Anal. Calcd. for $C_{33}H_{52}O_7S$: C, 66.86; H, 8.84; O, 18.92. Found: C, 66.21; H, 8.50; O, 18.90.

Dimethyl $\Delta^{2,12}$-Oleadiene-28,30-dioate (XIIIb).—The mesylate XIId (600 mg.) was heated under reflux with pyridine for 19 hr., and the product was isolated in the usual way including chromatography and elution with benzene. Recrystallization from methanol gave 255 mg. of colorless plates of the olefin, m.p. 192–195°, $[\alpha]D$ +134°.

Anal. Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74. Found: C, 76.88; H, 9.33.

Hydroxylation of Methyl $\Delta^{2,12}$-Oleadien-28-oate (XIIIa).—The methyl ester XIIIa (390 mg.) in 16 cc. of dioxane was left at room temperature for 8 days with 700 mg. of osmium tetroxide. The osmate ester was decomposed by passing hydrogen sulfide[28] into the solution and filtering the insoluble sulfide. The solvent was removed, and the residue was chromatographed carefully on 20 g. of alumina deactivated with 5% of 10% acetic acid. After removing 80 mg. of unreacted starting material with benzene, the benzene–ether (3:1 and 1:1) eluates afforded 147 mg. of solid, presumably **methyl Δ^{12}-oleanene-2α,3α-diol-28-oate (XIVa)**, which crystallized as needles from methanol–chloroform; m.p. 258–262°, $[\alpha]D$ +85°.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36; O, 13.15. Found: C, 75.96; H, 10.30; O, 13.82.

Further elution with 1:1 benzene–ether gave 28 mg. of the **2β,3β-isomer XVa**, which crystallized as plates from methanol–chloroform, m.p. 276–284° (depressed to 245–253° on admixture with XIVa), $[\alpha]D$ +72°.

Anal. Found: C, 75.85; H, 10.00.

Hydroxylation of Dimethyl $\Delta^{2,12}$-Oleadiene-28,30-dioate (XIIIb).—The reaction was carried out exactly as described above for XIIIa except that 450 mg. of dimethyl ester and 1.0 g. of osmium tetroxide was used. Benzene removed 190 mg. of unreacted olefin and the diols were eluted with ether. The initial material (37 mg., m.p. 227–229°, $[\alpha]D$ +112°) was followed by 70 mg. of an intermediate fraction (m.p. 248–260°) and finally by 60 mg. of a second diol XVb, m.p. 274.5–276°, $[\alpha]D$ +95°.

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50; O, 18.09. Found: C, 72.18; H, 9.40; O, 17.92.

Both diols showed resolution of the ester bands in Nujol mull to the same extent (5.72 and 5.77 μ) which differed very considerably from the shift observed with dimethyl medicagenate (Id). A 20-mg. sample of the mixed diols was oxidized in the manner described above for medicagenic acid (Ia) and the oxidation product was treated with base. The oil could not be crystallized but the position of its ultraviolet absorption maximum (λ_{max}^{EtOH} 266 mμ, $\lambda_{max}^{KOH-EtOH}$ 306 mμ) is in good agreement with expectation since this diosphenol contains one less substituent on the double bond as compared with Va.

Methyl 2-Hydroxymethyleneoleanonate (XVI).—Dry sodium methoxide (from 200 mg. of sodium), 15 cc. of ether and 1.8 cc. of dried and freshly distilled ethyl formate were stirred for 30 min. in an atmosphere of nitrogen followed by addition of 1.45 g. of methyl oleanonate in 30 cc. of benzene. After stirring under nitrogen for 17 hr., dilute hydrochloric acid was added, the organic layer was separated and washed well with water and dried. Recrystallization from methanol–chloroform afforded 1.2 g. of plates, m.p. 205–207.5°, $[\alpha]D$ +109°, dark brown color with ferric chloride.

Anal. Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74; O, 12.88. Found: C, 76.69; H, 9.79; O, 13.10.

A 300-mg. sample was ozonized in methylene chloride solution at −70° for 5 minutes in the earlier described fashion[29] and the ozonide was decomposed with ferrous sulfate. Separation into acidic (120 mg.) and neutral (148 mg., brown color with ferric chloride) fractions followed by warming of the latter with alkali and chromatography on deactivated alumina led to only 15 mg. of oil. Its ultraviolet absorption spectrum (λ_{max}^{EtOH} 270 mμ) indicated that it probably contained some of the desired diosphenol, but this approach was not pursued due to the poor yield. Ozonolysis in ethyl acetate solution at room temperature produced material which on the basis of its infrared spectrum seemed to be largely anhydride.

