The Modification of 11β-Hydroxy-11α-methyl-5β-pregnane-3,20-dione Dioxime under Acidic Conditions

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Under simulated physiological conditions in 0.1 *N* HCl at 37°, 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) is rapidly hydrolyzed to 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione 20-monooxime (II) which then is further cleaved to 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione (III). The rate constant, k_1, for the conversion of I to II could not be measured because of the rapid rate of hydrolysis, while the rate constant, k_2, for the hydrolysis of II to III was approximately 5.5×10^{-4} sec.$^{-1}$ as determined by both radioactive and spectrophotometric assays. Upon prolonged exposure of III to 0.1 *N* acid, an acid-catalyzed dehydration occurs forming three isomeric olefins, the exocyclic 11-methylene-5β-pregnane-3,20-dione (IV) and the endocyclic 11-methyl-$\Delta^{9(11)}$-5β-pregnene-3,20-dione (V) (the major dehydration product) and 11-methyl-$\Delta^{11(12)}$-5β-pregnene-3,20-dione (VI). The pseudo-first-order rate constant, k_3, for the dehydration of III to the mixture IV, V, and VI appears to be at least two orders of magnitude less than k_2.

The steroid 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I)[1] has been shown to exhibit significantly greater CNS depressant activity[2] than the parent 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione (III),[3] when administered orally to the mouse or rat, even though both steroids showed equivalent activity upon parenteral administration. Possible explanations for the increased biological potency of I upon oral administration include a facile hydrolysis in the stomach to yield either an active intermediate with a higher efficacy or a more easily absorbed physical state of the parent diketone III. It appeared that determination of the gastric stability of I could assist in establishing the mechanism for the observed differences in oral efficacy. Therefore, the kinetics of hydrolysis of I have been investigated under conditions approximating those of the stomach. In addition, the modification products of I formed at this pH have been isolated and identified.

Experimental

Product Studies.—Paper chromatographic examination of aliquots from the acid-catalyzed hydrolysis of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) revealed the presence of four zones detectable with 2,4-dinitrophenylhydrazine. These acid modification products have been designated components a–d in the order of increasing chromatographic mobility. Components a, c, and d migrated in a manner similar to that of authentic I, 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione (III), and 11-methyl-$\Delta^{9(11)}$-5β-pregnene-3,20-dione (V),[4] respectively. Component b possessed a mobility slightly greater than that of authentic 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione 3-monooxime (VII). The reaction products were chromatographed on paper in the Bush B-3,[5] the FBC,[6] and FBF[6] systems. Hydrolysis products were located by exposure of the developed paper chromatograms to HCl vapor for 2 hr. to hydrolyze oximes followed by spraying with 2,4-dinitrophenylhydrazine and silver nitrate.[6]

One gram of I was dissolved in 20 ml. of acetone and added with stirring to 6 l. of 0.1 *N* HCl which had been prewarmed to 37°. The acidic solution was maintained at 37° throughout the experiment. Approximately one-half of the reaction mixture was removed after 0.5 and 120 hr., respectively, and each aliquot was extracted four times with ethyl acetate. The ethyl acetate extracts were washed with small quantities of water and quickly evaporated to dryness *in vacuo* with a minimum of heating. Smaller aliquots of the reaction mixture were removed at 1, 2, 24, 48, 72, and 96 hr. and extracted similarly. Aliquots of the various extracts were spotted on a 20-cm.^{2} plate of aluminum oxide G (Brinkman Instrument, Inc., Great Neck, N. Y.) along with various reference steroids, the plate then was developed in a 50% mixture of ethyl acetate, and cyclohexane and the spots were visualized by spraying with 50% H_2SO_4 and heating.

Figure 1 shows a replica of the analytical thin layer chromatography (t.l.c.) plate. It may be seen that the dioxime I (component a) very rapidly disappeared with the production of a possible monooxime component b which in turn was converted to component c (with a mobility identical with that of authentic III). In addition, after prolonged exposure of the steroid mixture to 0.1 *N* HCl, nonpolar modification products began to appear in increasing amounts. These products migrated as a single zone (component d) on paper in the Bush B-3, FBC, or FBF systems; however, when component d was chromatographed on alumina in ethyl acetate–cyclohexane (1:3) it was resolved into three components (designated components d_1, d_2, and d_3). Under these conditions component d_3 was shown to exhibit the same mobility as that of authentic V.

