Such plots were also linear as shown in Fig. 3. The values of the second-order catalytic rate constants due to the H_2A^- and HA^{2-} buffer species for the compounds **II** and **III** have been calculated from plots in Fig. 3 and presented in Table 2.

Effect of temperature

The effect of temperature on the hydrolysis of these prodrugs were determined by measuring the rate constants at 40, 50, 60 and 70 °C in 0.01 M phosphate, acetate and borate buffers over the pH range of 6–10 ($\mu = 0.5$). The logarithm of the observed rate constants were plotted against the reciprocal of the absolute temperature according to the Arrhenius Eqn. 3:

$$\log k_{obs} = \log A - \frac{E_a}{2.303\,RT} \qquad (3)$$

The term log A, the intercept of the plots represents the logarithm of the collision frequency and E_a is the activation energy required for the reaction. Fig. 4 exhibits such plots for compounds **II** and **III** at pH 7. The activation energies of the compounds **II** and **III** at different pH values were calculated from the slopes of similar individual plots as shown in Fig. 4 and are given in Table 3.

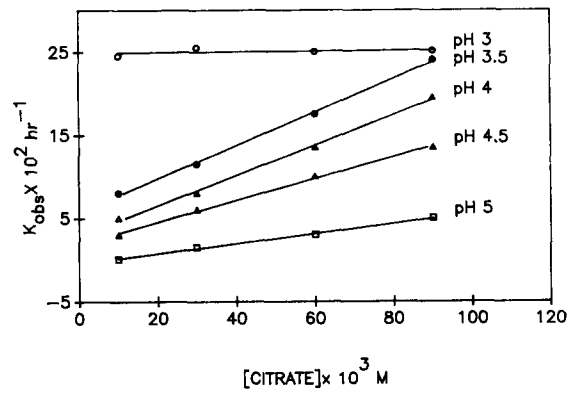


Fig. 2. The effect of citrate buffer concentration on the observed rate constants for the degradation of compound **III** at various pH values. (25 °C, $\mu = 0.5$).

pH–rate profile

The pH dependence of the overall rate of degradation of compounds **II** and **III** at 25 °C and ionic strength of 0.5 M is shown in Fig. 5. Com-

TABLE 2

Catalytic rate constants for the mono- and dihydrogen citrate ions for compounds **II** *and* **III**

Compound	$k_{H_2A^-}$ (M^{-1} h^{-1})	$k_{HA^{2-}}$ (M^{-1} h^{-1})
II	1.36	0.19
III	2.17	0

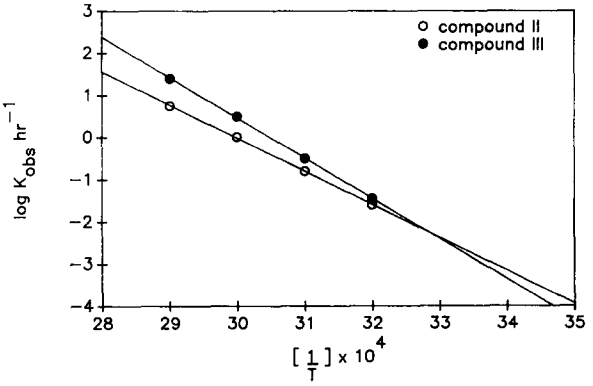


Fig. 4. Arrhenius-type plot of the logarithm of the observed pseudo-first-order rate constants against the reciprocal of absolute temperature for the overall degradation of compounds **II** and **III** at pH 7 ($\mu = 0.5$).

TABLE 3

Activation energies of compounds **II** *and* **III** *at various pH values*

pH	E_a in kcal/mol	
	II	**III**
6	46.0	32.50
7	46.2	32.58
8	46.05	32.52
9	46.02	32.48
10	45.89	31.00

pound IV was very stable at pH 4–10 even at elevated temperatures; therefore the rate profile for compound **IV** in Fig. 6 has been omitted

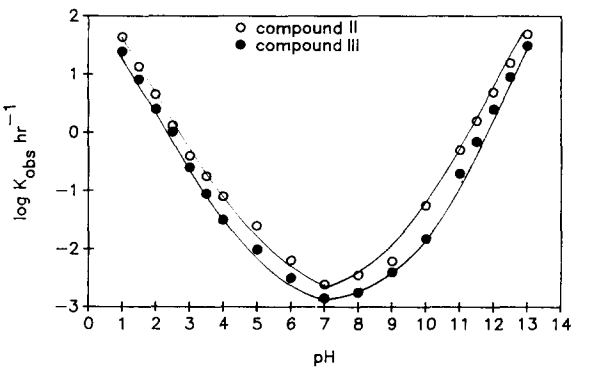


Fig. 5. Log k_{obs}-pH profile for the degradation of compounds **II** and **III** in aqueous solution at 25 °C ($\mu = 0.5$) where k_{obs} is the pseudo-first-order rate constant for decomposition in buffer free solutions.

