marily to Nujol absorption. There is marked similarity between the modifications of the same compound in the 3–8 micron region with noticeable differences occurring among the skeletal frequencies, particularly in the range of 12.5 to 14.5 microns.

α-Copper phthalocyanine, when suspended in cyclohexanol, undergoes transition to the β-form. This was followed, at room temperature, by the change in infrared absorption as recorded in Table I.

TABLE I

Time in minutes	β-form by I.R., %
0	20
30	75
60	90
90	100

Because of the insolubility of the phthalocyanines, we have not been able to obtain satisfactory spectra of their solutions in the infrared. However, copper phthalocyanine shows a limited solubility in α-chloronaphthalene so that the visible and a portion of the ultraviolet region could be scanned. The spectra obtained in this region were identical for both the α- and β-modifications in solution.

The infrared spectra of the crystal modifications of 2-chloro-4-nitrobenzoic acid, allylthiourea and anthranilic acid were also investigated (Figs. 5 and 6). Each modification was scanned in suspension in Nujol as well as in solution. Marked differences are apparent between the spectra of the different modifications in Nujol of any one compound. The solution spectra are not complete because of the limited choice of solvents available. For each compound, an effort was made to obtain a solvent to cover that region of the spectrum where the crystal modifications in suspension showed the greatest differences. In that region, the spectra were found to be identical for all the modifications of the compound in solution.

WILMINGTON, DELAWARE

[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Preparation of Δ^{20}-Enol Acetates from 20-Ketosteroids[1a]

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Three 20-ketosteroids, 3α,12α-dihydroxypregnane-20-one, 3β-hydroxy-Δ^5-pregnene-20-one and 3β-hydroxyallopregnane-20-one, when treated with isopropenyl acetate and sulfuric acid yielded Δ^{20}-20-enol acetates. The structure of the products was shown by color reaction with tetranitromethane, ozonolysis and the preparation of derivatives, and by infrared spectrometry.

When 20-ketosteroids are heated with acetic anhydride in the presence of *p*-toluenesulfonic acid, enol acetates are formed with the unsaturated bond between carbons 17 and 20. The structure of these enol esters has been firmly established by ozonolysis to 17-ketosteroids[2] and by perbenzoic acid oxidation in high yield to 17,20-epoxy-20-acetates.[3] Isopropenyl acetate has been recommended as especially suitable for the preparation of enol acetates,[4] affording excellent yields of product in a smooth reaction, and it was therefore of interest to investigate this reagent with steroid ketones. The first studies were carried out with 3α,12α-diacetoxypregnane-20-one (I) since the enol acetate of this compound prepared by the method of Bedoukian[5] is obtained in high yield as a single geometric isomeride that can be readily crystallized. When I was heated with isopropenyl acetate in the presence of catalytic amounts of sulfuric acid, a new compound was obtained in very good yield. From its infrared spectrum, elementary analysis and saponification equivalent, the product was unquestionably an enol acetate. It was initially believed that this compound was the hitherto undescribed *cis*-geometric isomer since upon treatment with acetic anhydride in the presence of *p*-toluenesulfonic acid it was readily converted to the higher melting enol acetate previously described. In order to test this supposition the same reaction was applied to 3β-hydroxy-Δ^5-pregnane-20-one (III), since this substance affords a crystalline mixture of the two geometric isomerides of $\Delta^{5;17,20}$-pregnadiene-3β,20-diol acetate both of which have been separated and well characterized.[6] These known geometric isomers have the following constants: enol acetate "A," m.p. 147°, $[\alpha]_D$ −50°; enol acetate "B," m.p. 171–172°, $[\alpha]_D$ −52°. The enol acetate obtained with isopropenyl acetate had m.p. 125–126°, $[\alpha]_D$ −47°, and the analysis was correct for $C_{25}H_{36}O_4$. Since the rotation of all three products was so similar it was possible that one or more was a polymorphic crystal modification of another but infrared spectrometry in solution proved conclusively that all three were different substances and that all were enol acetates.[7] From this evidence the conclusion was inescapable that one of the products must be the Δ^{20}-enol ester.