The n.m.r. spectrum of the 120-hr. extract indicated that this fraction was a mixture containing 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione (III), 11-methylene-5β-pregnane-3,20-dione (IV), and 11-methyl-$\Delta^{9(11)}$-5β-pregnene-3,20-dione (V), with a smaller amount of 11-methyl-$\Delta^{11(12)}$-5β-pregnene-3,20-dione (VI). The n.m.r. assignments for compounds V and VI have been reported previously[4] and those for compounds I, III, IV, and VII and 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione 20-monooxime (II) are shown in Table I. It appeared that components d_1, d_2, and d_3 represented olefinic products formed by dehydration of the methylcarbinol III. The similarity in chromatographic mobility of component d_3 and V suggested that component d_3 was the $\Delta^{9(11)}$-dehydration product while components d_1 and d_2 probably represented the 11-methylene (IV) and $\Delta^{11(12)}$ (VI) isomers.

One-half of the 120-hr. extract was separated on a column (1.8 × 10 cm.) made up of 22 g. of aluminum oxide (t.l.c. grade, Merck, Germany) using a mixture of ethyl acetate–cyclohexane (1:1) as the eluting solvent. Fractions (5 ml.) were collected and aliquots of the various fractions were then examined *via* t.l.c. The fifth fraction, containing the bulk of the less polar modification products, was recrystallized from acetone–Skellysolve B (Darco treatment) to yield 44 mg. of colorless needles,

(1) G. S. Fonken, U. S. Patent 3,019,243 (1962); W. J. Wechter and G. S. Fonken, to be published.

(2) Motor activities were determined by the method of P. B. Dews [*Brit. J. Pharmacol.*, **8**, 46 (1953)].

Compd.	ED_{50} (i.p.), mg./kg.	ED_{50} (*p.o.*), mg./kg.
I	100	>800
III	75	200

The detailed testing data will appear in a forthcoming publication of W. J. Wechter, P. H. Seay, and R. J. Matthews.

(3) G. S. Fonken, *J. Org. Chem.*, **23**, 1075 (1958).

(4) W. J. Wechter and G. Slomp, *ibid.*, **27**, 2549 (1962).

(5) I. E. Bush, *Biochem. J.*, **50**, 370 (1952).

(6) L. M. Reineke, *Anal. Chem.*, **28**, 1853 (1956).

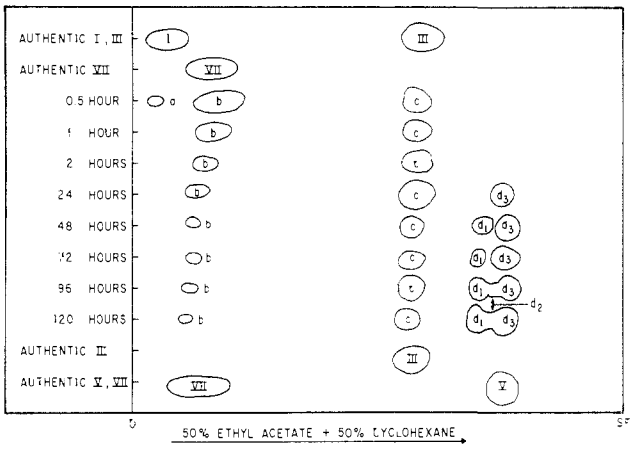


Figure 1.—Alumina t.l.c. of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) acid hydrolysis mixture.

TABLE I

N.M.R. ASSIGNMENTS[a]

Compd.	11-substituent	18H	19H	21H
I	90	52	78	127
II	90	53	79.5	114
III	90.5	52	79	127
IV	297, 304	35	80	127
VII	89.5	49.5	75	127

[a] All n.m.r. spectra were recorded on a Varian Model A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as internal standard and peaks are reported as cycles per second downfield. The authors are indebted to G. Slomp and F. MacKellar, Physical and Analytical Research, The Upjohn Company, for recording and in most cases interpreting these spectra.

m.p.[7] 141–143° (authentic V m.p.[4] 143–144°). This material had a chromatographic mobility on thin layer identical with that of component d_3 and the authentic $\Delta^{9(11)}$ compound V. N.m.r. spectroscopy confirmed this assignment.[4] The infrared spectrum[7] of this material also was identical with that of authentic V.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.83. Found: C, 80.22; H, 10.28.

