of androstane-3,17-dione in 25 ml. of methanol was added portionwise, with initial cooling to a solution of 44 mg. (4.6 meq.) of sodium borohydride in 1 ml. of water and 5 ml. of methanol. After 20 minutes, water was added and the resulting gelatinous mixture was extracted with ether, and the product was isolated in the usual manner. The white solid residue, 1.09 g., was separated by chromatography on alumina and recrystallization, yielding 319 mg. of pure isoandrosterone, m.p. 172–174°, and 131 mg. of less pure material, m.p. 163–170° (45% yield), together with 30% of unchanged starting material and 5% of androstane-3β,17β-diol, m.p. 161–163°. No androsterone was obtained.

Reduction of Androstane-3,17-dione with Sodium Borohydride in Pyridine.—A solution of 288 mg. (1.0 mmoles) of androstane-3,17-dione in 5 ml. of pyridine was added to a solution of 14 mg. (1.25 meq.) of sodium borohydride in 20 ml. of pyridine. This solution was allowed to stand for 2 hours, and then was worked up in the usual manner. The white crystalline residue was separated by chromatography on alumina and recrystallization yielding 55% of isoandrosterone and 4% of androsterone without regard for the unchanged starting material.

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[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Oxidation of Enol Ethers of 20-Ketosteroids by Perbenzoic Acid[1]

BY B. BELLEAU AND T. F. GALLAGHER

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A mixture of Δ^{17}- and Δ^{20}-enol ethers was prepared from three 20-ketosteroids by treatment with ethyl orthoformate followed by heating in xylene solution. Upon oxidation with perbenzoic acid this mixture of enol ethers was converted to two products: (1) the corresponding 17-ketosteroid and (2) the ethyl ester of the corresponding etianic acid. A mechanism for the results has been suggested.

As a portion of current studies in progress in this Laboratory we wished to adapt the method recently reported[2] for the preparation of 17α-hydroxy-20-ketosteroids to the synthesis of 11β-hydroxy adrenocortical hormones. The usual procedure starting from the enol acetate of a 20-ketosteroid is inapplicable in the presence of an 11β-hydroxyl group because the vigorous condition would unquestionably eliminate the C-11 oxygen function. We therefore investigated the enol ethers of 20-ketosteroids with the expectation that an 11-keto group which is known not to react with mercaptans[3] would be incapable of forming an enol ether when treated with ethyl orthoformate and might thus be reduced with lithium aluminum hydride without attack on the 20-enol ether. Subsequent treatment of the 11β-hydroxy-20-enol ether with perbenzoic acid was expected to yield the 17,20-epoxy-20-ether in analogy with the behavior of cyclohexanone enol ether[4] and the enol acetates of 20-ketosteroids.[2] As will be apparent the reactions did not follow the anticipated course. However, since this investigation was undertaken Wendler, Graber, Jones and Tishler[5] have synthesized compound F by an ingenious modification of Sarett's[6] procedure for the synthesis of the cortisone side chain and Wendler, Huang-Minlon and Tishler[7] have prepared compound F by the reduction of cortisone bis-semicarbazone.

The reaction of ethyl orthoformate with 20-ketosteroids has not been described. Treatment of 3α-hydroxypregnane-11,20-dione with ethyl orthoformate followed by refluxing the reaction product with xylene gave an amorphous mixture (II) which remained amorphous after chromatography. However, the product gave a positive tetranitromethane test and infrared spectrometry demonstrated the presence of a carbonyl group in the new material. Proof that the 11-ketonic group had not reacted was obtained by reduction of the amorphous mixture with lithium aluminum hydride followed by mild acid hydrolysis to yield 3α,11β-dihydroxypregnane-20-one (III).

The amorphous and, as will be clear, inhomogenous enol ether II from 3α-hydroxypregnane-11,20-dione was reduced with lithium aluminum hydride and without isolation, the product was treated with perbenzoic acid in benzene solution. The reaction with perbenzoic acid was unusually vigorous in comparison with 20-enol acetates. The reaction product was hydrolyzed with acid under mild conditions and after chromatography two compounds were obtained. One of these melted at 237–239° and its identity as 3α,11β-dihydroxyetiocholane-17-one (VII) was revealed by infrared spectrometry. This substance was previously synthesized by Sarett[8] and has been isolated from the urine of cancer patients by Dobriner and his associates.[9] The other product melted at 201–202° and elementary analysis agreed with the formula $C_{22}H_{36}O_4$. Infrared spectrometry demonstrated the absence of a 17α-hydroxy-20-ketone structure in the side chain since the band at 1693 to 1697 cm.$^{-1}$ characteristic of this grouping was not apparent. The compound did not react with bromine in chloroform solution confirming the absence of the ketol structure and was not altered by dilute hydrochloric acid in aqueous ethanol over a period of 24 hours, a result which strongly sug-

(1) 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.

