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Hydrolysis of Steroid Oximes: Mechanism and Products

RICHARD E. HUETTEMANN and ARVIN P. SHROFF[x]

Abstract □ The conversion of 17α-acetoxy-6α-methyl-4-pregnen-3,20-dione 3-oxime to the corresponding diketone in acidic media was found to be a first-order reaction at 37°. The effects of incorporating ester groupings at the oxime function or at the C-17 position and modifications in ring B were also investigated. Only the length of the ester chain at the oxime function had a profound effect on the rate constant. From these observations, it is proposed that two competing mechanisms of hydrolysis are involved for the oxime esters.

Keyphrases □ Steroid oximes—hydrolysis mechanisms and products, effects of ester substituents □ 17α-Acetoxy-6α-methyl-4-pregnen-3,20-dione 3-oxime—conversion to diketone, effects of ester substituent at oxime function and C-17 position, modifications in ring B, mechanisms of hydrolysis □ Oximes, steroid—hydrolysis mechanisms and products, effects of ester substituent and ring B modifications □ Hydrolysis—steroid oximes, mechanisms and products

Buhler *et al.* (1) reported that the acid hydrolysis of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime proceeds rapidly to the corresponding 3,20-diketone by the stepwise mechanism shown in Scheme I. The rate of hydrolysis for the 3-oxime could not be measured, but they reported the rate to be 5.5×10^{-4} sec^{-1} for the 20-oxime. The half-life for the overall hydrolysis (Scheme I) was about 21 min in gastric juice.

Several compounds with the 3-oximino function were synthesized in this laboratory (Table I) and reported (2) to be potent progestational agents. The observation of Buhler *et al.* (1) suggested that these oximes might be hydrolyzed rapidly in gastric fluid and that the resulting 3-keto compounds would be responsible for the observed biological activity. Therefore, a study was initiated to measure the kinetic rate of 3-oximino hydrolysis at pH 1.5 and 37° and to determine if other modifications at the oxime function or around the steroid nucleus affected the rate of hydrolysis.

EXPERIMENTAL[1]

Apparatus—A constant-temperature water bath and a spectrophotometer[2] were used.

Buffers—A pH 1.5 buffer, prepared according to USP XVIII (3), was used.

Procedure for Hydrolysis—A stock solution of each compound (Table I) at a concentration of 300 μg/ml was prepared in purified dioxane. A 30-ml aliquot of this solution was further diluted with 20 ml of buffer solution, and then a 5-ml aliquot was placed in each of three 10-ml glass ampuls. The ampuls were sealed and placed in a constant-temperature water bath maintained at 37°. At specified time intervals, the ampuls were removed and each was extracted separately with methylene chloride. The organic layers were transferred to another vial and evaporated, and the residues were saved for their respective assays. The first ampul residue was assayed by the oxime colorimetric method, and the second ampul residue was assayed by the carbonyl colorimetric method. The residue from the third ampul was used for TLC.

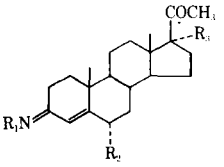
Oxime Colorimetric Assay Method—*Sulfanilamide Solution*—Transfer 200 mg of sulfanilamide into a 100-ml volumetric flask, and add approximately 75 ml of distilled water. Heat the flask on a steam bath until solution occurs, and dilute to volume with distilled water.

Iodine–Acetic Acid Solution—Transfer 1.3 g of iodine into a 100-ml volumetric flask, and add approximately 75 ml of acetic acid. Shake the flask until all of the solid dissolves, and dilute to volume with acetic acid.

Sodium Thiosulfate Solution—Transfer 26 g of sodium thiosul-

[1] Some compounds were obtained from the Division of Chemical Research, Ortho Research Foundation, and others were synthesized by published procedures (2).
[2] Shimadzu QV-50.

Table I—Oximino Steroids Studied at pH 1.5 and 37°



Compound	R_1	R_2	R_3
1	OH	CH_3	$OCOCH_3$
2	CH_3COO	CH_3	$OCOCH_3$
3	C_2H_5COO	CH_3	$OCOCH_3$
4	$CH_3(CH_2)_4COO$	CH_3	$OCOCH_3$
5	OH	CH_3	$OCOCH_3$ (6,7 unsaturated)
6	OH	H	$OCOCH_3$ (6,7 unsaturated)
7	OH	CH_3	$OCOC_2H_5$

fate and 200 mg of sodium carbonate into a 1000-ml volumetric flask, and dilute to volume with distilled water.

