contaminated with 24-dehydrocholesteryl acetate, for if this were the case the further addition of 25-dehydrocholesteryl acetate should have raised the melting point. Infrared spectra were run on all three preparations and there was no evidence for any significant absorption at 885 cm.$^{-1}$ characteristic of terminal unsaturation in the earlier preparations. The absorption at 1637 cm.$^{-1}$ for terminal unsaturation found in our preparation of 25-dehydrocholesterol (Fig. 1) and 24-methylenecholesterol was also absent.[1,25,26] We must conclude that the earlier products are not 25-dehydrocholesterol but are predominately the previously undescribed 24-dehydrocholesterol arising by dehydrohalogenation proceeding in the expected manner. The incorrect assignment of structure by the earlier workers may be explained by the apparent non-specificity of the formaldehyde determination when applied to sterols of this type; bond migration under the acid conditions of the periodate oxidation might offer an explanation. The formation of acetone when basic permanganate was used lends support to this hypothesis.

(25) R. B. Barnes, R. C. Gore, R. W. Stafford and V. Z. Williams, *Anal. Chem.*, **20**, 402 (1948).

(26) H. W. Thompson and D. H. Whiffen, *J. Chem. Soc.*, 1412 (1948).

Acknowledgment.—We are indebted to Dr. Byron Riegel, G. D. Searle and Co., Chicago, Ill., for a sample of synthetic 24-ketocholesterol; to Drs. Eugene P. Oliveto and W. Gebert, Schering Corp., Bloomfield, N. J., for synthetic 25-ketonorcholesterol and 25-dehydrocholesterol; to Dr. H. B. MacPhillamy, Ciba Pharmaceutical Products, Inc., Summit, N. J., for 3-acetoxy-5-cholenic acid; and to Dr. W. G. Dauben, University of California, for 25-dehydrocholesterol. Dr. R. H. Wright, British Columbia Research Council, kindly recorded infrared spectra. Mr. A. P. Ronald of our instrumentation section obtained many of the analytical data.

VANCOUVER 2, B. C.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Action of Lead Tetraacetate on an Enol Acetate. The Epimeric 16-Acetoxy Derivatives of Epiandrosterone Acetate, their Interconversion and Rearrangement

BY WILLIAM S. JOHNSON, BERNARD GASTAMBIDE[1] AND RAPHAEL PAPPO[2]

RECEIVED OCTOBER 12, 1956

Lead tetraacetate has been found to react stereoselectively with the enol acetate I of epiandrosterone acetate to produce the 16β-acetoxy compound IV. Evidence for the mechanism of the rearrangement of the epoxy acetate III to the 16α-acetoxy ketone II has been advanced. A previous postulate that the 16β-isomer IV serves as an intermediate has been invalidated. It now appears that rearrangement of such epoxyacetates involves two competing reactions, one proceeding by retention and the other by inversion of the configuration of the carbon accepting the acetoxy group. Treatment with dilute sulfuric acid followed by reacetylation converted IV into the 17β-acetoxy-16-keto compound V but did not effect rearrangement of II. Theoretical implications of this difference are discussed. The two epimeric acetates II and IV were unaffected by heating at 190° or by treatment with silica gel but could be equilibrated with potassium acetate in acetic acid as demonstrated by infrared spectroscopy. By the aid of ultraviolet spectroscopy and polarimetry the position of this equilibrium was shown to lie between 44 and 56% of the β-form.

