[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Preparation and Reactions of Steroidal $\Delta^{17,(20)}$-Enol Acetates

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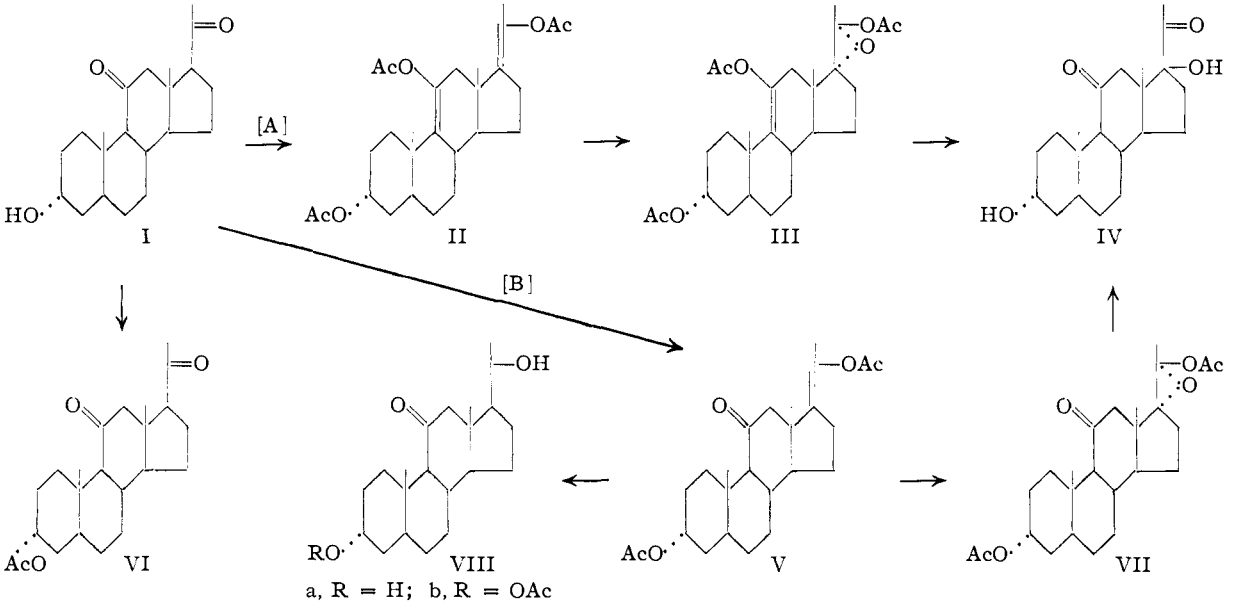
A technique for the determination of the relative rates of enol acetylation of steroidal carbonyl groups has been developed. By the selective enol acetylation of 3α-hydroxypregnane-11,20-dione (I), 3α,20-diacetoxy-17(20)-pregnen-11-one (V) has been prepared. Some of the reactions of V have been studied.

In the course of studies in these laboratories directed toward the synthesis of the adrenal hormones, a study was made of the excellent procedure described by Koechlin, Kritchevsky, Garmaise and Gallagher[1,2] for the introduction of the 17α-hydroxyl group into 3α-hydroxypregnane-11,20-dione (I). Since these investigators reported the formation and isolation of the dienol triacetate II in their reaction sequence A (I → II → III → IV), it was of interest to prepare the $\Delta^{17,(20)}$-monoenol acetate (V) for an evaluation of its possible superiority over II in the parallel sequence B (I → V → VII → IV). The $\Delta^{17,(20)}$-monoenol acetate was also of interest as a possible intermediate for the synthesis of 11β-hydroxysteroids.

reaction occurred beyond this time. From this reaction 3α,20-diacetoxy-17(20)-pregnen-11-one (V) was isolated easily and its structure was established by reaction with peracetic acid followed by basic hydrolysis to form 3α,17α-dihydroxypregnane-11,20-dione (IV). The rate constants for the acylation of the 3α-hydroxy group and the 20-keto group were determined. These are given in Table I.

Studies with pregnane-3,11,20-trione indicated that the 3-keto group reacted at about the same rate as the 3α-hydroxy group in I. Again no appreciable reaction was observed after two moles of acetic anhydride had been consumed, indicating reaction only at the 3- and 20-positions.