(1) (a) This investigation was supported by grants from the Anna Fuller Fund, the Lillia Babbit Hyde Foundation, and the National Cancer Institute, United States Public Health Service. (b) Fellow of the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Belgium; (c) deceased March 10, 1952.

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

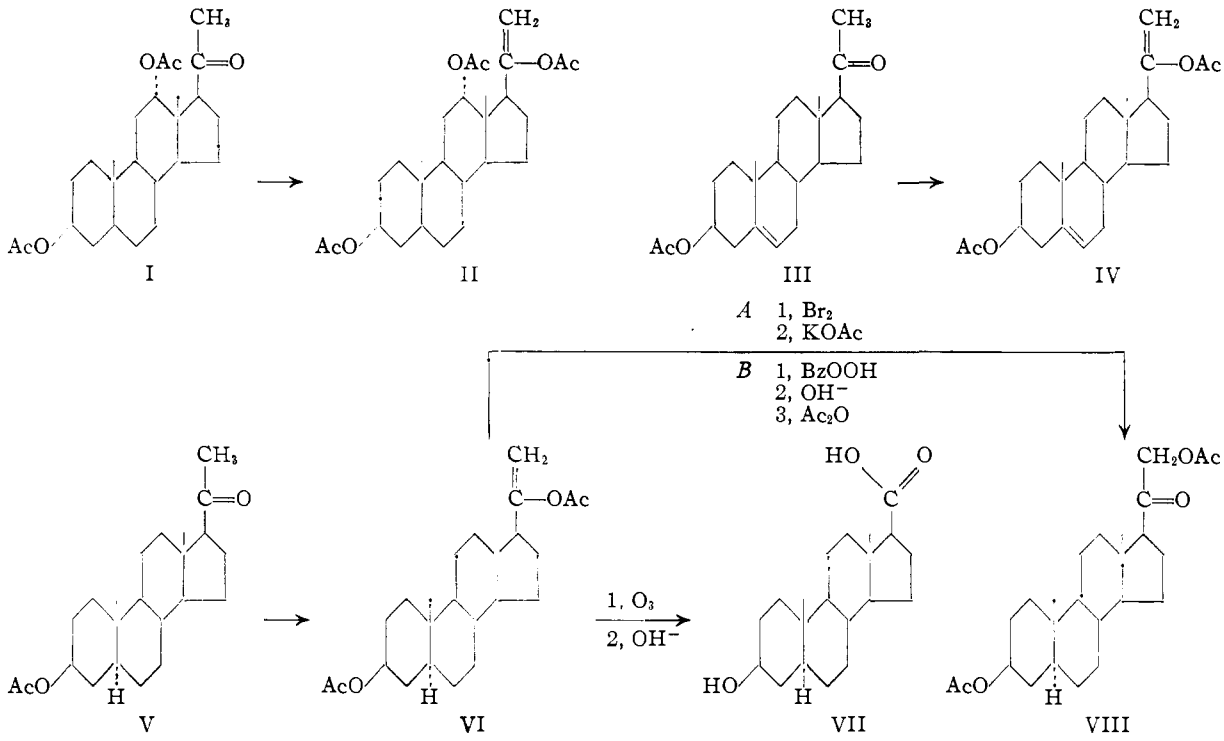
(3) T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 184 (1951); B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 189 (1951).

(4) H. J. Hagemeyer and D. C. Hull, *Ind. Eng. Chem.*, **41**, 2920 (1949).

(5) P. Z. Bedoukian, THIS JOURNAL, **67**, 1430 (1945).

(6) L. F. Fieser and Huang-Minlon, *ibid.*, **71**, 1840 (1949).

(7) We are indebted to Dr. Fieser and Dr. Huang-Minlon for supplying us with an authentic sample of enol acetate "B" for this comparison.