(2) T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951); B. Koechlin, D. Garmaise, T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **71**, 3262 (1949).

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(4) M. Mousseron and R. Jacquier, *Bull. soc. chim. France*, 698 (1950).

(5) N. L. Wendler, R. P. Graber, R. E. Jones and M. Tishler, THIS JOURNAL, **72**, 5793 (1950).

(6) L. H. Sarett, *ibid.*, **70**, 1454 (1948).

(7) N. L. Wendler, Huang-Minlon and M. Tishler, *ibid.*, **73**, 3818 (1951).

(8) L. H. Sarett, *J. Biol. Chem.* **173**, 185 (1948).

(9) S. Lieberman, D. K. Fukushima and K. Dobriner, *ibid.*, **182**, 299 (1950).

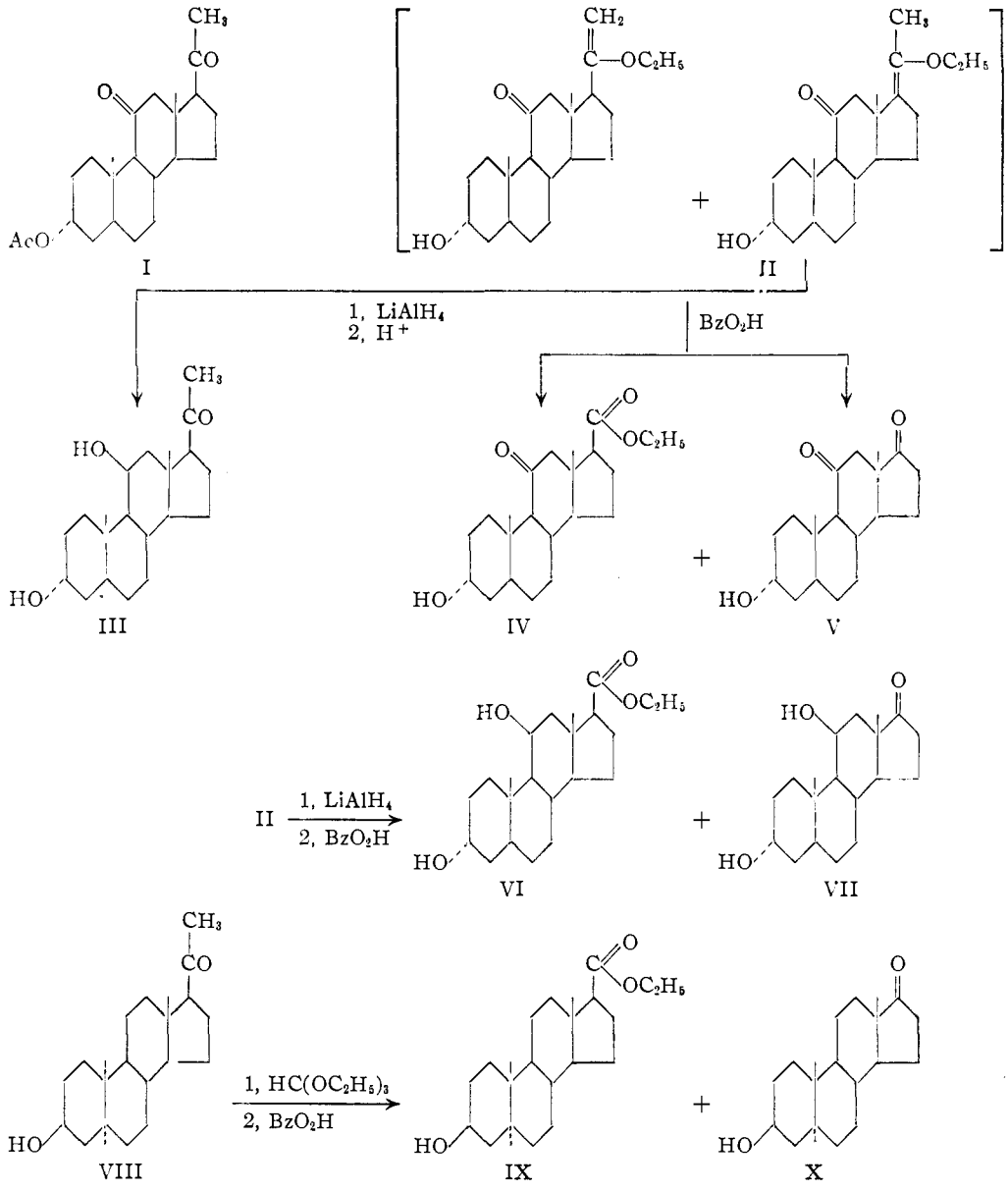

Fig. 1.