The relative reactivities of the three acylable groups in 3α-hydroxypregnane-11,20-dione were studied in a mixture of toluene, acetic anhydride and a strong acid catalyst. The course of the reaction was followed by measuring the acetic acid as it was formed (see Experimental). After one-half hour under reflux conditions, a full molar equivalent of acetic anhydride was consumed. When the reaction was terminated at this point, 3α-acetoxypregnane-11,20-dione was isolated. A second molar equivalent of acetic anhydride reacted during the next 12 hours and no apreciable

An attempt was made to study the reaction rates for the enol acetylation of 17α-hydroxy-21-acetoxypregnane-3,11,20-trione (dihydrocortisone acetate) and 17α-hydroxy-21-acetoxypregnane-3,20-dione.[3] In both cases two equivalents of acetic anhydride reacted within one hour. Our method of analysis did not permit quantitative determination of the separate reaction rate constants.

The increased catalytic power of sulfosalicylic acid (SSA) over toluenesulfonic acid (TSA) in the enol acetylation reaction is demonstrated (see Table I). It is also indicated that the concentration of catalytically effective solvated protons is not increased by increasing concentrations of sulfo-

(1) T. H. Kritchevsky and T. F. Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).

(2) B. A. Koechlin, D. L. Garmaise, T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **71**, 3262 (1949); T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

(3) R. B. Moffett and H. V. Anderson, *ibid.*, **76**, 747 (1954). The chemistry of these diacylations is discussed.

TABLE I

CONDITIONS AND FIRST ORDER RATE CONSTANTS OF STEROID ACYLATION AT 1.0 *M* ACETIC ANHYDRIDE CONCENTRATION IN REFLUXING TOLUENE (k = MIN.$^{-1}$)

Run no.	Steroid	k, first equiv.[a]	k, second equiv.[b]	Steroid, moles	Acetic anhydride, moles	Catalyst,[c] moles	Toluene, ml.
1	3α-Hydroxypregnane-11,20-dione	0.023	0.0030	0.010	0.145	0.00517	127
2	3α-Hydroxypregnane-11,20-dione	.025	.0030	.020	.143	.00525	127
3	3α-Hydroxypregnane-11,20-dione	.042	.0057	.010	.140	.00052	140
4	3α-Hydroxypregnane-11,20-dione	.069	.0058	.020	.140	.00104	140
5	3α-Hydroxypregnane-11,20-dione	.072	.0054	.020	.140	.00052	140
6	21-Acetoxy-17α-hydroxy*allo*pregnane-3,20-dione	...	....	.015	.140	.00104	140
7	21-Acetoxy-17α-hydroxypregnane-3,11,20-trione	...	....	.010	.140	.00059	140
8	Pregnane-3,11,20-trione	.065	.0053	.010	.140	.00104	140

[a] Calculated on the premise that the reaction and not the distillation was rate determining. [b] Estimated error: 10%. [c] Toluenesulfonic acid used for runs 1 and 2, sulfosalicylic acid for runs 3 through 8.

salicylic acid within the ranges studied. Doubling the concentrations of this catalyst had no significant effect on first-order rate with respect to acylable steroid concentrations.

When reaction sequences A and B were carried out without isolations under conditions identical beyond the enol acylation step, comparable yields of 3α,17α-dihydroxypregnane-11,20-dione were obtained.

Pure crystalline 3α,20-diacetoxy-17(20)-pregnen-11-one (V) when subjected to reaction with peracetic acid, followed by alkaline hydrolysis, resulted in 82% of 3α,17α-dihydroxypregnane-11,20-dione (IV). Under the same conditions 78.5% of IV was obtained from the pure dienol acetate II prepared according to Gallagher, *et al.*[2] These results indicate that the extent of attack at the 9(11)-double bond by peracetic acid under these conditions is not great and that the use of the monoenol acetate rather than the dienol acetate offers only a slight advantage in yield. Recently, Hirschmann and Wendler[4] have demonstrated that the double bond in the enol acetate of an 11-oxygenated steroid (methyl 3α-hydroxy-11-ketoetiocholanate) is in the 9(11)-position and that this bond can be epoxidized with peracids under more strenuous conditions than used in this work.

Reduction of V with lithium borohydride in dry tetrahydrofuran resulted in a mixture from which 3α,20β-dihydroxypregnan-11-one (VIIIa) and its 3-acetate VIIIb were isolated. Gallagher and Belleau[5] and Dauben and co-workers[6] have shown that enol acetates in aqueous solvents are first hydrolyzed to the ketone and then reduced to the corresponding alcohol by sodium borohydride. Since we wished to preserve the 20-enol acetate structure, an attempt was made to reduce V at C-11 in an anhydrous medium. However, when V was treated in dry pyridine at room temperature with excess sodium borohydride for two hours no reduction occurred.