In connection with another investigation we had occasion to treat the enol acetate I of 3β-acetoxyandrostane-17-one with lead tetraacetate and discovered that the reagent was consumed after a few hours at room temperature. The major product, produced stereoselectively and isolated in 57% yield, corresponded in composition and spectral properties to a diacetoxy ketone which we presumed to be a 3β,16-diacetoxyandrostane-17-one formed by attack of acetoxy free radicals or cations at the nucleophilic 16-position of the enol acetate I. Our product existed in two polymorphic forms, m.p. 139° and 159°, and was clearly different from the 16α-acetoxy ketone II, m.p. 185°, of known configuration obtained by Leeds, Fukushima and Gallagher[3] by rearrangement of the epoxy acetate III. The inference that our isomer was the 16β-epimer IV was since confirmed by a recent disclosure of Fajkos[4] who prepared the 16β-acetoxy ketone IV from 16α,17α-epoxyandrostane-3β-ol acetate by cleavage of the epoxide group with acetic acid, followed by chromic acid oxidation. The reported properties of Fajkos' material are in good agreement with those of our compound.[5]

With the 16β-acetoxy ketone IV in hand we found ourselves in a position to shed some light on the mechanism of the rearrangement of the epoxy acetate III, which has been effected by chromatography on silica gel, or by heat alone.[3] One of two mechanisms previously proposed[3] involved the postulation of the 16β-acetoxy ketone IV as an intermediate which was assumed to be unstable, readily undergoing epimerization (*via* the enol) to the 16α-isomer (see chart B of reference 3). When we submitted the 16β-acetoxy ketone IV to these

(1) On leave from the Centre National de la Recherche Scientifique, Paris, on funds provided by the International Coöperation Administration, Washington, D. C.

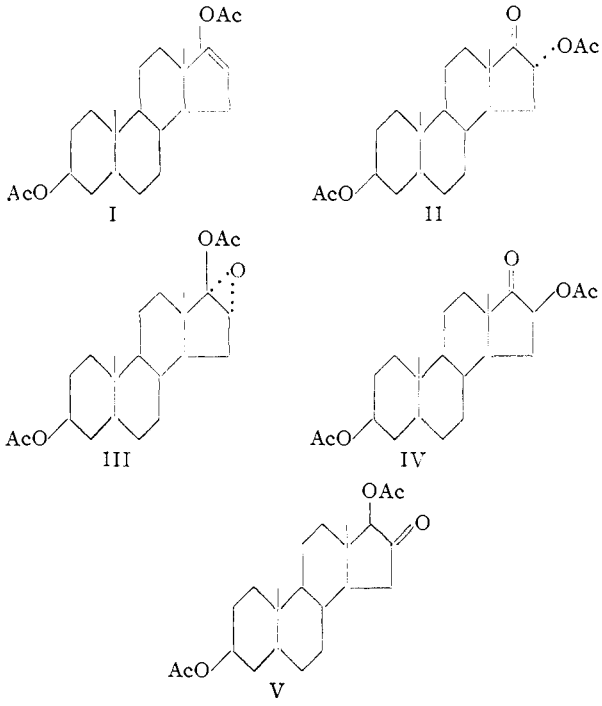
(2) Lecturer in Chemistry supported by the Research Committee of the Graduate School on funds provided by the Wisconsin Alumni Research Foundation, 1954–1955. On leave from the Weizmann Institute of Science.

(3) N. S. Leeds, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **76**, 2943 (1954).

(4) J. Fajkos, *Coll. Czechoslovak. Communs.*, **20**, 1478 (1955).

(5) The action of lead tetraacetate on enol acetates is receiving further attention. In preliminary work we have found that the enol acetates of coprostanone and cholestanone react only slowly. The bis-enol acetate from pregnane-3,20-dione (isopropenyl acetate method) afforded the 21-acetoxy derivative. The reaction is similar to the reaction of lead tetraacetate with enols (see R. Criegee, "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 8) or with their ethers (see, for example, H. O. L. Fischer, *et al.*, *Ber.*, **63**, 1732 (1930); **65**, 345 (1932)).