Hagemeyer and Hull[3] have shown that isopropenyl acetate converted methyl ethyl ketone into 2-butene-2-ol acetate in 96% yield whereas methyl isobutyl ketone afforded a 92% yield of 4-methyl-1-pentene-2-ol acetate. Enolization of a carbonyl group toward a methyl rather than a methylenic carbon is therefore not an unexpected reaction, and this seemed the most reasonable explanation for the existence of three different enol acetates. The enol diacetate of 3β-hydroxyallopregnane-20-one was therefore prepared with isopropenyl acetate and, in complete agreement with the prior finding, a new enol acetate, differing in melting point, rotation and infrared spectrum from the two previously described[2] *cis*- and *trans*-$\Delta^{17,20}$-enol acetates, was obtained.

It was possible to establish the structure of these new enol acetates prepared with isopropenyl acetate from three separate types of evidence. First, in agreement with older observation[8] that tetranitromethane has little tendency to form colored complexes with terminal double bonds, these enol acetates failed to develop a yellow color in the presence of the reagent whereas *cis*- and *trans*-$\Delta^{17,20}$-enol acetates gave pronounced positive reactions. In the instance of the enol acetates of 3β-hydroxy-Δ^5-pregnene-20-one the substance presumed to be the Δ^{20}-enol acetate exhibited a much lighter yellow color than an equal amount of either $\Delta^{17,20}$-isomer. Second, chemical evidence based upon ozonolysis and the preparation of derivatives of Δ^{20}-enol acetates proved that the structure was correctly assigned. Third, consideration of the absorption bands found in the infrared spectrum clearly indicated two types of enol acetates and it was possible to correlate these bands with the structural details of the compounds investigated. The studies made are outlined in the following sections.

(8) A. Werner, *Ber.*, **42**, 4324 (1909).

Ozonolysis of the sirupy product obtained directly from the reaction of isopropenyl acetate with 3β-hydroxyallopregnane-20-one yielded predominantly acidic products containing considerable 3β-hydroxyalloetianic acid (VII) and very little 17-ketosteroid was obtained. The sirupy reaction product was chosen for investigation rather than the purified Δ^{20}-allopregnene-3β,20-diol diacetate because our aim was to study the products obtained with isopropenyl acetate and a typical 20-ketosteroid rather than a single compound obtained by fractionation. When purified Δ^{20}-allopregnene-3β,20-diol diacetate (VI) was brominated in carbon tetrachloride solution, followed by treatment of the bromoketone with potassium acetate in acetone for 24 hours, a product still containing halogen was obtained. After reduction with zinc in acetic acid an equal mixture of starting material V and 3β,21-diacetoxyallopregnane-20-one (VIII) resulted. The conclusion reached from the ozonolysis was thus verified by an independent procedure. Similar confirmation was afforded by perbenzoic acid oxidation of Δ^{20}-allopregnene-3β,20-diol diacetate followed by brief alkaline hydrolysis under very mild conditions which yielded VIII together with a small amount of 3β-acetoxy-17α-hydroxyallopregnane-20-one ("Compound L" acetate) and a larger amount of a compound tentatively identified but not characterized as 20,21-epoxyallopregnane-3β,20-diol diacetate. The chemical evidence was therefore in agreement with the formula VI for the enol acetate obtained with isopropenyl acetate.