Compound V reacted readily with hypobromous acid to produce 3α-acetoxy-17-bromopregnane-11,20-dione (IX).[7]

(4) R. Hirschmann and N. L. Wendler, THIS JOURNAL, **75**, 2361 (1953).

(5) B. Belleau and T. F. Gallagher, *ibid.*, **73**, 4458 (1951).

(6) W. G. Dauben and J. F. Eastham, *ibid.*, **73**, 4463 (1951); W. G. Dauben, R. A. Michaeli and J. F. Eastham, *ibid.*, **74**, 3852 (1952).

(7) This addition of hypobromous acid to steroid enol acetates to give α-bromo ketones was developed by Dr. B. J. Magerlein in these laboratories and will be described in a future publication.

Experimental[8]

Apparatus.—A 250-ml. flask was equipped with a 41 × 1.3 cm. column packed with glass helices. The column was fitted with a still head which permitted total reflux. The flask was equipped with a thermometer and a graduated dropping funnel so that a known volume of toluene containing a known concentration of acetic anhydride could be added during the distillation process.

Measurements.—Acetic anhydride and acid catalyst were dissolved in the toluene and heated under reflux. The rate of evolution of acetic acid was measured both before and after the addition of the steroid. Any acetic anhydride or toluene removed by distillation and reaction was replaced after each measurement of volume and composition of the distillate, so as to maintain both constant volume and constant acetic anhydride concentration in the reaction medium. The net result is a pseudo unimolecular reaction which may be treated by first-order reaction kinetics.

The method of analysis was a modification of a technique developed by Smith and Bryant.[9]

The toluene distillate at no time comprised more than 3% of the total volume of the reaction and contained the formed acetic acid and some acetic anhydride. It was diluted with anhydrous methanol and titrated with a standard methanolic solution of 0.1 *N* sodium methylate using phenolphthalein indicator according to the equations

$$NaOCH_3 + CH_3COOH \longrightarrow CH_3COONa + CH_3OH$$
$$NaOCH_3 + (CH_3CO)_2O \longrightarrow CH_3COOCH_3 + CH_3COONa$$

The solutions were then diluted with water and saponified completely with a measured excess of aqueous standard 0.1 *N* sodium hydroxide by heating on a hot plate and distilling the toluene so that

$$NaOH + CH_3COOCH_3 \longrightarrow CH_3COONa + CH_3OH$$

The cooled solutions were then titrated with standard 0.1 *N* hydrochloric acid. Thus the total acetic anhydride and acid originally present in the distillate could be calculated. The validity of this procedure was checked against standard toluene solutions of acetic anhydride.

The amount of acetic acid present in the distillate (*i.e.*, the amount of esterification occurring in the reaction for a given time interval) was determined. The data from a representative run are plotted in Fig. 1. The amount of acetic anhydride distilled also was determined. The sum of these molar quantities was the amount of anhydride necessary to add back to the reaction mixture to maintain a constant concentration of acetic anhydride. The volumes removed were measured and sufficient toluene was added to maintain a constant volume for the reaction.

The reaction mixture was initially distilled without steroid present until the acetic acid in the distillate approached a constant rate of removal. In this fashion the water of hydration of the catalyst and water present in the solvent and equipment were removed. The constant rate allowed

(8) All melting points were taken on a Fisher–Johns block and are uncorrected unless otherwise stated. Analyses were by Mr. William Struck and associates of our Microanalytical Laboratory. Spectra were determined by Dr. J. L. Johnson and his associates in our Physics Department.

(9) D. M. Smith and W. M. D. Bryant, THIS JOURNAL, **58**, 2452 (1936).

the determination of the error introduced by irrelevant reactions or introduction of water or acetic acid. This background acetic acid production usually averaged 0.003 ± 0.001 millimole per minute which introduced a maximum of 5% error in the total stoichiometry at the half-life of the slowest acylated steroid function (see Fig. 1). Table I presents the reaction conditions for the various studies.