Thin layer chromatography of the seventh fraction from the above column showed it to contain primarily component c. This fraction was recrystallized twice from acetone–isooctane (Darco treatment) to yield 10 mg. of material, m.p. 170–171° (authentic III, m.p.[2] 171–173°). The infrared spectrum, n.m.r. spectrum (Table I), and chromatographic mobility of this material were identical with that of authentic III.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 76.26; H, 9.89. Found: C, 76.72; H, 9.97.

The second half of the 120-hr. extract was streaked at the origin of two thick alumina plates (1 mm. × 20 cm.[2]) and chromatographed in a mixture of ethyl acetate–cyclohexane (1:3). The steroid bands were located by a methanolic iodine (I_2) spray of a portion of the plate, and appropriate areas were scraped off, combined, and eluted with acetone–chloroform. The eluate from the area corresponding to component d_1 was again streaked at the origin of a 20-cm.[2] alumina plate and redeveloped in ethyl acetate–cyclohexane (1:3) in order to separate this material from a small amount of component d_2. After locating the steroid band by spraying with I_2 along one edge, the purified component d_1 was eluted and recrystallized twice from ethyl acetate–isooctane to yield 5 mg. of crystals, m.p. 135–136°. The infrared spectrum of the purified steroid showed a spectrum which was characteristic of 11-methylene compound IV (ν_{max} 3100, 1705, 1635, and 900 cm.$^{-1}$). Elks[8] has found that such infrared bands at 3100, 1640, and 898 cm.$^{-1}$ were characteristic of an exocyclic methylene group in 11-methylenetigogenin acetate.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.83. Found: C, 79.40; H, 10.37.

The eluate from the area corresponding to component d_2 also was streaked at the origin of a 20-cm.[2] alumina plate which was developed in a mixture of ethyl acetate–cyclohexane (1:3) in order to separate component d_2 from a small amount of component d_3. The component d_2 band was located by spraying one edge of the plate with I_2 and then eluting the unsprayed zone with acetone–chloroform to yield approximately 1 mg. of material. The infrared spectrum of this modification product was quite similar to that of the known $\Delta^{11(12)}$ olefin VI.[3]

Examination of an aliquot of the reaction mixture obtained after 0.1 *N* HCl hydrolysis of I for 30 min. by n.m.r. spectroscopy showed that this material was a mixture of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione 20-monooxime (II) and III plus a smaller amount of unhydrolyzed I. One-half of the 30-min. hydrolysis mixture was fractionated on a column (1.8 × 11 cm.) made up from 30 g. of alumina (t.l.c. grade) using ethyl acetate–cyclohexane (3:1) for elution. Fractions of 4 ml. were collected and an aliquot from each fraction was examined *via* t.l.c. Fractions 7 through 9 were combined and recrystallized twice from ethyl acetate–isooctane to yield 24 mg. of material, m.p. 169.5–170°, which proved identical with authentic III by infrared, n.m.r., elemental, and chromatographic analyses.

Fractions 11 through 19 were pooled and recrystallized twice from ethyl acetate–isooctane to give 29 mg. of crystalline material, m.p. 159.5–162°, with a chromatographic mobility on paper and t.l.c. identical with that of component b but slightly greater than that of authentic VII. The infrared spectrum of this compound was characteristic of a monooxime (ν_{max} 3400, 1708, 1645, 1250, 1225, 1205, 1165, 1105, 1090, and 1060 cm.$^{-1}$). The n.m.r. spectrum was identical with that assigned to the 20-monooxime II (Table I), exhibiting displaced 21H resonances and normal 11-substituent, 18H, and 19H resonances characteristic of a 20-monooxime; O.R.D. (c 0.38, dioxane) $[M]_{366} = +490°$, $[M]_{345}^{315} = -693°$, and $[M]_{335} = -199°$.

Anal. Calcd. for $C_{22}H_{33}NO_3$: C, 73.09; H, 9.75; N, 3.87. Found: C, 73.36; H, 10.05; N, 3.99.