N-1-Naphthylethylenediamine Dihydrochloride Solution—Place 100 mg of *N*-1-naphthylethylenediamine dihydrochloride into a 100-ml volumetric flask, and dilute to volume with distilled water. This solution should be prepared fresh daily.

Sodium Acetate Solution—Transfer 50 g of sodium acetate into a 100-ml volumetric flask, and add approximately 80 ml of distilled water. Heat to effect solution, and then dilute to volume with distilled water.

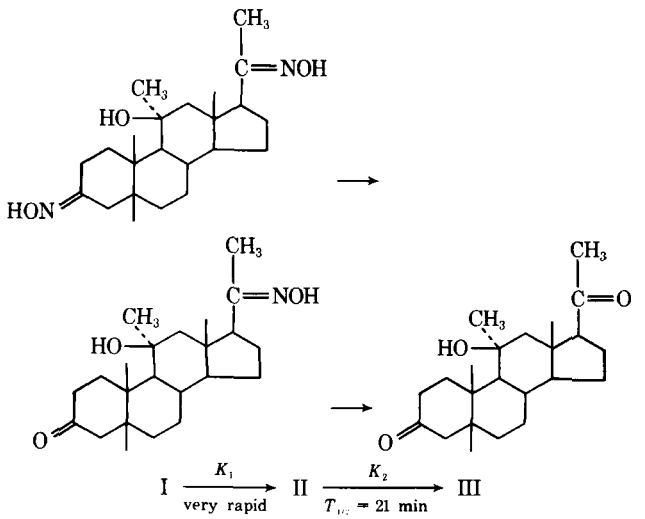
Assay—Evaporate 3.0 ml of the methylene chloride stock solution of a steroid oxime being studied (standard). To both this residue and the residue from the hydrolysis experiment, add 1.0 ml of ethanol and 2.0 ml of 2% aqueous sulfuric acid. Heat both vials at 85° for 30 min and allow them to come to room temperature. To each vial, add 2.5 ml of sulfanilamide solution, 2.5 ml of iodine solution, 5.0 ml of sodium acetate solution, 2.5 ml of sodium thiosulfate solution, and 5.0 ml of *N*-1-naphthylethylenediamine dihydrochloride solution. (*Note:* Stopper and shake the vial after the addition of each solution.) Quantitatively transfer the contents of each vial into 100-ml volumetric flasks, and dilute to volume with distilled water. Determine the absorbance of the standard and the sample at 505 nm, using water as the reference, and calculate the amount of oxime in the sample.

Carbonyl Colorimetric Assay Method—*Isoniazid Solution*—Transfer 500 mg of isoniazid into a 1-liter volumetric flask. Add 800 ml of methanol and 0.63 ml of concentrated hydrochloric acid. Swirl to dissolve, and dilute to volume with methanol.

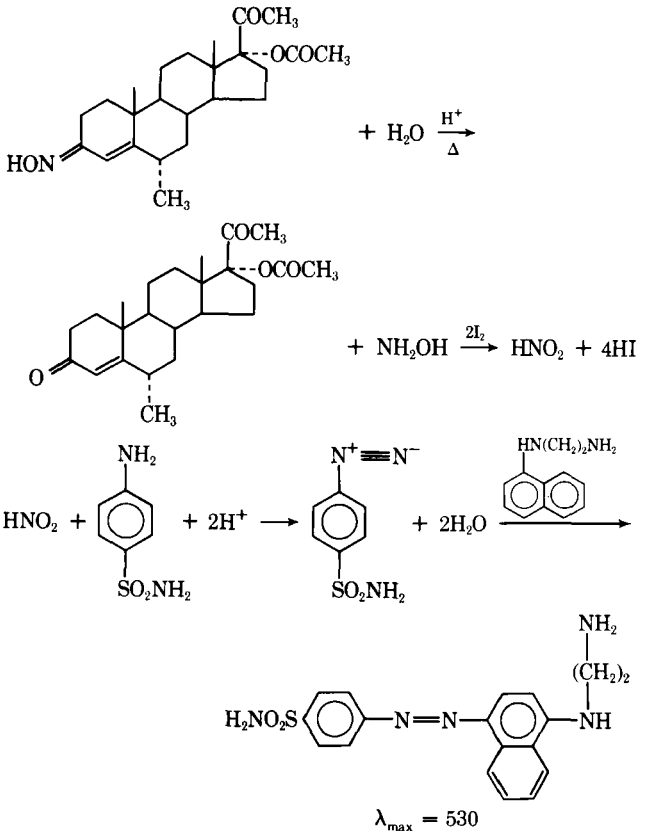
3-Ketosteroid Standard Solution—Weigh 100 mg of the appropriate 3-ketosteroid (compounds listed in Table I) into a 100-ml volumetric flask, and dilute to volume with methanol.