Infrared spectrometry strongly supported the structure assigned to these various enol acetates. Steroid enol acetates are characterized by strong bands in the "carbonyl region" and in the "acetate region." These appear at 1736–1739 cm.$^{-1}$ and 1749 to 1754 cm.$^{-1}$ ("carbonyl region") in carbon disulfide solution and at 1236 to 1242 cm.$^{-1}$ and at

TABLE I

CHARACTERISTIC ACETATE, CARBONYL AND C=C STRETCHING BANDS OF ENOL ACETATES OF 20-KETOSTEROIDS

	Compound	Position of maxima "Carbonyl" (CS_2)	"Acetate" (CS_2)	C=C stretching ($CHCl_3$)
1	$\Delta^{17,20}$-Pregnene-3α,12α,20-triol triacetate	1756, 1739	1242, 1225	..
2	$\Delta^{17,20}$-Allopregnene-3β,20-diol diacetate (*"cis"*)	1750, 1739	1239, 1221	..
3	$\Delta^{17,20}$-Allopregnene-3β,20-diol diacetate (*"trans"*)	1750, 1739	1239, 1219	..
4	$\Delta^{5,6;17,20}$-Pregnadiene-3β,20-diol diacetate ("enol acetate A")	1750, 1739	1239, 1225	..
5	$\Delta^{5,6;17,20}$-Pregnadiene-3β,20-diol diacetate ("enol acetate B")	1749, 1736	1239, 1223	..
6	$\Delta^{20,21}$-Pregnene-3α,12α,20-triol triacetate (II)	1755, 1736	1239, 1225	1660
7	$\Delta^{20,21}$-Allopregnene-3β,20-diol diacetate (VI)	1752, 1736	1242, 1223	1660
8	$\Delta^{5,6;20,21}$-Pregnadiene-3β,20-diol diacetate (IV)	1752, 1736	1239, 1225	1660
9	Isopropenyl acetate	1760	1196	1675

1219 to 1225 cm.$^{-1}$ ("acetate region") in carbon disulfide solution. The bands at 1736 to 1739 cm.$^{-1}$ and at 1236 to 1242 cm.$^{-1}$ have been ascribed to the usual acetoxy group such as that at C-3 in the compounds under discussion.[9] The strong bands at 1749 to 1754 cm.$^{-1}$ and at 1219 to 1225 cm.$^{-1}$ have been observed only in enol acetates and ketol acetates (*e.g.*, 21-acetoxy-20-ketosteroids) in the course of extensive investigations[10] in these laboratories on the relation between chemical structure and infrared absorption. The presence of a strong absorption band at 1219 to 1225 cm.$^{-1}$ together with the band in the carbonyl region at 1749 to 1754 has therefore been related specifically to the presence of an enol acetate or a ketol acetate in the molecule under examination. When, as in the present examples, a ketol acetate can be excluded from consideration *it is possible from these characteristic absorption bands to deduce that a compound is an enol acetate.* All the compounds listed in Table I possessed these bands and are therefore enol acetates by the criterion of infrared spectrometry.

Absorption bands in the region characteristic of the C=C stretching vibrations (1580–1680 cm.$^{-1}$) were studied. When the substances listed in Table I were examined in chloroform solution in this region of the spectrum a number of weak absorption bands were noted, the significance of which will be dealt with elsewhere. Notable, however, was the fact that only enol acetates of 20-ketosteroids prepared with isopropenyl acetate exhibited an intense band at 1660 cm.$^{-1}$; other enol acetates earlier demonstrated to have the $\Delta^{17,20}$-20-acetoxy structure lacked this band. It was thus possible to divide these enol acetates into two groups of compounds according to the presence or absence of the 1660 cm.$^{-1}$ band together with the three other "enol acetate absorption bands." Since the chemical evidence for the structures of both types of enol esters seems to be without objection, the tentative conclusion can be drawn that the band at 1660 cm.$^{-1}$ is associated with the C=C stretching vibrations of an enol acetate with a terminal ethylenic group. In agreement with this conclusion the infrared spectrum of isopropenyl acetate, a simpler analog of Δ^{20}-20-acetoxysteroids, is characterized by an intense absorption band at 1675 cm.$^{-1}$ in chloroform solution.

(9) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951).

(10) R. N. Jones and K. Dobriner, *Vitamins and Hormones*, **7**, 293 (1949); R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952).

We gratefully acknowledge the assistance of Miss Friederike Herling in the determination of the infrared spectra.