11β-Hydroxy-11α-methyl-5β-pregnane-3,20-dione 3-Monooxime. To a sample of I (3.46 g., 0.01 mole) dissolved in 75 ml. of alcohol was added hydroxylamine hydrochloride (695 mg., 0.01 mole) dissolved in 5 ml. of water. The resulting solution was heated at reflux for 0.5 hr. and allowed to cool to room temperature overnight. The reaction mixture was poured into 200 ml. of water, whereupon the product separated and solidified. The product was isolated by filtration, washed thoroughly with water, and dried (60° *in vacuo*) giving 3.0 g., m.p. 90–105° dec. Recrystallization from ethanol afforded colorless leaves, m.p. 110–115° dec., yield 1.67 g. A sample was recrystallized once again for analysis: m.p. 110–115° dec.; infrared ν_{max} 3500, 3220, and 1700 cm.$^{-1}$; n.m.r. (Table I) consistent with structure. This material exhibited a single spot on Bush B-3[5] paper chromatography and upon t.l.c. on silica gel.

Anal. Calcd. for $C_{22}H_{35}NO_3 \cdot 0.5H_2O$: C, 71.56; H, 9.79; N, 3.78; H_2O, 2.50. Found: C, 71.49; H, 9.61; N, 3.75; H_2O (Karl Fischer), 2.65.

Preparation of Tritium-Labeled 11β-Hydroxy-11α-methyl-5β-pregnane-3,20-dione Dioxime (I).—To 31.0 mg. (89.5 μmoles, 4.92×10^8 d.p.m.) of tritium-labeled 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione (III), specific activity 1.59×10^8 d.p.m./mg.,[9] was added 55.6 mg. (800 μmoles) of hydroxylamine hydrochloride, 0.064 ml. (792 μmoles) of pyridine, and 5 ml. of absolute ethanol. The reaction mixture was heated at reflux for 7 hr. The cooled reaction mixture was then poured slowly with stirring into 20 ml. of ice-cold water. The resulting suspension was allowed to stand for 1 hr. The crude dioxime I was removed by filtration and thoroughly washed with water. After drying over phosphorus pentoxide *in vacuo*, 25.7 mg. of material was obtained with a specific activity of 1.48×10^8 d.p.m./mg. The specific activity of I was determined by adding suitable aliquots of the steroid to counting vials containing 10 ml. of scintillator solution made up of 2 parts of toluene and 1 part of absolute ethanol, 0.4% 2,5-diphenyloxazole (PPO), and 0.01% 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP). Counting was performed in a Tri-Carb Model 314X liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Ill.). Absolute radioactivity was

(7) KBr pellet infrared spectra were obtained on a Perkin-Elmer Model 421 spectrophotometer. Melting points, determined in a Thomas–Hoover apparatus, are corrected.

(8) J. Elks, *J. Chem. Soc.*, 3333 (1960).

(9) The 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione-5H[3] (III) was prepared by catalytic reduction with tritium gas of 11β-hydroxy-11α-methyl-pregnene-3,20-dione (R. C. Thomas, D. R. Buhler, G. Ikeda, W. J. Wechter, and J. A. Campbell, to be published).

determined by recounting with added tritium-labeled toluene internal standard.

The purity of the labeled I preparation was determined by chromatography in the Bush B-3 system and scanning the resulting chromatograms in the Forro 4π radiochromatogram scanner (Forro Scientific Co., Evanston, Ill.). Inspection of the resulting scans showed the presence of two radioactive components. The mobility of the major radioactive zone (7.0 cm. from the origin) corresponded to that of authentic I while the minor product migrated 23.0 cm. from the origin, a mobility similar to that of the II produced during the acid-catalyzed hydrolysis of nonradioactive I. No unchanged starting material III was detected. Integration of the areas under the two radioactive peaks indicated that the major component accounted for 95% of the total radioactivity while the less polar radioactive zone contained only 5% of the radioactivity.

When the two radioactive areas were cut from the chromatograms, eluted, and rechromatographed, the major component again gave rise to two radioactive spots (a major component with a mobility similar to that of authentic I and a minor component with the mobility similar to that of II). Rechromatography of the eluted minor component, however, resulted in only one radioactive zone which migrated in a manner indistinguishable from that of the original minor component. Infrared analysis of the radioactive I preparation produced a spectrum which was identical with that of authentic I.[1] These results confirm that the radioactive I preparation was radiochemically pure and that a small amount of a minor component with a mobility similar to that of II was produced during paper chromatography.