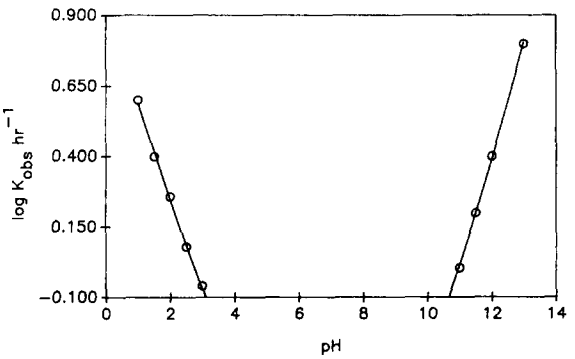


Fig. 6. Log k_{obs}–pH profile for the degradation of compound **IV** in aqueous solution at 25 °C ($\mu = 0.5$) where k_{obs} is the pseudo-first-order rate constant for decomposition in buffer free solutions.

within that pH range. There was no observable catalytic effect of the acetate, borate or the phosphate buffer species. However, catalysis by citrate buffer species in the pH range of 3–6 was observed for compounds **II** and **III** as shown in Figs. 1 and 2. The rate constants used in the construction of the rate profile in this pH region have been obtained from the intercepts of the plots of k_{obs} vs total buffer concentrations. Results from studies performed in dilute hydrochloric acid (pH < 3) and sodium hydroxide (pH > 10) solutions have also been incorporated in the pH–rate profiles.

The shape of the pH rate profiles for the compounds suggest that the following reactions contribute to the overall velocity of degradation;

Reactions	Rate constants	
$A + H^+ \rightarrow$ products	k_1	(4)
$A + H_2O \rightarrow$ products	k_2	(5)
$A + OH^- \rightarrow$ products	k_3	(6)

where A represents quaternized progesterone prodrugs **II**, **III** and **IV**. The total experimental rate equation for the degradation becomes:

$$\text{rate} = k_{obs}[A] \tag{7a}$$

Also,

$$\text{rate} = k_1[A][H^+]^a + k_2[A][H_2O] + k_3[A][OH^-]^b \tag{7b}$$

Equating equations 7a and 7b,

$$k_{obs} = k_1[H^+]^a + k_2[H_2O] + k_3[OH^-]^b \quad (8)$$

where a and b are the orders of hydrogen ion and hydroxide ion concentration, respectively, and k_1, k_2, and k_3 are the rate constants for the specific acid-catalyzed, water-catalyzed and specific base-catalyzed reactions, respectively.

At low pH, $[H^+]$ is very large, therefore

$$k_{obs} = k_1[H^+]^a \quad (9)$$

or

$$\log K_{obs} = \log k_1 - a\ pH \quad (10)$$

A plot of log k_{obs} against pH was linear for all compounds **II** and **III** below pH 4 with slopes (a) of negative unity suggesting single order dependance of the reaction rate on hydrogen ion concentration. The values of k_1 for the compounds **II**, **III** and **IV** have been calculated according to Eqn 10 and found to be 4.57×10^2, 2.45×10^2 and 8.1 $M^{-1}\ h^{-1}$, respectively.

Within the pH range of 4–10, water-catalysed spontaneous degradation reaction dominates the degradation pathway. Eqn. 11 describes the degradation reaction

$$k_{obs} = k_2[H_2O] \quad (11)$$

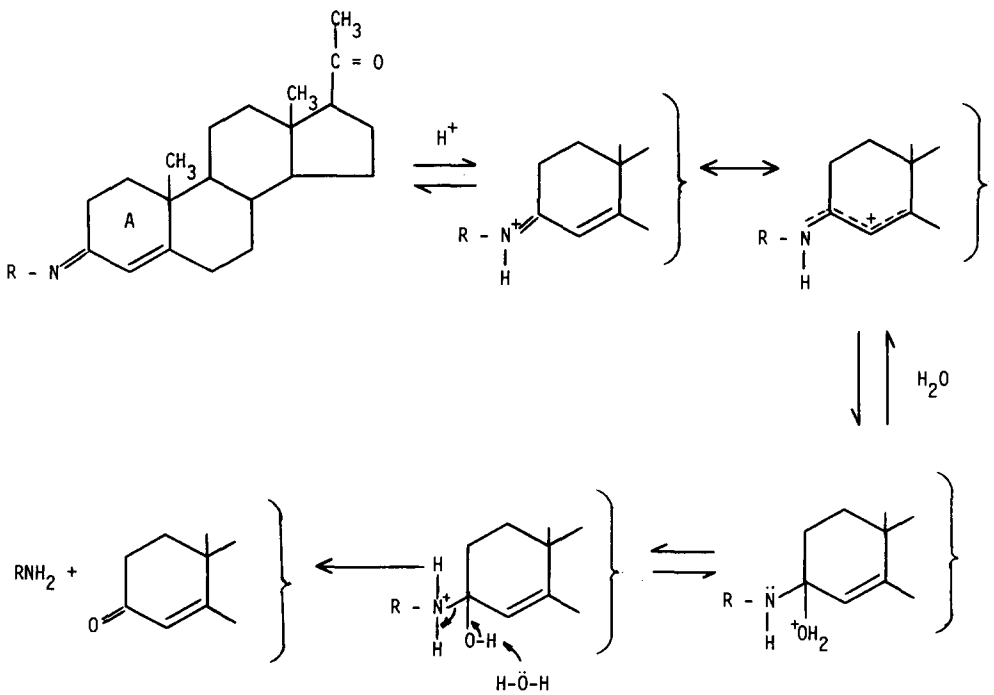
The values of k_2 for compounds **II** and **III** have been found to be $4.42 \times 10^{-5}\ M^{-1}\ h^{-1}$ and $2.55 \times 10^{-5}\ M^{-1}\ h^{-1}$, respectively. The observed rate constant for the degradation of the compounds above pH 10 may be written as:

$$k_{obs} = k_3[OH^-]^b \quad (12)$$

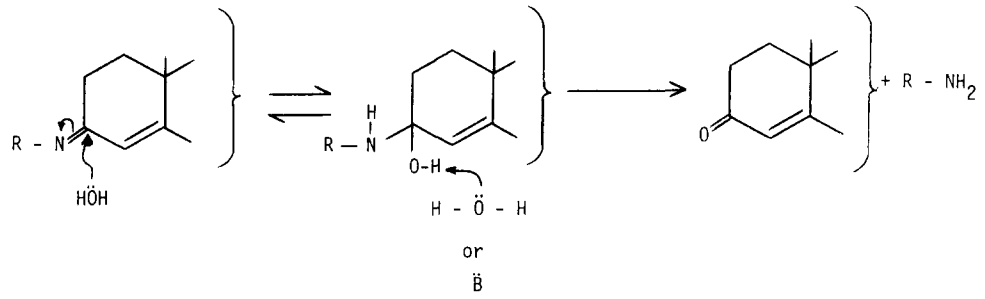
Eqn. (12) can be written as a function of proton concentration as shown in Eqn. (13):

$$k_{obs} = k_3 \frac{(K_w)^b}{[H^+]^b} \quad (13)$$

$$\log k_{obs} = \log K_w + b \log K_3 + b\ pH \quad (14)$$



Scheme 2.



Scheme 3.

k_w is the ion product of water. A plot of log k_{obs} vs pH above pH 10 was linear for the compounds **II** and **III** with slope equal to positive unity.

The ion product of water, K_w, is a function of temperature. The value of K_w at 25 °C was calculated by Eqn. 15 (Ho, 1972):

$$-\log k_w = \frac{4470.99}{T} + 0.01706T - 6.0875 \qquad (15)$$

and was found to be 1.0007×10^{-14}.

The k_3 values have been determined from the portion of the pH-rate profile above pH 10 and were found to be 6.61×10^2, 3.39×10^2 and 16.20 M^{-1} h^{-1} for the compounds **II**, **III** and **IV** respectively.

The pH–rate profile for compound **IV** as depicted in Fig. 6 does not indicate a slope of negative unity in the acidic region and positive unity in alkaline pH. The slope in the acidic region is approximately −0.3 and that in the alkaline region is about +0.4. Such non-unity in the slopes of log k vs pH data tends to suggest that the kinetic descriptions of these reactions for compound **IV** is more complex and can not be adequately described by a second-order model.

The possible mechanism of decomposition of the prodrugs in acidic solutions has been depicted in Scheme 2. The hydrolysis of **II**, **III** and **IV** may be initiated by the protonation of the nitrogen forming the iminium ions which can undergo resonance stabilization. The water attack followed by elimination of neutral amine resulted in the formation of the ketone.

The decomposition in neutral to slightly alkaline solutions can take place according to Scheme 3. The degradation may be initiated by the nucleophilic attack by the water molecule or the hydroxide ion on the 3-carbon of the A-ring of the steroid with simultaneous delocalization of π electrons towards the nitrogen. Subsequent proton abstraction by any base however gives the ketone back.

The variation in the stability of **IV** as compared to **II**, and **III** may be due to the difference in the relative ease of protonation of the nitrogens. Compound **IV** where nitrogen is attached to oxygen is less basic than compound **II** or **III** where nitrogen is attached to an amide grouping. As a consequence compound **IV** requires a high proton concentration for protonation and thereby it can be hydrolysed only at lower pH values.

Further studies on the stability of these prodrugs in plasma and liver homogenates of rat and rabbit are in progress and will be reported in a future communication.

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