Experimental[11]

Δ^{20}-Pregnene-3α,12α,20-triol Triacetate (II).—A solution of 2.1 g. of 3α,12α-diacetoxypregnane-20-one (I) in 10.5 ml. of isopropenyl acetate containing 0.01 ml. of concd. sulfuric acid was heated under reflux for 3 hours. An additional 0.5 cc. of isopropenyl acetate containing 0.01 ml. of concentrated sulfuric acid was added to the solution and approximately half of the solvent was removed. The light brown solution was cooled in ice and after dilution with ether was extracted with sodium carbonate solution and with water, the ether was dried over anhydrous sodium sulfate and the solvent was removed. The brown viscous residue was evacuated until there was no longer any odor of isopropenyl acetate. The residue was dissolved in petroleum ether, filtered through a short alumina column and the colorless filtrate was crystallized from petroleum ether yielding 1.3 g. of product, m.p. 125–127°. The mother liquor was chromatographed on alumina and 300 mg., m.p. 115–117° after recrystallization from ethanol, was obtained. The remainder of the product was discarded. Two recrystallizations from ethanol yielded Δ^{20}-pregnene-3α,12α,20-triol triacetate (II) as needles, m.p. 131–132°, $[\alpha]^{23}D$ +106°. The substance was colorless when treated with tetranitromethane in ether solution.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.41; H, 8.75. Found: C, 70.66; H, 8.91.

Hydrolysis of 230 mg. of II with 5 meq. of 0.1 *N* NaOH in 70% methanol solution at room temperature resulted in the removal of 2 acetoxy groups within 60 minutes. After reflux for an additional hour, the third acetoxy group was removed; sapon. equiv., calcd. 153; found 145.

Very similar results were obtained when 2.1 g. of I was refluxed in 10 ml. of isopropenyl acetate containing 1 g. of *p*-toluenesulfonic acid monohydrate. After heating for 3 hours and slowly removing half of the solvent in the course of an additional hour, purification in the usual manner yielded 1 g. of material, m.p. 115–120°, and a second crop of 0.18 g., m.p. 120–125°, resolidified and melted again at 140–145°. Chromatography of the mother liquors yielded 0.42 g., m.p. 105–110°, which after a single recrystallization from methanol melted at 125–127°. While there was some indication of the presence of $\Delta^{17,20}$-isomer in this preparation the major portion of the material was the Δ^{20}-isomer.

A solution of 360 mg. of II in 40 ml. of acetic anhydride was slowly distilled for 5 hours until approximately 35 ml. of distillate was collected. After the usual isolation procedure II was recovered unchanged, m.p. 131–132°. A sample of II was heated slowly in a capillary tube to 170° and held at this temperature for 5 minutes. After cooling and standing for one week the melt resolidified and again melted at 130–131°.

Conversion of Δ^{20}-Enol Acetate II to $\Delta^{17,20}$-Enol Acetate.—A solution of 460 mg. of II, m.p. 125–127°, in 40 ml. of acetic anhydride containing 190 mg. of *p*-toluenesulfonic acid monohydrate was distilled until 35 ml. of solvent had been removed. After the usual isolation procedure 238 mg. of crystalline product, m.p. 170–172°, was obtained. A second

(11) All melting points are corrected. The optical rotations were measured in chloroform solution.

crop of 60 mg. melted at 165–167°. Both substances were identical with the $\Delta^{17,20}$-enol acetate described by Marshall, *et al.*,[2] and gave a yellow color when treated with tetranitromethane in ether solution. The residual oil also was essentially identical in the "fingerprint region" of the infrared spectrum with the $\Delta^{17,20}$-isomer. The reverse change could not be realized. $\Delta^{17,20}$-Pregnene-3α,12α,20-triol triacetate was recovered unchanged when heated for 3 hours with isopropenyl acetate and sulfuric acid under the same conditions used for the preparation of II.