Kinetic Studies.—Radioactive I (1.15 mg., 1.69×10^8 d.p.m.) was dissolved in 0.5 ml. of acetone and added to 20 ml. of 0.1 *N* HCl. The acetone was rapidly removed in a stream of N_2 and the solution was filtered free of undissolved I. Determination of the radioactivity of the resultant filtrate indicated that it contained 1.43×10^8 d.p.m. (0.97 mg.) of labeled dioxime. At this time (5 min. following addition of 0.1 *N* HCl to the steroid solution), a 2-ml. aliquot was removed from the reaction mixture and immediately extracted with 6 ml. of ethyl acetate. The completeness of the extraction was verified by measurement of the radioactivity in suitable aliquots of the extract and aqueous residues. The major portion of the ethyl acetate extract then was removed, rapidly evaporated to dryness, and spotted on Whatman No. 1 paper.

The HCl solution contained in a glass-stoppered flask was placed in a water bath at 37° and subjected to continuous shaking. Periodically, further aliquots were removed, extracted, and spotted in the manner described above. Following development in the Bush B-3 paper chromatographic system, the dried chromatograms were cut into strips and the distribution of radioactivity along the strips was measured by means of a Forro radiochromatogram scanner. The areas under the peaks on the scans were measured by planimetry and the per cent distribution for each component was determined by calculation. Hydrolysis products also were located by spraying with 2,4-dinitrophenylhydrazine.[6]

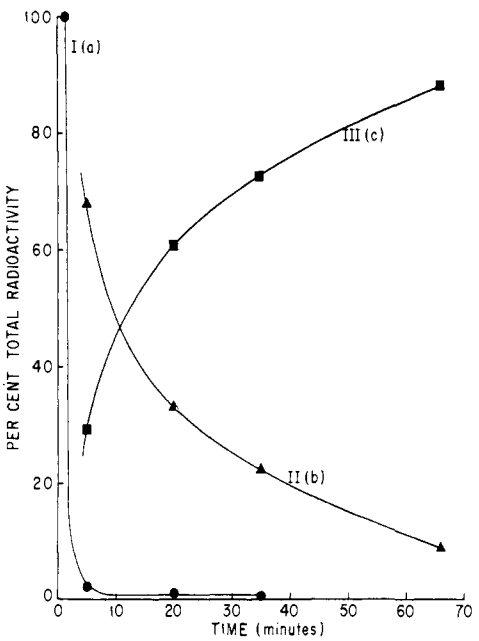


Figure 2.—Hydrolysis of 1.53×10^{-4} *M* tritium-labeled 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) in 0.1 *N* HCl at 37°.

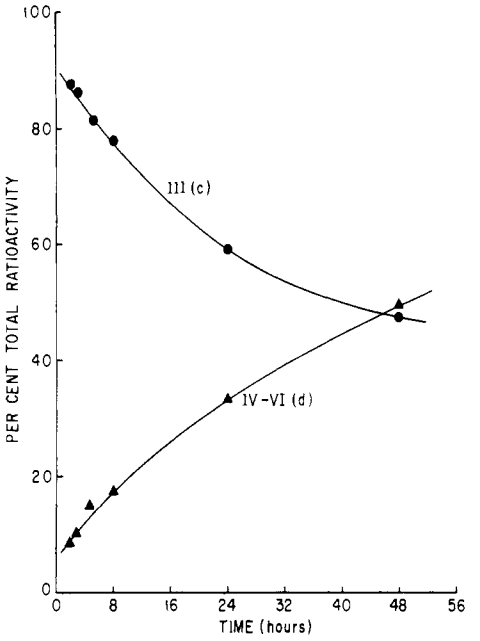


Figure 3.—Hydrolysis of 1.53×10^{-4} *M* tritium-labeled 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) in 0.1 *N* HCl at 37°.

Paper chromatographic examination of aliquots from the acid-catalyzed hydrolysis of radioactive I showed four radioactive zones which reacted with 2,4-dinitrophenylhydrazine. The radioactive areas, corresponding to the previously described components a–d, represented compounds I, II, III, and IV–VI (not resolved by paper chromatography), respectively. Two minor radioactive zones (mobility 32.4 and 48.2 cm., respectively) were detected in certain of the chromatograms. These materials which did not react with 2,4-dinitrophenylhydrazine will not be considered further in the present investigation. The distribution of radioactivity in the acid modification products with respect to time is presented in Table II and is shown graphically in Figures 2–4.