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[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC.]

Identification and Synthesis of 4,17-Pregnadien-20-ol-21-al-3,11-dione Acetate[1]

BY R. E. BEYLER AND FRANCES HOFFMAN

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The synthesis and reactions of 4,17-pregnadien-20-ol-21-al-3,11-dione acetate and 4,17-pregnadiene-11β,20-diol-21-al-3-one 20-acetate are described.

In the course of an investigation of steroids related to adrenocortical hormones, we encountered a compound of unusual chemical and biological properties. This material was isolated in very small yield from mother liquors obtained in the synthesis of cortisone *via* the 3-dinitrophenylhydrazone. The most distinctive property it possessed was its high extinction coefficient in the ultraviolet, λ_{max}^{MeOH} 241 mμ, $E\%$ 617. The source of the compound,[2] together with the ultraviolet absorption and elemental analysis, suggested that it might be 4,16-pregnadien-21-ol-3,11,20-trione acetate, which we desired for further synthetic studies. That this was not the correct structure was conclusively shown when an authentic sample of this compound was made available to us.[3] The ultraviolet spectra of the two compounds are similar but all other properties are different.[4]

Alternative structures for steroids with two chromophoric groups (presumed to be conjugated carbonyl groups) are not plentiful. The 20-enol acetate-21-aldehyde[5] structure (VIa) was a logical candidate, particularly in view of the following properties which the compound possessed: 5.70 (Nujol) or 5.68 ($CHCl_3$) μ band in the infrared typical of an enol acetate; Porter–Silber test very rapid (similar to "cortisone aldehyde"); instability on acid-washed alumina; positive blue tetrazolium test (hereafter abbreviated "BT test"). A negative Schiff test (other 21-aldehydes are positive) was somewhat disturbing, however.

(1) Presented in part at the Ninth Annual Meeting-in-Miniature of the North Jersey Section at Seton Hall University, South Orange, N. J., on January 28, 1957.

(2) H. Reich and B. K. Samuels, *J. Org. Chem.*, **19**, 1041 (1954), have obtained Δ^{16}-steroids from 17-hydroxy-20-dinitrophenylhydrazones by treatment with acid.

(3) We are grateful to Dr. W. F. McGuckin for providing us with a sample and physical constants prior to the publication.[4]

(4) W. F. McGuckin and H. L. Mason, THIS JOURNAL, **77**, 1822 (1955).

(5) G. A. Fleisher and E. C. Kendall, *J. Org. Chem.*, **16**, 573 (1951), have reported a 20-enol acetate 21-aldehyde in another series.

The first attempts to convert the presumed VIa to a known structure employed acidic and alkaline hydrolysis in aqueous methanol. The product was not 4-pregnen-21-al-3,11,20-trione but in *both* cases was 21,21-dimethoxy-4-pregnene-3,11,20-trione (IX), as proven by infrared comparison with an authentic sample.[6]

The formation of IX in the acid hydrolysis seemed reasonable but under alkaline conditions it was not anticipated. Furthermore, reaction of dehydrocorticosterone 21-aldehyde hydrate (IVa) or its anhydrous form under the identical conditions (sodium bicarbonate in methanol–water) did not produce any detectable amount of IX; only starting material and adrenosterone were obtained.[7] It is noteworthy that a recent paper[8] reports the preparation of dimethylacetals of aldehydes containing a strong electronegative substituent in the presence of aqueous sodium hydroxide.

Reaction of the presumed VIa with acetic anhydride in the presence of sulfuric acid afforded a triacetate with loss of the high molecular extinction value. This product was 4,17-pregnadiene-20,21,-21-triol-3,11-dione triacetate (VIII), whose analogous structure was similarly obtained by Fleisher and Kendall.[5]

With this much evidence for the structure in hand, its synthesis was undertaken. Dehydrocorticosterone (Ia) was converted to the corresponding 21-aldehyde hydrate IVa *via* the pyridinium tosylate II and nitrone III as previously reported with desoxycorticosterone,[9] Substance S,[10] cortisone and hydrocortisone.[11] Direct oxidation of the ketol with copper acetate was far more productive, however.[12]

Enolization of the glyoxal side chain was carried out in pyridine–acetic acid (1 to 1) at 60°. The enol V was never purified but ultraviolet data indicated the presence of 30 to 40% in the oil which was obtained. Acetylation of the mixture, followed by chromatography, afforded 4,17-pregnadien-20-ol-21-al-3,11-dione acetate (VIa) identical in every respect with the compound isolated from cortisone mother liquors. Simultaneous enolization and acetylation of IVa was more convenient and gave a similar yield.