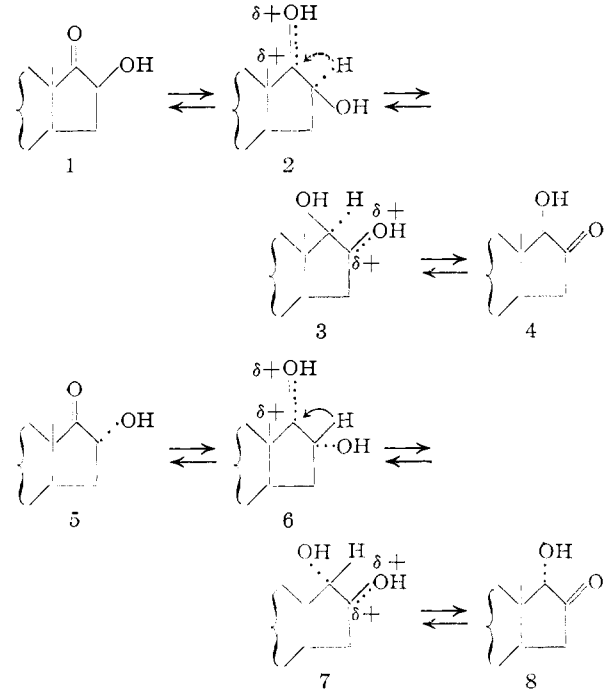
treatments, it was recovered largely unchanged, and none of the 16α- compound could be detected. These facts clearly show that the mechanism in question is untenable. The alternative proposal (chart C of reference 3) provides a reasonable pathway for rearrangement of the acetoxy group with retention of configuration at C_{16}. The yield in the rearrangement was not high, and we were not surprised to find, on repeating the silicic acid rearrangement experiment, that the residual material contained the 16β-acetoxy ketone, produced undoubtedly by a competing process involving the inversion mechanism that was postulated for the



rearrangement of 17α,20β-epoxyallopregnane-3β,-20-diol diacetate.[6] Similarly, the unidentified residues in the latter case may contain the C_{17}-epimer produced by the retention mechanism; thus the apparent striking difference in behavior in the two cases[3] may be reconciled.

The 16α-acetoxy ketone II has also been produced in good yield from the epoxy acetate III by the action of dilute sulfuric acid followed by reacetylation,[3] a process which undoubtedly involves hydrolysis of the epoxide ring. In order to see whether these acidic conditions would effect equilibration of the C_{16} epimers, the 16β-acetoxy ketone IV was submitted to this treatment, whereupon it was isomerized, not to the 16α-epimer, but to a product recognized as the 17β-acetoxy-16-keto isomer V, m.p. 181°,[3,7] identified by comparison with an authentic specimen prepared by alkaline treatment of II followed by acetylation.[3] The stability of the 16α-hydroxy ketone to dilute sulfuric acid was confirmed by treatment of the acetate II with this reagent, which readily effected hydrolysis of the acetate group to a hydroxy ketone. Acetylation of this substance without any purification gave back the 16α-acetoxy ketone II in high over-all yield.

The fact that the 16α-hydroxy ketone is stable to dilute sulfuric acid while the 16β-epimer undergoes isomerization to the 17β-hydroxy-16-ketone has interesting theoretical implications. If the isomerization of the 16β-epimer proceeds *via* the enol, one is forced to the conclusion that under these acidic conditions the rate of enolization of the 16α-epimer is negligible. Such a difference in rate of enolization could be due either to a greater steric availability of the 16α- than of the 16β-hydrogen atom, the abstraction of which is involved in the rate expression,[8] or to a driving force in the 16β-epimer resulting from a relief of strain (due to non-bonded interactions) as C_{16} becomes trigonal. While this type of an argument is applicable to certain cases,[9] molecular models of the epimeric hydroxy ketones in question do not show pronounced differences in the steric requirements mentioned above. The enolization mechanism, if not invalidated, is thus weakened, and we prefer the alternative hydride shift interpretation presented below. The enolization mechanism is further weakened by the fact that the 16β-acetoxy ketone was recovered unchanged after treatment with hydrochloric acid in acetic acid. Had this substance enolized under these conditions, it would have been converted, in part, into the 16α-epimer (see equilibration studies below).