TABLE II

RELATIVE DISTRIBUTION OF RADIOACTIVITY IN THE ACID-MODIFICATION PRODUCTS OF 11β-HYDROXY-11α-METHYL-5β-PREGNANE-3,20-DIONE DIOXIME-H³ FOLLOWING CHROMATOGRAPHY IN THE BUSH B-3 PAPER CHROMATOGRAPHIC SYSTEM[a]

	Total radioactivity, % — Components			
Time, min.	a (I)	b (II)	c (III)	d (IV-VI)
5	2.4	68.9	25.5	3.3
20	0.74	34.6	61.4	3.3
35	0.34	22.6	72.3	4.8
65	*b*	8.1	89.6	2.4
125	*b*	1.7	87.5	8.5
190	*b*	1.7	86.5	10.4
5 hr.	*b*	2.0	80.2	15.2
8 hr.	*b*	1.3	78.5	17.2
24 hr.	*b*	0.2	59.0	33.8
48 hr.	*b*	*b*	47.5	49.0

[a] Mobilities of compounds in Bush B-3 system were: component a, 9.1 cm.; component b, 23.0 cm.; component c, 41.3 cm.; and component d, 55.5 cm. Mobilities of standard steroids were: I, 9.0 cm.; VII, 20.1 cm.; III, 42.0 cm.; and V, 56.1 cm. [b] Not detected.

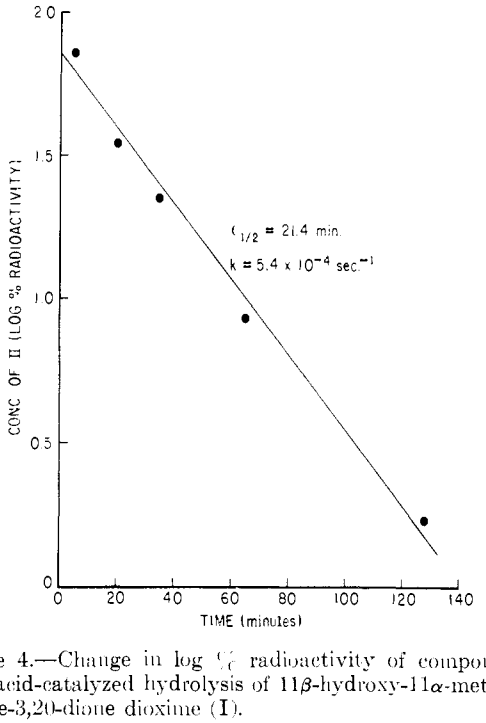
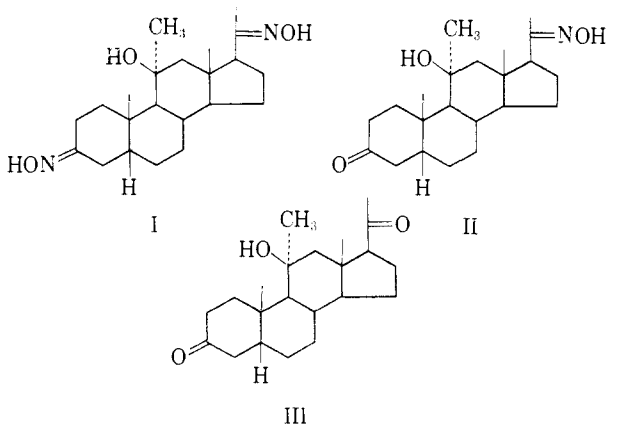


Figure 4.—Change in log % radioactivity of component II during acid-catalyzed hydrolysis of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I).

Spectrophotometric Studies.—Examination of the ultraviolet absorption spectra of synthetic I and III indicated at least a 25-fold greater absorption of I at 230 mμ. Therefore, the rate of hydrolysis of I could be measured by following the loss of absorption at 230 mμ. Approximately 75 mg. of the dioxime I was dissolved in a few drops of 95% ethanol and added to 1 l. of 0.1 *N* HCl. An aliquot of this solution was placed in 1-cm. silica cuvette in a Beckman Model DU spectrophotometer equipped with a cell compartment maintained at 37° by circulation of water from an electronically controlled bath. The decrease of absorption at 230 mμ was determined with respect to time, and a pseudo-first-order plot of the spectrophotometric data is presented in Figure 5.