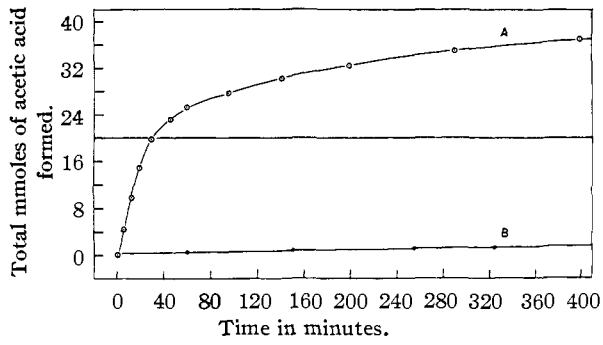


Fig. 1.—A, plot against time in minutes of the total number of millimoles of acetic acid formed on reaction of 20 millimoles of 3α-hydroxypregnane-11,20-dione in 140 ml. refluxing toluene containing 0.140 mole of acetic anhydride and 0.00525 mole of SSA (run no. 5); B, similar plot for the same system not containing steroid.

The logarithm of the calculated millimoles of unreacted steroid concentration was plotted against time. The rate of acylation of the first equivalent was approximated by multiplying 2.303 by the initial slope of this plot. The apparent fast rate of acylation of the first function (see Table I) does allow the possibility that the first steroid function (the 3α-hydroxy group) undergoes acylation at a faster rate than the technique can follow. Thus these first-order rate constants would at least be minimum rate values. Since the production of acetic acid was so much slower for the second equivalent, its calculated acylation rate can no longer be considered an artifact of distillation but a true measure of reaction rate.

There was no observable formation of a third equivalent of acetic acid that could not be readily explained by the low rate of formation of the blank runs.

The first-order rate constants for acylation of the second steroid function were calculated by means of the Guggenheim expression[10]

$$\log [(m)_{t'} - (m)_t] = kt/2.303 + K$$

where

$(m)_{t'}$ = mmoles of acetic acid produced at time t'
$(m)_t$ = mmoles of acetic acid produced at time t
k = first-order rate constant (min.$^{-1}$)
K = constant

and $t' - t$ should exceed the half-life of the reaction.

A smooth curve was drawn between the experimental points (see Fig. 1 for run no. 5) and the logarithms of the difference between millimoles of acetic acid produced at a constant time interval was plotted against the initial times of the interval (see Fig. 2 for run no. 5 where $[t' - t] = 160$ minutes). The slope of this plot multiplied by 2.303 is the first-order rate constant (k). This method minimizes the error that would be involved in extrapolation of the curve of Fig. 1 to infinite time.

Preparation of 3α,20-Diacetoxy-17(20)-pregnen-11-one (V).—A solution of 7.14 g. (0.07 mole) of acetic anhydride and 0.132 g. (0.0006 mole) of sulfosalicylic acid in 70 ml. of toluene was slowly distilled through a 48 cm. × 1.3 cm. column packed with 1/8-inch Pyrex helices and equipped with a variable take-off head. The reflux–distillation ratio was set so that 6.0 ml. of distillate was collected during the course of about two hours. The acetic acid and acetic anhydride collected were determined by differential titration (see description of method for rate measurements), and the acetic anhydride and toluene removed were replaced. Then

(10) F. A. Guggenheim, *Philosophical Mag. and J. of Sci.*, **2**, 538 1936).

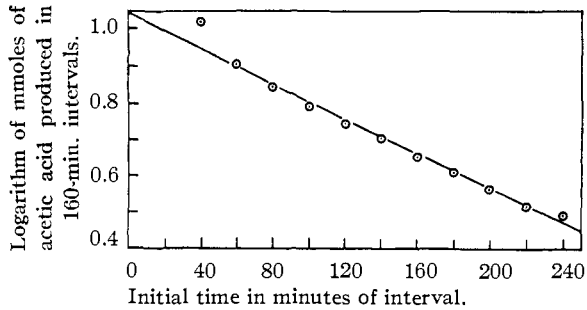


Fig. 2.—First-order plot of logarithm of millimoles of acetic acid produced in 160-minute intervals against the initial time of the interval. The plot is based on the smooth curve A in Fig. 1 for acylation of 3α-hydroxypregnane-11,20-dione in refluxing toluene.

3.29 g. (0.01 mole) of 3α-hydroxypregnane-11,20-dione was added, and the solution boiled under total reflux, with periodic removal of small amounts of distillate (7 portions of about 2.0 ml.) and corresponding replacement of the acetic anhydride and toluene, for 12 hours. The amounts of acetic anhydride and toluene added after each removal of distillate were estimated from the amounts required in the rate-determination measurements.

The toluene solution, after cooling, was agitated thoroughly with ice-water for one hour, the water was extracted with ether and the combined ether and toluene solution was washed with aqueous sodium bicarbonate. Evaporation under reduced pressure left 4.82 g. of gummy residue which crystallized readily when triturated with 20 ml. of methanol. The crystals dissolved in the methanol at about 50° and recrystallized on cooling to room temperature; yield 3.28 g., m.p. 75–110°.