$\Delta^{5,20}$-Pregnadiene-3β,20-diol Diacetate (IV).—The enol ester was prepared from 1 g. of 3β-hydroxy-Δ^5-pregnene-20-one (III) precisely as described for II and 382 mg. of crystalline material, m.p. 118–120°, was obtained from petroleum ether. Recrystallization from methanol and from petroleum ether yielded IV as platelets, m.p. 125–126°; $[\alpha]^{27}D$ −47°. The test with tetranitromethane was positive but the color was lighter than given by an equal amount of the *cis*-$\Delta^{17,20}$-isomer.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.10; H, 9.03.

The oily residue and the material from the mother liquors were combined and chromatographed on alumina yielding products melting in the range of 110–120° which were not depressed in melting point upon admixture with the purified product. The infrared spectra of these fractions from the chromatogram in the "fingerprint region" were essentially identical with pure IV. Recrystallization of the chromatographed fractions gave products melting from 121–123°. Repeated fractional crystallization failed to yield any other product. Three careful chromatograms on silica gel together with infrared spectrophotometric examination of the individual eluates failed to reveal evidence of the separation of another component. These crystalline eluates melted somewhat lower than the purest sample, ranging in m.p. from 113–120° and were undoubtedly contaminated with some of the $\Delta^{17,20}$-isomer as evidenced by the following experiment. To 100 mg. of enol acetate, m.p. 120–121°, in 5 ml. of acetic acid was added 2 ml. of 0.25 *M* bromine in acetic acid and the mixture was stored at room temperature for 0.5 hour. The solution was diluted with water and extracted with ether. After recrystallization from acetone–petroleum ether 82 mg. of product was obtained, m.p. 145–147° (decomposition). Three recrystallizations from acetone or chloroform–petroleum ether mixtures yielded a few milligrams of 3β-acetoxy-5,6,17-tribromoallopregnane-20-one, m.p. 165–166°, presumably identical with the product obtained by Fieser and Huang-Minlon.[6] The major portion of the bromo compound was not further investigated.

Upon heating with *p*-toluenesulfonic acid in acetic anhydride as previously described, IV was converted to a mixture of isomers similar to that obtained by Fieser and Huang-Minlon, from which about 25% of the *cis*-$\Delta^{17,20}$-isomer, m.p. 148–149°, was isolated. This material was not depressed in melting point upon admixture with an authentic sample.

Δ^{20}-Allopregnene-3β,20-diol Diacetate (VI).—From 1 g. of 3β-hydroxyallopregnane-20-one (V) 1.07 g. of sirupy enol acetate was obtained by the procedure described for (II). Chromatography upon silica gel followed by recrystallization from petroleum ether yielded 800 mg. of product, m.p. 70–78°. Recrystallization from aqueous ethanol yielded VI as needles, m.p. 88–89°, $[\alpha]^{27}D$ +12.6°. No color developed upon treatment with tetranitromethane in ether solution.

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51. Found: C, 74.30; H, 9.66.