Results and Discussion

Six components sensitive to 2,4-dinitrophenylhydrazine were detected by paper and thin layer chromatography of aliquots from the acid-catalyzed hydrolysis of I. These components have been identified as unreacted dioxime I, 20-monooxime II, diketone III, and three isomeric 11-dehydration products, the exocyclic olefin IV and the endocyclic olefins V and VI. Each of these acid modification products has been isolated by column chromatography or preparative t.l.c. and was identified by a variety of chemical and physical analyses and by comparison with authentic standards.



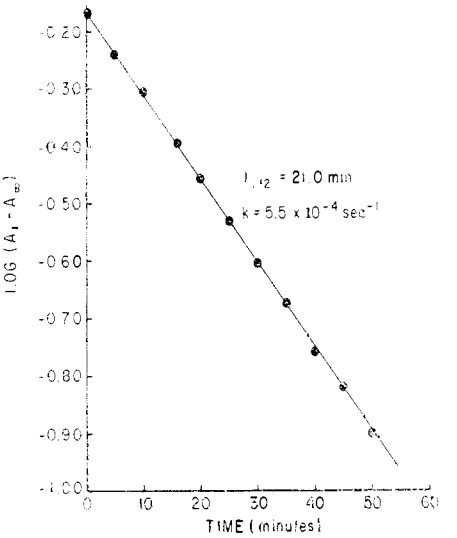
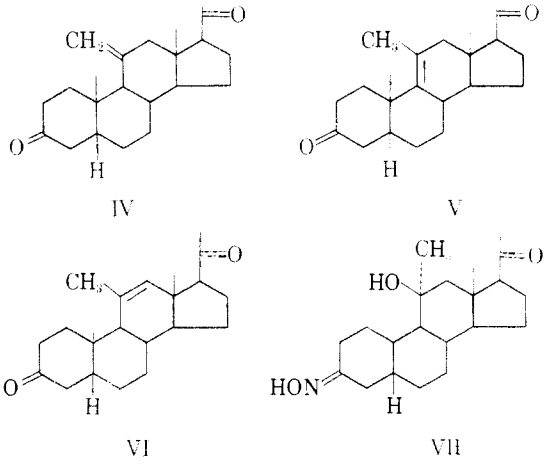


Figure 5.—Pseudo-first-order plot for hydrolysis of 2 × 10^{-5} *M* 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime (I) in 0.1 *N* HCl at 37° as measured by absorbance (A_t) changes at 230 mμ; A_∞ is an asymptotic absorbance.

After incubating I in 0.1 *N* acid for 30 min., the reaction mixture contained primarily hydrolysis products, mainly II with smaller amounts of I and III. Unreacted dioxime I (component a) and diketone III (component c) were identified by relative chromatographic mobilities, elemental analysis, infrared spectra, and n.m.r. spectra in comparison with authentic standards. Component b was identified as the 3-keto 20-monooxime II by a negative Cotton effect in its O.R.D. spectrum characteristic of a 3-keto steroid,[10] an n.m.r.



spectrum characteristic of the 20-monooxime II, the elemental analysis, an infrared spectrum consistent with a monooxime structure, and chromatographic mobility similar to that of authentic 3-monooxime 20-ketone VII. In addition, the optical rotatory dispersion and infrared and n.m.r. spectra of the authentic 3-monooxime 20-ketone VII differed appreciably from those of compound II.

Exposure of I to dilute acid for 120 hr. resulted in a significant amount of acid-catalyzed dehydration of the methylcarbinol III hydrolysis product. The major reaction product at this time was the $\Delta^{9(11)}$ olefin V but smaller amounts of III, IV, and VI and traces of II also were present. Component d migrated as a

[10] C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, Chapter IV.