(6) A. H. Soloway, W. J. Considine, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **76**, 2941 (1954). That the 16β-acetoxy ketone was not formed by isomerization of the 16α-epimer was shown by recovery of the latter unchanged after submission to the silicic acid treatment.

(7) *Cf.* M. N. Huffman and M. H. Lott, *ibid.*, **73**, 878 (1951).

(8) P. D. Bartlett in Gilman's "Organic Chemistry," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 87.

(9) For example, H. E. Zimmerman, THIS JOURNAL, **78**, 1168 (1956), has reported that *cis*-2-methyl-3-phenylindanone undergoes acid-catalyzed bromination readily compared with the *trans*-isomer. This behavior is, in our opinion, best rationalized by the steric approach and/or relief of strain argument.

It seems probable that the acid-catalyzed rearrangement of the 16β-hydroxy ketone (1) proceeds by the sequence 1–4 involving a reversible stereospecific hydride shift 2 ⇄ 3 in the protonated form. Loss of a proton from form 3 affords the 17β-hydroxy-16-ketone (4) which, being more stable than (1),[10] predominates at equilibrium. A similar reversible sequence 5–8 is postulated for the 16α-hydroxy ketone (5), but in this case the equilibrium evidently lies strongly in favor of (5); hence there is no apparent reaction. If this hypothesis is correct, the 17α-hydroxy-16-keto isomer (8) should undergo acid-catalyzed isomerization to (5). We hope to test this hypothesis.

Some preliminary observations have been made on the epimerization of the 16-acetoxy ketones.[11] Neither isomer was affected appreciably by heating at 190° for 16 hr. Treatment with potassium acetate in acetic acid,[12] however, did effect epimerization. Some of the 16α-isomer was isolated from equilibration of the 16β-form by chromatography, but this separation was far from quantitative. More definitive information was obtained by infrared and ultraviolet spectroscopy. Distinguishing minor maxima in the long-wave region of the infrared spectrum, exhibited by one but not the other epimer, were found, for the 16β-acetoxy ketone, at 9.5, 10.25, 10.83, 10.92 and 12.1 μ and, for the 16α-epimer, at 8.35 μ. After heating each epimer with the reagent for 30 hr. or more at 115°, the infrared spectra of the two products were practically identical and indistinguishable from that of a 1:1 mixture of the pure isomers. All of the characteristic maxima mentioned above were detectable at weakened intensity in these spectra of the mixtures, and no spurious bands were apparent. For quantitative estimation the carbonyl absorption band observed at high concentration in the 300 mμ ultraviolet region proved useful because of differences in the extinction coefficient: ε 46 for the 16β-acetoxy ketone, 80 for the 16α-epimer and 63 (calculated 63) for a 1:1 mixture of the two. After the 16-hr. treatment with potassium acetate ε for the 16β-acetoxy ketone was 57 and for the 16α-epimer 69 (average value). After 45 hours ε for the 16β-isomer was 61 and for the 16α-isomer 65. At equilibrium in this medium at 115° the mixture, therefore, contains between 44 and 56% of the β-form. Longer heating resulted in some decomposition with the appearance of spurious absorption in the 250-260 mμ region; therefore the equilibrium position was not determined with greater accuracy.

Treatment of the 16-hr. equilibration mixture from the 16α-acetoxy ketone with dilute sulfuric acid followed by acetylation gave a product with $[\alpha]^{27}D$ +3.2°. Since the hydrolysis–acetylation sequence regenerates the 16α-epimer, $[\alpha]^{27}D$ + 53.5° (+49° in control experiment) but effects rearrangement of the 16β-epimer to the highly levorotatory 17β-acetoxy ketone V, $[\alpha]^{27}D$ −118° (−109.5° in control experiment), the specific rotation of the equilibrated product shows that the composition is approximately 29% of the 16β-isomer, which is in good agreement with the results of the ultraviolet spectroscopic experiments described above. The 45-hour equilibration product from the 16β-acetoxy ketone similarly gave, after sulfuric acid treatment, material with $[\alpha]^{27}D$ −42.6° which corresponds to 58% of the β-isomer in the original mixture.