Assay—Transfer 1.0 ml of the appropriate 3-ketosteroid standard solution into a vial, and evaporate to dryness. To this residue and to the residue obtained from the hydrolysis experiment, add 25 ml of isoniazid solution. Heat both vials at 60° for 5 min, and then allow them to come to room temperature. Determine the absorbance of the standard and the sample at 370 nm *versus* reagent blank, and calculate the amount of ketosteroid in the sample.

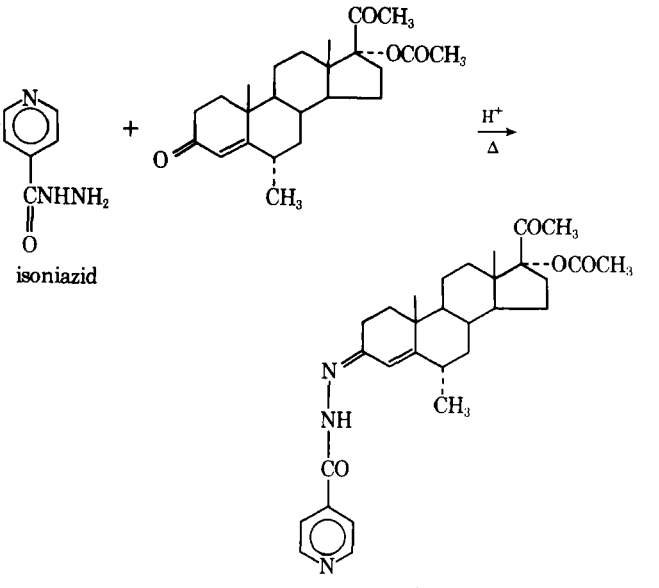
TLC Procedure—The steroid residue from the hydrolysis experiment was dissolved in 1.0 ml of methylene chloride, and a 5.0–10.0-μl sample was spotted on a 20 × 20-cm TLC plate[3], 250



Scheme I—Stepwise hydrolysis of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime



Scheme II—Mechanism for the oxime chromophore formation



Scheme III—Mechanism for the carbonyl chromophore formation

[3] Analtech, Wilmington, Del.

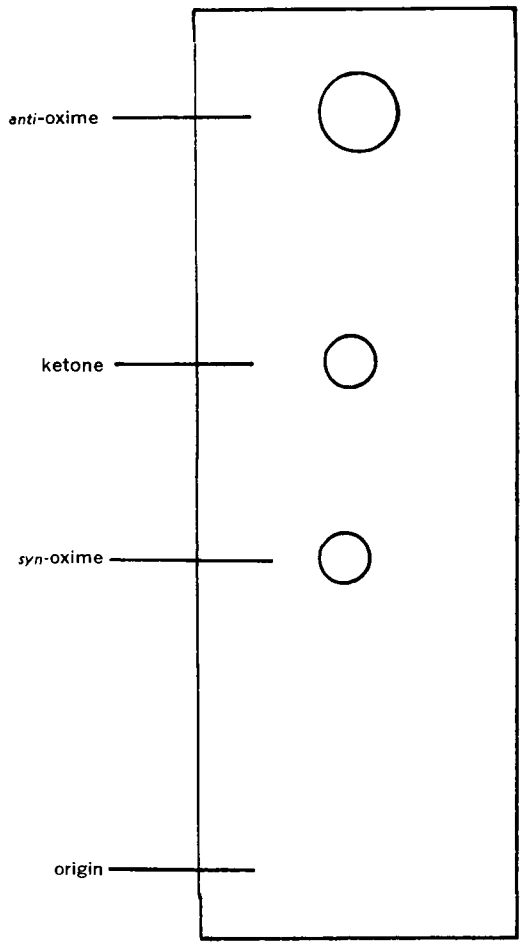
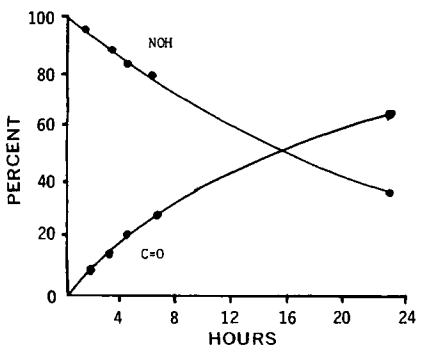


Figure 1—*Thin-layer chromatogram depicting the separation of oxime from its corresponding ketone.*

μm thick. Chromatograms were developed in 7 × 22 × 22-cm glass tanks containing 200 ml of hexane–ethyl acetate (1:1). After development the plates were air dried, sprayed with 5% methanolic sulfuric acid, and heated in an oven at 100° for 15 min. The plates were then examined under UV light (366 nm) for qualitative identification of various steroids. Reference steroids were used wherever necessary.