Ozonolysis of Δ^{20}-Allopregnene-3β,20-diol Diacetate.—A solution of 450 mg. of amorphous VI, obtained directly from enol acetylation, in 300 ml. of a 1:1 methanol–ethyl acetate mixture was chilled to −30° and treated with 6.25 mmoles of ozone during the course of 12 minutes. The solution was poured on 2.5 g. of palladium-on-barium carbonate catalyst and shaken in an atmosphere of hydrogen until uptake ceased. The catalyst was filtered off and the solution was evaporated to dryness. The residue was suspended in ether and washed twice with 5% sodium hydroxide. An insoluble sodium salt precipitated and was retained with the aqueous alkali. After washing the ether with water the neutral residue weighed 367 mg. Colorimetric determination of 17-ketosteroid by the Callow[12] modification of the Zimmerman reaction showed 52 mg. in terms of dehydroisoandrosterone as the standard. The aqueous alkaline solution was acidified and extracted with ethyl acetate. The solvent was removed and the residue was heated for 2 hours under reflux with 15 ml. of 0.25 *N* potassium hydroxide in 50% aqueous methanol. Isolation of the acid in the usual manner followed by crystallization from ethyl acetate yielded 45 mg., m.p. 246–247°, identical in all respects with an authentic sample of 3β-hydroxyalloetianic acid (VII), m.p. 245–246°, obtained from Professor Reichstein. The mother liquors (37 mg.) were esterified with diazomethane and acetylated with pyridine and acetic anhydride. After recrystallization, methyl 3β-acetoxyalloetianate, m.p. 145–149°, was isolated and identified by infrared spectrometry and by the melting point of mixtures with an authentic sample. The neutral fraction was dissolved in 25 ml. of aqueous ethanol and refluxed for 2 hours with 3 ml. of 1 *N* KOH. The solvent was removed and the residue was partitioned between ether and dilute base. The aqueous layer was acidified and extracted with ethyl acetate and after removal of the solvent 124 mg. of oily acid fraction was obtained. This product could not be brought to crystallization and esterification with diazomethane failed to yield a crystalline ester. The neutral ether extract, after removal of the solvent, followed by crystallization from acetone petroleum ether yielded 42 mg. of 3β-hydroxyallopregnane-20-one, m.p. 190–195°. The semi-crystalline mother liquors (100 mg.) contained 31 mg. of 17-ketosteroid in terms of dehydroisoandrosterone as the standard estimated colorimetrically by the Callow modification of the Zimmerman reaction.

3β,21-Diacetoxyallopregnane-20-one from VI.—A solution of 67 mg. of $\Delta^{20,21}$-allopregnene-3β,20-diol diacetate (m.p. 86–88°) in 2 ml. of carbon tetrachloride was treated with 3.1 ml. of 0.054 *M* bromine in carbon tetrachloride. The solution was rapidly decolorized and after 5 minutes at room temperature the solvent was removed and the residue was heated under reflux for 24 hours with a suspension of 150 mg. of potassium acetate in 25 ml. of acetone. After dilution with brine, extraction with ether yielded an oil which gave a positive Beilstein test. The product was therefore dissolved in 30 ml. of acetic acid and treated with zinc dust for 1 hour on a steam-bath. Dilution with ether and extraction in the usual manner yielded 45 mg. of a product m.p. 125–135° which after recrystallization from methanol melted 145–147°. Chromatographic separation of the total material and infrared analyses of the eluates showed that this product was an approximately equal mixture of 3β-acetoxyallopregnane-20-one (V) and 3β,21-diacetoxyallopregnane-20-one (VIII).

A 3.1 *M* solution of perbenzoic acid in benzene was added to 400 mg. of sirupy VI, directly from the reaction with isopropenyl acetate. After 1 hour at room temperature the solution was diluted with ether and extracted with 5% sodium hydroxide. After washing with water the ether was removed and the residue was dissolved in 75 ml. of methanol. A stream of nitrogen was bubbled through the solution and 25 ml. of aqueous 0.2 *N* sodium hydroxide was added. After 15 minutes at room temperature the solution was acidified and extracted with ether. The residue from the ether extract was acetylated with acetic anhydride in pyridine at room temperature. After the usual isolation procedure the product was separated by chromatography and recrystallization into three components: (1) 51 mg. of slightly impure 3β-acetoxy-17α-hydroxyallopregnane-20-one (Compound "L" acetate) m.p. 183–186°; (2) 62 mg. of 3β,21-diacetoxyallopregnane-20-one, m.p. alone and admixed with an authentic product 150–152°; (3) 145 mg. of a product, m.p. 120–125°, not further characterized in this investigation.

NEW YORK 21, N. Y.

(12) N. H. Callow, R. K. Callow and C. W. Emmens, *Biochem. J.*, **32**, 1312 (1938).