(10) Compare the alkali-catalyzed isomerization of (1) and also of (5) to (4) (see above and also ref. 3).

(11) Compare the 16-bromo-17-ketones, in which the 16β-form is favored, J. Fajkos, *Coll. Czechoslovak Communs.*, **20**, 312 (1955).

(12) Compare R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, THIS JOURNAL, **77**, 661 (1955).

Experimental[13]

3β,16β-Diacetoxyandrostane-17-one (IV).—A 3.74-g. sample of the 3β-acetoxyandrostane-17-one enol acetate (I),[3] m.p. 174–176°, was dissolved in 60 ml. of glacial acetic acid containing 2 ml. of acetic anhydride, 4.43 g. of lead tetraacetate was added and the mixture shaken occasionally until dissolution was complete. After 6 hr. at room temperature the tetraacetate was consumed as indicated by a negative starch–iodide test, and the solvent was removed at 20° under reduced pressure. The residue was treated with benzene, filtered to remove lead acetate and evaporated. Crystallization of the residue from petroleum ether (65–68°) gave 1.30 g. (first crop), m.p. 153–155.5°, and 0.70 g. (second crop). The later crops were oily and contained some acidic material extractable with bicarbonate. A sample of the first crop was sublimed at 180° (0.05 mm.) and the sublimate, m.p. 150–155°, chromatographed on Florex. A center cut melted at 158–160°. Successive recrystallizations from ether eventually gave a low-melting form as colorless prisms, m.p. 138–140°. On resublimation this form changed over to colorless needles, melting at 156–158°. The product gave a positive "tetrazolium" test.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.77. Found: C, 70.9; H, 8.81.

Another experiment was carried out as described above except that the reaction mixture was allowed to stir for 24 hr. at room temperature, then diluted with water and extracted with ether. The ether extracts were washed with water, dilute sodium hydroxide, again with water and dried over anhydrous sodium sulfate. The slightly tacky solid residue obtained upon evaporation of the solvent amounted to 3.87 g. Crystallization from isopropyl ether gave 2.14 g. of crystals, m.p. 146–155°. Repeated recrystallizations from dilute methanol and from isopropyl ether gave a total of 1.31 g. of fairly pure material, m.p. 156.5–158°. After two more recrystallizations from 95% ethanol, a sample was obtained as colorless crystals of mixed form, m.p. 158.2–158.8° with some previous melting about 137° and resolidification; $[\alpha]^{27}D$ +60° (*c* 5 in $CHCl_3$); $\lambda_{max}^{95\%\ EtOH}$ 306 mμ, ε 46; $\lambda_{max}^{CS_2}$ 5.72–5.80, 8.1–8.2 μ, and characteristic bands mentioned in discussion; λ_{max}^{KBr} 5.74, 5.80, 5.84 μ. The reported properties are, m.p. 156–157°, $[\alpha]^{20}D$ +60° (*c* 2.33 in $CHCl_3$).[4]