An analytical sample was obtained by chromatography of the crude product from a similarly conducted run. Adsorption on Florisil and elution with 5% acetone in Skellysolve B gave crystals melting at 130.5–131.5°, $[\alpha]_D$ +56° in chloroform.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71; acetyl, 20.7. Found: C, 71.97; H, 8.21; acetyl, 20.30.

When the crude crystalline enol acetate, melting at 75–110°, was dried in vacuum at 78° for two hours or at room temperature for two weeks, it lost about 5.9% of its weight (calcd. for 1 molecule of CH_3OH, 7.15% weight loss) and melted at 120–127°.

Hydrolysis.—A sample of the crude crystalline enol acetate, dissolved in methanol and treated with dilute aqueous hydrochloric acid, was hydrolyzed at room temperature to give 3α-hydroxypregnane-11,20-dione, which after one recrystallization from a mixture of ethyl acetate and Skellysolve B, melted at 172–173° and did not depress the melting point of authentic 3α-hydroxypregnane-11,20-dione.

3α-Acetoxypregnane-11,20-dione (VI).—The above experiment was repeated but was stopped by pouring onto crushed ice 25 minutes after the steroid had been added. The aqueous phase was extracted with ethyl acetate. The combined organic phase was distilled to dryness under reduced pressure. The residue was recrystallized from hexane–ethyl ether and then methanol–water to give material of m.p. 134.5–136° (cor.).[11] The infrared spectrum was identical with that of an authentic sample of 3α-acetoxypregnane-11,20-dione.

3α,17α-Dihydroxypregnane-11,20-dione (IV). A. From Crystalline Monoenol Acetate.—A solution of 400 mg. of 3α,20-diacetoxy-17(20)-pregnen-11-one in 2.2 ml. of chloroform was shaken for 2.5 hours at room temperature with 1.0 ml. of a 40% solution of peracetic acid[12] to which had been added 21 mg. of sodium acetate. Ten milliliters of chloroform was added and the mixture extracted with 5% sodium hydroxide and water. After drying over anhydrous sodium sulfate, the chloroform solution was evaporated to a pale amber glass. Crystallization from methanol gave 17α,20-

(11) J. v. Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944). The m.p. was reported by these authors to vary between 132–133° and 138°, depending upon the rate of heating.

(12) Buffalo Electro Chemical Company, Buffalo, New York.

oxido-3α,20-diacetoxypregnan-11-one (VII) as needles, m.p. 80–100°. After two recrystallizations from Skellysolve B, rosettes were obtained, m.p. 128–130°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.49; H, 8.49.

A solution of 300 mg. of the total crude 17α,20-oxido-3α,-20-diacetoxypregnan-11-one (VII) in 10 ml. of methanol was treated in portions, with shaking, with 6 ml. of 0.73 *N* aqueous sodium hydroxide. After standing 55 minutes at room temperature, the product was separated by extraction with methylene chloride. The crude material obtained upon evaporation was crystallized from benzene giving 207 mg. of IV (82% yield from V), m.p. 201–204°, $[\alpha]_D$ +62° (acetone).

B. From Crystalline Dienol Acetate.—Crystalline 3α,11,20-triacetoxy-9(11),17(20)-pregnadiene (0.75 g.), m.p. 203–204°, prepared according to the procedure of Gallagher and co-workers,[13] was epoxidized and saponified essentially as described in Part A. After crystallization from benzene, there was obtained 445 mg. of IV as shiny plates, m.p. 201–203° (78.5% yield).

C. From Crude Monoenol Acetate.—3α-Hydroxypregnane-11,20-dione[14] (6.65 g., 0.02 mole) was enol acetylated by the toluene azeotrope technique described above. The cooled reaction mixture was washed twice with an excess of iced 4% sodium bicarbonate solution, washed with water, and dried with anhydrous sodium sulfate. The crude monoenol acetate obtained by evaporation was dissolved in 50 ml. of chloroform and treated with cooling with 21 ml. of 40% peracetic acid in which had been dissolved 0.5 g. of sodium acetate. After stirring this mixture at room temperature for 2.5 hours, methylene chloride was added and the solution was washed with iced 5% sodium hydroxide and water. The crude epoxide obtained on evaporation of the dried organic phase was saponified in 540 ml. of 0.25 *N* sodium hydroxide in 50% ethanol for 40 minutes at room temperature. The product was extracted with methylene chloride. After the usual work-up, IV crystallized from benzene in two crops: 4.10 g. as plates, m.p. 200–202°, and 0.46 g., m.p. 194–198° (65.5% yield).