An analogous synthesis of 4,17-pregnadiene-11β,20-diol-3-one-21-al 20-acetate (VIb) was also carried out starting with corticosterone (Ib). Copper acetate oxidation and enol acetylation provided the product in two steps.

Both enol acetates VIa and b can exist in *cis* and *trans* forms about the C_{17-20} double bond. Chromatography invariably gave crystalline product distributed over a rather broad range of eluting solvents. Recrystallization gave only one component which did, however, exhibit an appreciable melting point range. Consequently, it is possible that our materials may be mixtures of the *cis* and *trans* isomers even though the paper strip chromatograms showed only a single spot.

The biological testing of these enol acetate aldehydes proved to be interesting.[13] In the liver glycogen test VIa was about 60% as active as hydrocortisone (11-dehydrocorticosterone aldehyde hydrate (IVa) was less than 10% as active as hydrocortisone). Compound VIa also possessed very good activity in the local granuloma assay but was inactive in the systemic granuloma test and in the sodium retention test in adrenalectomized rats. The 11-hydroxy analog VIb was somewhat less active than VIa in the liver glycogen test and also inhibited granuloma formation in the cotton pellet test when administered locally but not when administered systemically.

Acknowledgment.—The authors are indebted to Dr. L. H. Sarett for encouragement and suggestions during the course of this investigation.

Experimental[14]

Properties of 4,17-Pregnadien-20-ol-21-al-3,11-dione Acetate (VIa).—The melting point of different samples with essentially identical infrared spectra varied from 205–220° to 245–249°, $[\alpha]D$ +163° (*c* 1, $CHCl_3$); λ_{max}^{MeOH} 241 mμ, ε 23,700; λ_{max}^{ether} 238 mμ, ε 26,600; BT positive, Porter–Silber rapid positive, $FeCl_3$ negative, Schiff aldehyde test negative.

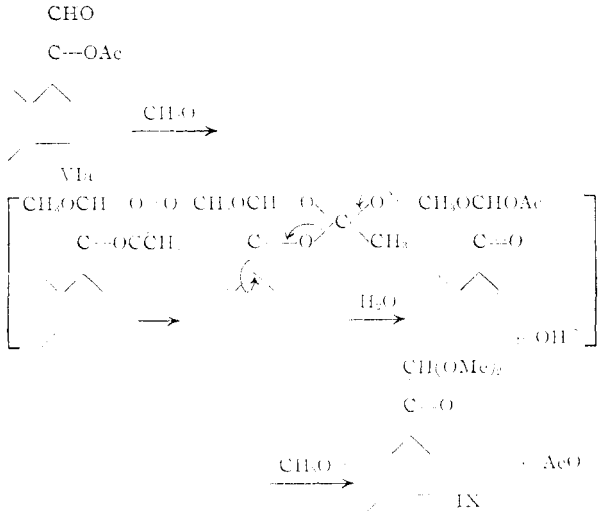
Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34; CH_3CO, 11.2. Found: C, 71.65, 71.89; H, 7.43, 7.09; CH_3CO, 9.0 (alkaline hydrolysis), 14.9 (acid hydrolysis).

Acid Hydrolysis of VIa.—Five hundred milligrams of VIa was dissolved in 35 ml. of methanol, 1.25 ml. of concentrated hydrochloric acid added, and the mixture allowed to stand overnight at room temperature. Water was added and the resultant crystals filtered giving 408 mg., m.p. 153–157°. Recrystallization from methylene chloride–ether gave a sample, m.p. 159–161°. The infrared spectrum was essentially identical with that of authentic 21,21-dimethoxy-4-pregenene-3,11,20-trione (IX).[6]

Alkaline Hydrolysis of VIa.—To 100 mg. of VIa in 5 ml. of methanol was added 100 mg. of sodium bicarbonate dissolved in 0.1 ml. of water. The mixture was allowed to stand at room temperature for two days. Concentration under reduced pressure of part of the methanol and addition of water gave crystals, 77 mg., m.p. 156–158°. Recrystalli-

(6) V. R. Mattox, THIS JOURNAL, **74**, 4340 (1952).