3β,16α-Diacetoxyandrostane-17-one (II) was prepared by the method of Leeds, Fukushima and Gallagher.[3] A 0.865-g. sample of the epoxy acetate III,[3] m.p. 149–150°, in 50 ml. of methanol was treated with 50 ml. of 6 *N* sulfuric acid in 50 ml. of methanol. After 5 days at room temperature, the crude product was isolated as described[3] and treated with 1 ml. of acetic anhydride and 4 ml. of pyridine for 2 days at 50°. The mixture was diluted with ether, then washed in turn with water, dilute sodium hydroxide, water, dilute hydrochloric acid, water and finally dried over anhydrous sodium sulfate. Evaporation of the solvent gave 0.788 g. of colorless needles, m.p. 172–183°. Recrystallization from a mixture of acetone, isopropyl ether and petroleum ether (66–69°) gave 0.419 g. (first crop), m.p. 184.5–186°, and 0.287 g. (second crop), m.p. 183–185°. The purest specimen obtained by recrystallization melted at 186–187.5°, $[\alpha]^{27}D$ +57.4° (*c* 5 in $CHCl_3$); $\lambda_{max}^{95\%\ EtOH}$ 302 mμ, ε 80; $\lambda_{max}^{CS_2}$ 5.72, 5.80, 8.1–8.2 μ, and in particular 8.35 μ, along with other bands in the characteristic region; λ_{max}^{KBr} 5.74, 5.80 μ. The reported properties are m.p. 184–185°, $[\alpha]^{23}D$ +57.1° ($CHCl_3$).[3]

Isomerization of the Epoxy Acetate III on Silica Gel.—When the epoxy acetate was simply chromatographed on

(13) All melting points are corrected for stem exposure.

silica gel as previously described,[3] it was recovered mainly unchanged, perhaps because our adsorbent was less active than that previously used.[3] The rearrangement could be effected, however, by prolonged treatment with silica gel as described below.

A paste of 6 g. of silicic acid (Mallinckrodt 2847—activated by heating at 100–110° for 4 days), 0.150 g. of the epoxy acetate, m.p. 149–150°, and 12 ml. of isopropyl ether was allowed to stand at 50° for 17 hr. The mixture was filtered, the adsorbent washed with 200 ml. of ethyl acetate and the combined filtrates evaporated. By a combination of fractional crystallization from aqueous methanol and chromatography on Florisil, there was separated a total of 0.016 g. of the 16α-acetoxy ketone II, m.p. 184–185.5°, undepressed on admixture with the material prepared as described above. Also isolated was 0.009 g. of crystalline material having all of the characteristic bands in the infrared spectrum of the 16β-acetoxy compound IV with no spurious absorption. This substance is difficult to characterize by melting point because of dimorphism. After careful recrystallization this specimen yielded material, m.p. 156.5–158°, undepressed on admixture with authentic 16β-acetoxy ketone IV.

When either the 16α- or the 16β-acetoxy ketone was submitted to treatment, like that described above, with silicic acid, it was recovered essentially unchanged.

Action of Sulfuric Acid on the Epimeric 3β,16-Diacetoxyandrostane-17-ones (IV).—A solution of 0.129 g. of the 16β-acetoxy ketone, m.p. 158.2–158.8°, in 7.5 ml. of methanol was admixed with 7.5 ml. of 6 *N* sulfuric acid in 7.5 ml. of methanol. After 5 days at room temperature the crude product was isolated (see above) and acetylated with 1.5 ml. of acetic anhydride and 6 ml. of pyridine for 2 days at room temperature. The crude product, isolated as described above under the preparation of II, amounted to 0.119 g. of somewhat tacky yellowish solid, $[\alpha]^{27}D$ −109.5° (*c* 4 in $CHCl_3$). Crystallization from 80% methanol gave 0.099 g. of almost colorless crystals, m.p. 178–181° with softening at 175°. Further recrystallization raised the m.p. to 180–181°, $[\alpha]^{27}D$ −118° (*c* 3.8 in $CHCl_3$). The m.p. was not depressed on admixture with 3β,17β-diacetoxyandrostane-16-one (V) prepared by the action of sodium hydroxide on II followed by reacetylation.[3]

When 0.086 g. of the 16α-acetoxy ketone II, m.p. 184.5–186°, was treated with sulfuric acid and reacetylated as described above, it was recovered essentially unchanged. The crude somewhat tacky product amounted to 0.085 g., $[\alpha]^{27}D$ +49° (*c* 5 in $CHCl_3$). Recrystallization from dilute methanol gave 0.073 g., m.p. 181–186°. Two more recrystallizations raised the m.p. to 184–186.5°, undepressed on admixture with starting material.