D. From Crude Dienol Acetate.[2]—A solution of 3.00 g. (0.009 mole) of 3α-hydroxypregnane-11,20-dione[14] and 0.87 g. of toluenesulfonic acid monohydrate in 60 ml. of acetic anhydride was distilled slowly for three hours[15] so that approximately 40 ml. of distillate was collected. Crushed ice was added to the cooled residue and when the excess acetic anhydride was essentially decomposed, the gummy product was extracted with methylene chloride. The extract was washed with iced 4% sodium bicarbonate, water and finally dried with sodium sulfate. The brown sirup obtained after evaporation was epoxidized and saponified as described in Part A. The product IV was crystallized from benzene in two crops: 2.04 g., m.p. 199–201°, and 0.09 g., m.p. 194–198° (67.9% yield).

Reduction of 3α,20-Diacetoxy-17(20)-pregnen-11-one with Lithium Borohydride.—A solution of 416 mg. of the monoenol acetate (purified by chromatography, m.p. 130–133°) in 5 ml. of dry tetrahydrofuran was added to a solution of 81 mg. of lithium borohydride in dry tetrahydrofuran. The solution was allowed to stand at room temperature overnight and then poured into 50 ml. of dilute acetic acid. A white gum separated; the gum and the solution were extracted with ether. The ether solution was washed with water, dried and evaporated to dryness to give 400 mg. of an oil.

When this was dissolved in hot benzene, 160 mg. of crystalline product separated on cooling, which after one recrystallization from ethyl acetate melted at 234–235°,[16] and was identified as 3α,20β-dihydroxypregnan-11-one (VIIIa) by mixed m.p. and comparison of the infrared absorption spectrum with that of an authentic specimen.

The filtrate was chromatographed over Florisil, eluting with increasing concentrations of acetone in Skellysolve B. An 8% acetone solution removed 69 mg. of a crystalline compound, m.p. 200–201°, whose infrared absorption spectrum showed bands attributable to hydroxyl (3543 cm.$^{-1}$), acetate (1250 and 1735 cm.$^{-1}$), and non-conjugated carbonyl oxygen (1685 cm.$^{-1}$). Sarett[16] gives 204–205° as the m.p. of the most likely product, 3α-acetoxy-20β-hydroxypregnan-11-one (VIIIb).

3α-Acetoxy-17-bromopregnane-11,20-dione (IX).—To a solution of 1.92 g. of 3α,20-diacetoxy-17(20)-pregnen-11-one in 50 ml. of *t*-butyl alcohol was added a solution of 855 mg. of N-bromosuccinimide in 100 ml. of *t*-butyl alcohol and 55 ml. of 1.0 *N* sulfuric acid. After standing at room temperature for 1.5 hours, the reaction was stopped by the addition of an aqueous solution of 4.66 g. of sodium sulfite. After 15 minutes the reaction mixture was diluted with an excess of water and extracted with methylene chloride. The extract was washed with water and dried over anhydrous sodium sulfate. Evaporation yielded a white, partially crystalline residue which crystallized from ether to give 1.31 g. (63% yield) of flat rods, m.p. 171–174°.[17] Recrystallization from an ethyl acetate–Skellysolve B mixture gave heavy needles, m.p. 174–175°, $[\alpha]_D$ −5° in chloroform.

Anal. Calcd. for $C_{22}H_{33}BrO_4$: Br, 17.63. Found: Br, 16.63.

KALAMAZOO, MICHIGAN

(13) C. W. Marshall, T. H. Kritchevsky, S. Lieberman and T. F. Gallagher, THIS JOURNAL, **70**, 1837 (1948).

(14) Prepared by reduction of pregnane-3,11,20-trione and subsequently found to be contaminated with 3α,20β-dihydroxypregnan-11-one (VIIIa).

(15) Infrared data indicated only approximately 25% of the 11-ketone to be enol acetylated under these conditions. Longer distillation periods caused a corresponding increase in acetylation of the 11-ketone.

(16) L. H. Sarett, THIS JOURNAL, **70**, 1690 (1948), gives the m.p. as 236–238°.

(17) P. L. Julian, private communication, reports a m.p. 171–172° for 3α-acetoxy-17-bromopregnane-11,20-dione.