(7) The acetal IX may have formed by the indicated mechanism:



(8) E. Schmitz, *Angew. Chem.*, **68**, 375 (1956).

(9) H. Reich and T. Reichstein, *Helv. Chim. Acta*, **22**, 1124 (1939).

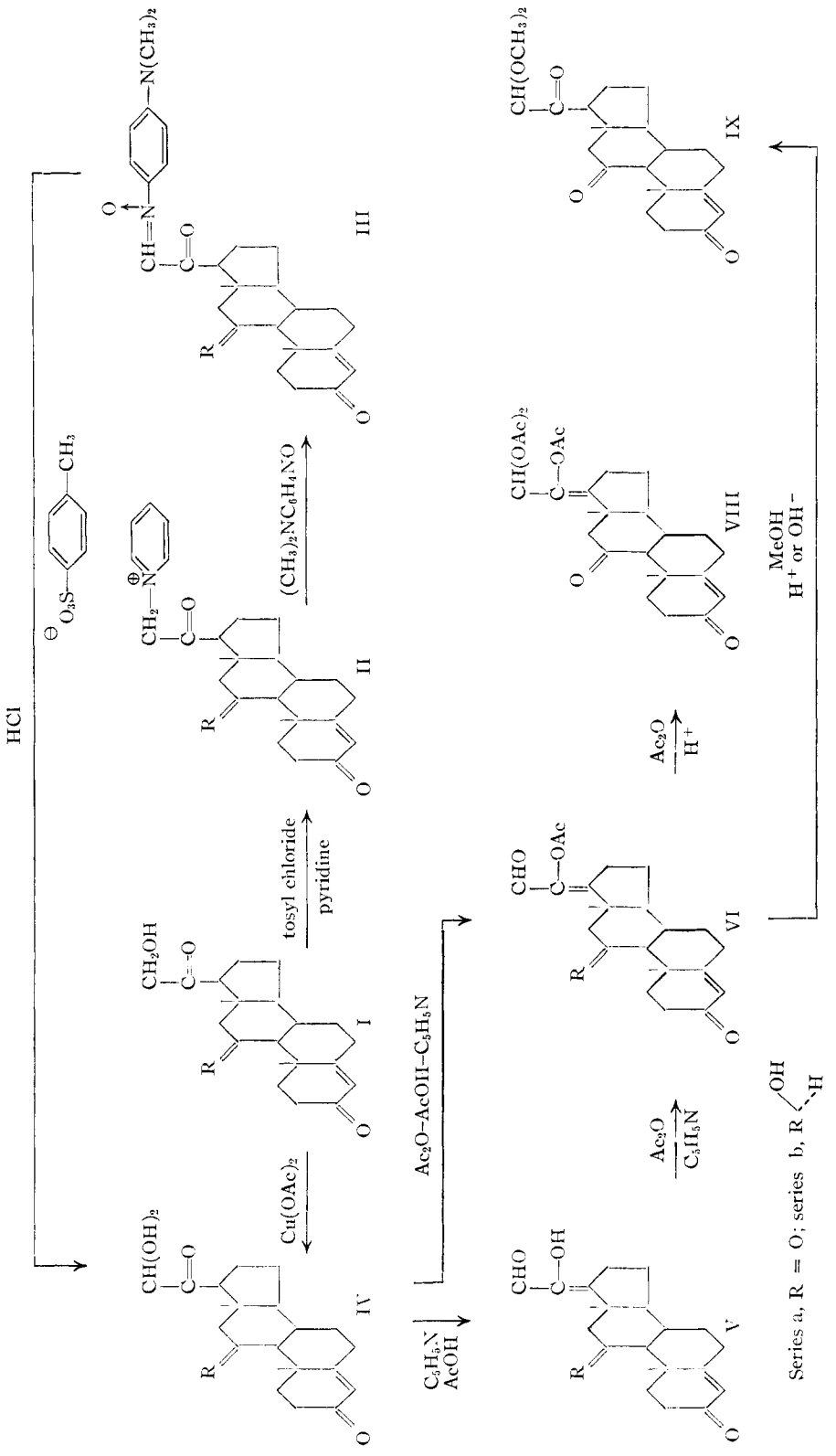
(10) K. Miescher and J. Schmidlin, *ibid.*, **33**, 1840 (1950).

(11) W. J. Leanza, J. P. Conbere, E. F. Rogers and K. Pfister 3rd, THIS JOURNAL, **76**, 1691 (1954).