DISCUSSION

The sensitivity of the oxime colorimetric method (10 μg/ml), the carbonyl colorimetric method (10 μg/ml), and the TLC procedure was satisfactory for detection of minor changes during hydrolysis. The mechanism of chromophore formation and separation on TLC is shown in Schemes II and III and Fig. 1. Both chromophores were stable for at least 3 hr.



Hours	NOH, %	C=O, %
0	100	0
1.5	96	8
3	88	13
4	84	21
6	80	28
23	37	64

Figure 2—*Plot of time* versus *oxime and ketone concentrations for hydrolysis of Compound 1.*

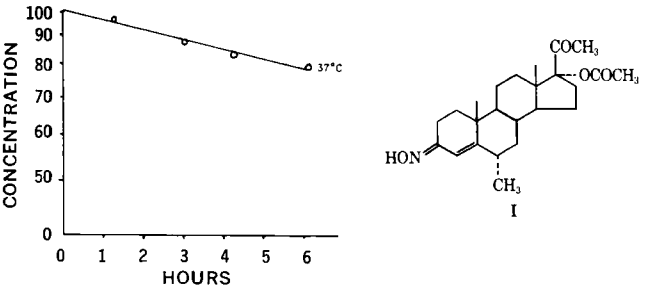


Figure 3—*Typical first-order plot obtained for Compound 1 at 37°.*

Initially, the kinetics of hydrolysis at pH 1.5 and 37° was investigated for 17α-acetoxy-6α-methylpregn-4-en-3,20-dione 3-oxime (Compound 1 of Table I). The rate was monitored quantitatively by TLC. The data are graphically depicted in Fig. 2. Spectroscopic identification of the hydrolyzed product, isolated by TLC, revealed it to be the 3-ketone, 17α-acetoxy-6α-methylpregn-4-en-3,20-dione. The first-order kinetics obeyed by this reaction are shown by Fig. 3.

Similar hydrolysis was carried out with Compounds 2–7 (Table I), which have modifications on the 3-oximino function at C-17 and in ring B of the steroid nucleus. It was found that changes at C-17 or in ring B did not influence the rate of hydrolysis of the 3-oxime (K in hr^{-1}: Compound 1, 0.038; Compound 5, 0.039; Compound 6, 0.032; and Compound 7, 0.035). However, ester substitutions at the 3-oximino function had a profound effect on the kinetics (K in hr^{-1}: Compound 1, 0.038; Compound 2, 0.068; and Compound 3, 0.163) (Fig. 4). The first-order plot reveals that ester groups initially increase the rate of hydrolysis of the 3-oxime.

For example, methyl and ethyl esters of Compound 1 showed a marked increase in the initial rate of hydrolysis over Compound 1 but after 6 hr the slope (Fig. 4) was parallel to that for Compound 1. On the other hand, the pentyl ester, Compound 4, had such a fast first-order rate of hydrolysis that its overall rate appeared to be identical with that of Compound 1. In fact, under experimental conditions, no pentyl ester could be detected after the first 30 min.

These results suggest that in the case of oxime esters there were two mechanisms by which the hydrolysis was proceeding. To study this in-depth, Compound 2 was chosen and the TLC technique was used because of its sensitivity and ability to separate

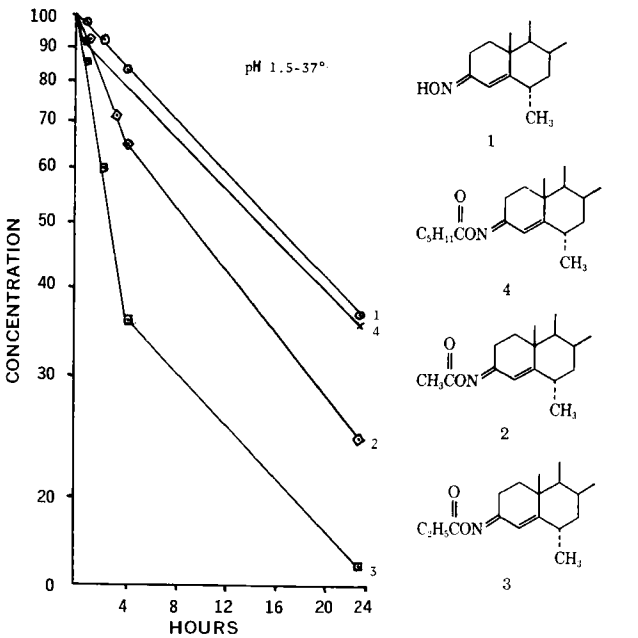


Figure 4—*Comparison of first-order plots for Compounds 1–4 showing the loss of total oxime with time (pH 1.5, 37°).*