single zone on paper, but was resolved into 3 subfractions, components d_1, d_2, and d_3 by t.l.c. Component d_1 was identified as the exocyclic 11-dehydration product IV by its characteristic infrared spectrum[8] and its elemental analysis. The n.m.r. spectrum of this material confirmed the loss of the 11-methyl resonance and the appearance of a doublet attributed to the 11-methylene hydrogens. Components d_2 and d_3 proved to be the endocyclic $\Delta^{11(12)}$ derivative VI and its $\Delta^{9(11)}$ isomer V as evidenced by infrared and n.m.r. spectra similar to those previously reported for these compounds.[4] It is interesting to note that Elks[8] reported that the acid-catalyzed dehydration of 3β-acetoxy-11β-hydroxy-11α-methyl-5α-25D-spirostane first formed a 1:1 mixture of the exocyclic 11-methylene and $\Delta^{9(11)}$ olefins, but that this ratio became 4:1 after several hours. On the other hand, 11α-methylhydrocortisone is apparently dehydrated to yield primarily the 11-methyl-$\Delta^{9(11)}$ product.[11] The present studies now demonstrate that the $\Delta^{9(11)}$ isomer V is the major acid-catalyzed dehydration product of the diketone III.

The distribution of radioactivity in the acid-catalyzed modification of radioactive I with respect to time is presented in Table II and shown graphically in Figures 2 and 3. Within 5 min. under conditions which simulated those found in the stomach, over 95% of the I was converted to products which, in turn, underwent further alteration. The over-all process suggested a simple sequential relationship (eq. 1).

$$\text{I (a)} \xrightarrow{k_1} \text{II (b)} \xrightarrow{k_2} \text{III (c)} \xrightarrow{k_3} \text{IV–VI (d)} \qquad (1)$$

Very likely the actual mechanism is much more complex and may include reverse reactions and other equilibria; however, the available kinetic data are not sufficient to characterize all such processes. The rate constant, k_1, for the conversion of I to the intermediate II, appears to be greater by an order of magnitude than the constant, k_2, which describes the conversion of II to III. The apparent first-order rate constant, k_2, was approximated as 5.4×10^{-4} sec.$^{-1}$ from a semi-logarithmic plot of the decrease in II radioactivity with respect to time (Figure 4). Essentially the same rate constant (5.5×10^{-4} sec.$^{-1}$) was obtained from a first-order plot of the decrease in concentration of I (determined spectrophotometrically) with respect to time (Figure 5). These results confirm that k_1 is very large and that k_2 is essentially the over-all rate-determining constant in the conversion of I to III. The acid-catalyzed dehydration of III to the olefins IV–VI occurs at a rate which is considerably slower, exhibiting an apparent half-life of about 55 hr. as determined from a first-order plot of the change in III radioactivity with respect to time.

These studies indicate that the 20-monooxime II would be the major species present in gastric fluid for the first 20 min. or so after ingestion of the dioxime I. The half-life for the conversion of the dioxime to the monooxime is probably less than 1 min. Therefore, it seems unlikely that appreciable amounts of the dioxime would be available for absorption from the gastrointestinal tract. The half-time for the over-all conversion I → II → III is estimated to be 21 min. in gastric fluid. Thus, significant concentration of the monooxime would be available for absorption for periods up to 1 hr. after administration. Experiments with mixtures largely composed of the monooxime II suggest that this compound has about the same CNS-depressant activity upon oral administration as the dioxime I.[12] The olefinic dehydration products IV–VI would not be expected to form in any appreciable concentration prior to discharge of the stomach contents. Moreover, these olefinic products show little oral CNS-depressant activity.[12]

Thus, under physiological conditions, the acid-catalyzed formation of substantial quantities of the 20-monooxime appears probable. It is not clear from the present studies if the 20-monooxime is the active species *per se* or if the observed activity resulted from the high concentrations of III which would also be formed from I within its retention time in the stomach. The increased efficacy upon oral administration of I could result from its conversion in the stomach to III in such a manner that gastrointestinal absorption of III is facilitated. For example, if the conversion resulted in the precipitation of the relatively insoluble diketone III in a finely divided state with a specific surface area significantly greater than that of a similar dose of crystalline III, the increased rate of solution of the finely divided III could result in an enhanced rate of absorption.

However, other possible explanations for the increased oral efficacy of I over the parent III also may be advanced. These include: the enzymatic formation of an active metabolite of I *in vivo*; a structural configuration of the dioxime I which permits a very rapid rate of gastrointestinal absorption; or a synergistic effect of the hydroxylamine formed by hydrolysis of I upon the pharmacological response to I. Since it is not yet known if the pharmacological effect of I is due to the intact molecule *per se* or to its breakdown products II and III, it is not possible to choose between these alternatives. An investigation of the relative rates of absorption of orally administered I and III and the characterization of the active species which appears in the plasma must be undertaken before the true mechanism can be established.

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