(12) J. P. Conbere, U. S. Patent 2,773,077 (Dec. 4, 1956).

(13) We are indebted to Drs. C. A. Winter, C. C. Porter, and H. C. Stoerk of the Merck Institute of Therapeutic Research for these results.

(14) Melting points were determined on the Kofler micro hot-stage. We are indebted to R. N. Boos and associates for elemental analyses, to F. A. Bacher and his group for ultraviolet spectra, and to R. W. Walker for infrared spectra.



zation from methylene chloride–ether gave a sample, m.p. 160–162°, infrared spectrum essentially identical with 21,21-dimethoxy-4-pregnene-3,11,20-trione. Another experiment conducted in aqueous methanolic sodium hydroxide yielded 40% of the same product.

4-Pregnene-3,11,20-trione-21-pyridinium Tosylate (IIa).—To 1.2 g. of dehydrocorticosterone (Ia) in 2 ml. of pyridine was added 795 mg. of *p*-toluenesulfonyl chloride. The mixture was warmed gently a few minutes and allowed to stand at room temperature for five hours. The crystals which had formed were separated and washed with acetone to give 930 mg., m.p. 235–245°. Recrystallization from ethanol provided the analytical sample, m.p. 253–256°; λ_{max}^{Nujol} 5.82 (shoulder), 5.86, 5.98, 6.09, 6.17, 6.65 μ.

Anal. Calcd. for $C_{33}H_{39}O_6NS$: C, 68.61; H, 6.81; N, 2.43. Found: C, 68.82; H, 6.30; N, 2.38.

4-Pregnen-21-al-3,11,20-trione Hydrate (IVa). A. From the Pyridinium Tosylate IIa.—Seventy milligrams of pyridinium tosylate IIa and 28 mg. of *p*-nitrosodimethylaniline were dissolved in 5 ml. of methanol and 1.3 ml. of water. To this was added 17 mg. of potassium bicarbonate dissolved in 0.5 ml. of water. After standing two hours at room temperature a large volume of water was added and the resulting solid centrifuged, water-washed and dried to give 48 mg. of crude nitrone IIIa, m.p. 110–117°.

The crude nitrone above was suspended in 5 ml. of ether and shaken with 5 ml. of 2.5 *N* hydrochloric acid. The layers were separated and the aqueous phase extracted several times with ether. The combined ether extract was washed with aqueous sodium bicarbonate and water. After drying and concentrating, there was obtained 20 mg. of yellow oil. This was dissolved in acetone, treated with Darco and filtered. Water was added to the filtrate and it was allowed to evaporate slowly on a watch glass. A small quantity of crystals was obtained, m.p. 95–105° (with loss of water). The infrared spectrum was essentially the same as that of IVa prepared below; Schiff test positive.

B. By Copper Acetate Oxidation of Dehydrocorticosterone.—To 500 mg. of dehydrocorticosterone (Ia) in 10 ml. of methanol was added a solution of 750 mg. of copper acetate in 10 ml. of 80% methanol containing two drops of acetic acid. This mixture was placed in an oil-bath maintained at 60° and the progress of reaction followed by periodic testing with BT reagent. In 20 minutes the BT test was very weak; after 30 minutes the solution was decanted from copper oxide, 100 mg. of Versene added, and concentrated under reduced pressure to a small volume. Addition of water gave 496 mg. of crude crystalline IVa. Recrystallization from aqueous methanol yielded 350 mg., m.p. 120–130° (gas evolved). Further recrystallization from the same solvents gave the analytical sample, m.p. 121–124° (clear at 133°), λ_{max}^{MeOH} 238 mμ, ε 15,300; λ_{max}^{Nujol} 2.90, 5.77, 5.89, 6.00, 6.18 μ; Schiff test positive, BT negative.

Anal. Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 69.67; H, 7.63.

4,17-Pregnadien-20-ol-21-al-3,11-dione (Va).—Three hundred milligrams of the aldehyde hydrate IVa was dissolved in 1 ml. of pyridine–1 ml. of acetic acid and heated at 60° for two hours. The mixture was poured into ice—2.5 *N* hydrochloric acid and extracted immediately with methylene chloride several times. The combined extract was washed with dilute hydrochloric acid, aqueous sodium bicarbonate and water. After drying, the methylene chloride was distilled leaving 282 mg. of oil; λ_{max}^{MeOH} 238 mμ, *E*% 420; 279 mμ, *E*% 150. This indicated the presence of approximately 35% of the desired enol Va.