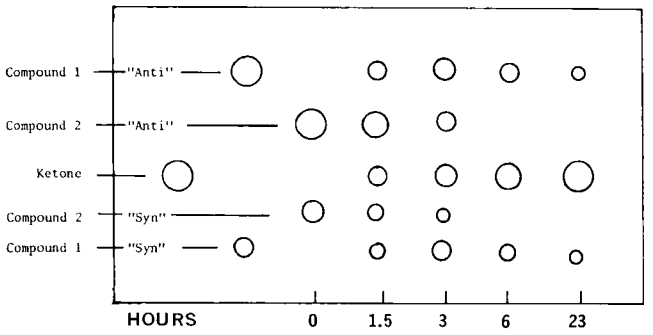


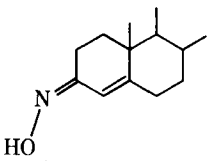
Figure 5—*Thin-layer chromatogram depicting the products obtained on acid hydrolysis of Compound 2.*

all possible hydrolysis products. Figure 5 illustrates a typical thin-layer chromatogram obtained for Compound 2 at various time intervals. It reveals that Compound 2 hydrolyzed rapidly to Compound 1 and its corresponding 3-ketone. After 6 hr, all ester was consumed and one could detect only Compound 1 and its 3-keto analog. The two spots observed on TLC for Compound 1 are attributed to *syn*- and *anti*-isomers (I and II, respectively).

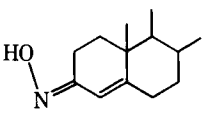
These TLC observations suggest that the mechanism of hydrolysis must be as depicted in Scheme IV. The time study also indicated that rapid hydrolysis of oxime ester to the corresponding ketone (K_1 of Scheme IV) took place in the early stages of the reaction but hydrolysis to Compound 1 (K_2 of Scheme IV) took place at a relatively slower rate. The overall conversion to the ketone, therefore, must be a combination of $K_1 + K_3$ (Scheme IV).

From the few oximes studied, it can be stated that the longer the alkyl chain of the ester group the faster is the rate of hydrolysis. The inductive effect is believed to play a significant role. The longer the ester group the greater is the +I effect on the nitrogen atom, making it more susceptible to attack by a proton and resulting in faster overall hydrolysis.

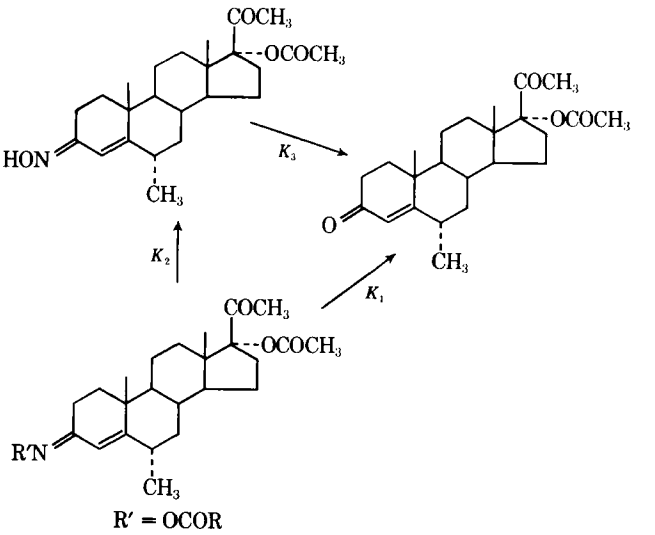
In conclusion, these experiments suggest that the oxime and its derivatives undergo hydrolysis at different but measurable rates. In view of the contrary findings of Buhler *et al.* (1) on other compounds, experience dictates that each compound should be individually evaluated; general prediction based on limited data may be erroneous. The rate of hydrolysis for the various compounds



I: *syn*-isomer



II: *anti*-isomer



Scheme IV—Proposed mechanism for the hydrolysis of 3-oximino esters

studied suggests that their *in vivo* biological performance is not necessarily dependent upon their conversion to ketones in gastric fluid.

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