The above experiment was repeated with 0.026 g. of II, except that the acid treatment was allowed to proceed for only 1 day and the product isolated without acetylation. Crystallization from ethyl acetate gave 0.014 g. of the 16α-hydroxy ketone, having an indefinite m.p. (dec.) above 180°, λ_{max} 2.9–3.0 (OH), 5.8 μ (C=O), with no acetate absorption at 8 μ.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found C, 74.6; H, 9.83.

A 0.050-g. sample of the 16β-acetoxy ketone IV was treated with methanolic aqueous sodium hydroxide and reacetylated as described for the 16α-epimer.[3] The crude product was recrystallized twice from isopropyl ether to give 0.023 g. of crystals, m.p. 180–182°, undepressed on admixture with the product V from the sulfuric acid treatment described above.

Equilibration Experiments. (a) With the 16β-Acetoxy Ketone IV.—A solution of 0.150 g. of the acetoxy ketone, m.p. 158.2–158.8°, and 0.750 g. of potassium acetate in 22.5 ml. of acetic acid was heated at 115° for 16 hr., then diluted with water and extracted with ether. The ether layer was washed with water, then with 10% potassium carbonate solution and finally again with water. The solution was dried over anhydrous sodium sulfate and evaporated to leave 0.150 g. of nearly colorless solid which was recrystallized once from 80% methanol giving 0.140 g. of colorless product, m.p. 131–142.5°, $[\alpha]^{27}D$ +58.3° (*c* 5 in $CHCl_3$), $\lambda_{max}^{95\%\ EtOH}$ 304.5 mμ (ϵ 57). In other runs as above: after 30 hr., λ_{max} 304 mμ (ϵ 59); after 45 hr., λ_{max} 303.5 mμ (ϵ 61).

A 0.037-g. sample of the product from the 45 hr. equilibration treatment was chromatographed on a column of 3 g. of Florisil. The early fractions eluted with benzene were combined and recrystallized from dilute methanol to give 2.5 mg. of the 16α-acetoxy ketone, m.p. 184–186°, undepressed on admixture with authentic material.

An attempt to effect equilibration with acid failed. A 0.050-g. sample of the 16β-acetoxy ketone was treated with 10 ml. of acetic acid and 0.5 ml. of concentrated hydrochloric acid. After 3 days at room temperature the product was isolated and crystallized from dilute ethanol; yield 0.035 g., m.p. 136–138°, resolidifying and remelting at 155–156°.

(b) With the 16α-Acetoxy Ketone II.—A 0.100-g. sample of the acetoxy ketone, m.p. 183.2–185°, was treated for 16 hr. with 0.5 g. of potassium acetate in 15 ml. of acetic acid just as described above. The once-crystallized material amounted to 0.090 g., m.p. 146–153°, $[\alpha]^{27}D$ +58.8° (*c* 5 in $CHCl_3$), $\lambda_{max}^{95\%\ EtOH}$ 303.5 mμ (ϵ 67) and 303 (72). In other runs as above: after 30 hr., λ_{max} 303.5 mμ (ϵ 65); after 45 hr., λ_{max} 303.5 mμ (ϵ 65).

A 0.086-g. sample of product ($\lambda_{max}^{95\%\ EtOH}$ 303 mμ, ϵ 72) from the 16 hr. treatment was dissolved in 5 ml. of methanol and treated with 5 ml. of 6 *N* sulfuric acid in 5 ml. of methanol for 5 days at room temperature. After acetylation as described above, the crude product amounted to 0.085 g., $[\alpha]^{27}D$ +3.2° (*c* 5 in $CHCl_3$). A sample of the product ($\lambda_{max}^{95\%\ EtOH}$ 303.5 mμ, ϵ 61) from a 45 hr. treatment of the 16β-acetoxy ketone was similarly treated to give material with $[\alpha]^{27}D$ −42.6°.

MADISON, WISCONSIN