4,17-Pregnadien-20-ol-21-al-3,11-dione Acetate (VIa).—The above enol was dissolved in a mixture of 1 ml. of pyridine and 1 ml. of acetic anhydride and kept at room temperature for 15 hours. It was poured into water–methylene chloride, separated, and the methylene chloride layer washed with dilute acid, bicarbonate, dried and concentrated to give 301 mg. of oil, λ_{max}^{MeOH} 240 mμ, *E*% 661. This was chromatographed on 18 g. of silica gel. Elution with ether–chloroform (9:1) gave 57 mg. of crude enol acetate aldehyde VIa. Recrystallization from methylene chloride–ether and acetonitrile gave a sample, m.p. 226–232°. The infrared spectrum and paper strip running rate were identical with that of VIa described above.

Simultaneous Enolization and Acetylation of IVa to VIa.—Fifty-four milligrams of the aldehyde hydrate IVa was dissolved in 0.5 ml. of pyridine, 0.5 ml. of acetic acid and 0.5 ml. of acetic anhydride. The mixture was heated at 60° for 2.5 hours. It was poured into water and extracted with methylene chloride. The methylene chloride was washed with water, acid, bicarbonate, dried and evaporated to 48 mg. of oil. Crystallization from acetone–ether gave 15 mg., m.p. 205–220°, λ_{max}^{MeOH} 237 mμ, ε 22,200. The infrared spectrum was essentially identical with VIa above.

4,17-Pregnadiene-20,21,21-triol-3,11-dione Triacetate (VIII).—One hundred milligrams of enol acetate aldehyde VIa was dissolved in 2 ml. of warm acetic anhydride. The mixture was cooled in an ice-bath and one drop of concentrated sulfuric acid was added before the steroid crystallized. It was kept in the ice-bath for 45 seconds and a large volume of water added. The acetic anhydride was allowed to hydrolyze slowly at room temperature, leaving a gum. This was extracted into ether, the ether washed with aqueous bicarbonate, dried and evaporated to 58 mg. of oil. Trituration with ether–petroleum ether gave 40 mg. of crystals, m.p. 145–151°. Two recrystallizations from ether gave 23 mg. of analytically pure VIII, m.p. 150–152°; λ_{max}^{MeOH} 237.5 mμ, ε 17,000; λ_{max}^{Nujol} 5.68 (shoulder), 5.72, 5.87, 5.99, 6.15, 8.05, 8.3, 8.4 μ.

Anal. Calcd. for $C_{27}H_{34}O_8$: C, 66.65; H, 7.04; CH_3CO, 26.6. Found: C, 66.77; H, 7.08; CH_3CO, 24.3.

4,17-Pregnadiene-11β,20-diol-21-al-3-one 20-Acetate (VIb).—To 100 mg. of corticosterone (Ib) in 5 ml. of methanol was added 5 ml. of 80% methanol containing 150 mg. of copper acetate and one drop of acetic acid. The mixture was heated for five minutes on the steam-bath (BT test essentially negative after four minutes) and decanted from copper oxide. The solution was concentrated under reduced pressure, water and ether added, and the ether extract washed once more with water. Evaporation of the ether left 102 mg. of oil. Trituration with acetone–ether gave 38 mg. of crystals, m.p. 132–138°. One recrystallization from acetone–ether yielded the aldehyde hydrate IVb, m.p. 137–141°, Schiff test positive; λ_{max}^{Nujol} 2.9–3.0, 5.75, 5.87, 6.0, 6.08, 6.18 μ. The infrared spectrum of the residue from the mother liquors was essentially identical so the entire product could be used in the next step.

To 333 mg. of crude aldehyde IVb, similarly prepared, was added 2 ml. of pyridine, 2 ml. of acetic acid and 2 ml. of acetic anhydride. The mixture was heated at 60° for three hours, poured into ice–dilute hydrochloric acid and immediately extracted with methylene chloride. The organic extract was washed with dilute hydrochloric acid, bicarbonate and water, dried and concentrated to give 298 mg. of oil. This was chromatographed on 10 g. of silica gel. The ether–chloroform (9:1) fractions gave 97 mg. of crystalline material. Recrystallization from acetone–ether several times provided an analytical sample of VIb, m.p. 213–217°; λ_{max}^{MeOH} 239 mμ, ε 26,500; λ_{max}^{Nujol} 2.95, 5.68, 5.94, 6.03, 6.15, 8.20 μ.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 70.97; H, 8.11.

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