gested the absence of a 17,20-epoxy-20-ether structure. Since it was clear from the isolation of the 17-ketosteroid VII that cleavage of an enolic double bond had occurred, it was possible that the enol ether oxidized was a mixture of $\Delta^{17,20}$- and $\Delta^{20,21}$-isomers which might in part have been cleaved to an ester. When hydrolyzed with alkali the second compound was converted to an acid subsequently identified as $3\alpha,11\beta$-dihydroxyetianic acid.[10] Esterification with diazoethane reconstituted the original material VI and thus both products of the reaction were conclusively identified. The structure of the ethyl ester VI was further verified by oxidation of the monoacetate to ethyl 3α-acetoxy-11-ketoetianate, isolated and characterized in this investigation.

In agreement with these results, the enol ether II, obtained from 3α-hydroxypregnane-11,20-dione with ethyl orthoformate followed by pyrolysis, when oxidized with perbenzoic acid yielded a mixture of 3α-hydroxyetiocholane-11,17-dione (V) and ethyl 3α-hydroxy-11-ketoetianate (IV). Similarly when 3α-hydroxyallopregnane-20-one (VIII) was subjected to the same reaction sequence isoandrosterone (X) and ethyl 3β-hydroxyalloetianate (IX) were produced.

Although the mixture of 20-enol ethers was not resolved into its components, it is apparent from the oxidation products that the presence of $\Delta^{17,20}$- and $\Delta^{20,21}$-enol ethers accounts satisfactorily for the results. It is noteworthy that the intermediate ketal produced in the first step can eliminate a molecule of alcohol by loss of hydrogen from either C-21 or C-17 to give rise to two enol ethers and that this elimination of alcohol from the ketal parallels the behavior of 20-hydroxy steroids toward dehydration as well as the 20-amino steroids toward nitrous

(10) The stem name "etianic acid" has been suggested by the Subcommittee on Steroid Nomenclature of the National Research Council as a replacement for "etiocholanic acid" in order to avoid the use of the same name for parent hydrocarbons of different carbon content.

acid treatment.[11] The observation[12] that 20-keto-steroids can enolize in both directions depending upon the experimental conditions does not bear upon the present discussion since the formation of steroid 20-enol acetates does not appear to involve an elimination reaction. The cleavage of the steroid 20-enol ethers constitutes a novel method for the degradation of steroid 20-methyl ketones to 17-ketosteroids as well as to etianic acids. Although the yields are relatively low, the method can be advantageously compared with a number of other known procedures.[13] The cleavage of enol ethers finds a parallel in the behavior of aromatic ethers which undergo fission to esters in similar manner when treated with perbenzoic acid.[14] In contrast is the stability of the corresponding enol acetates which are not readily oxidized with perbenzoic acid beyond the epoxide stage.

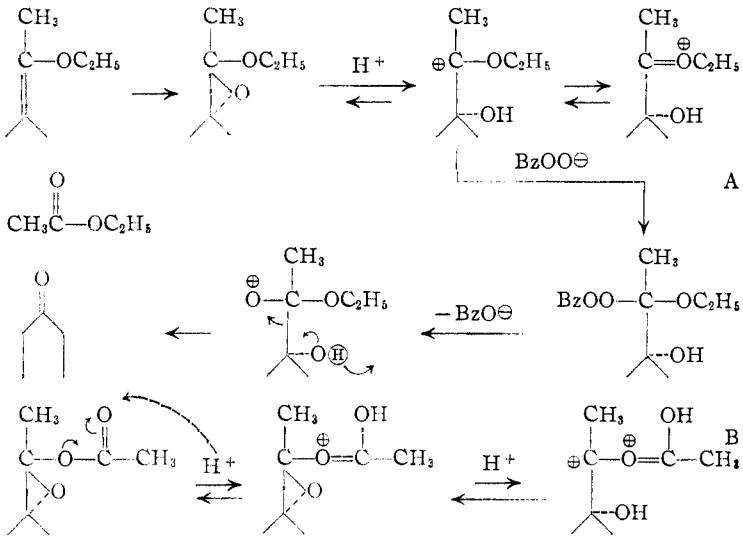

Fig. 2.

A plausible mechanism for the cleavage of steroid enol ethers is formulated in Fig. 2A. Attack of the intermediate ether epoxide XI by a proton would cause opening of the oxide bridge and create a positive charge at carbon atom 20, favored by the resonance stabilization of the carbonium ion thus formed. Addition of the perbenzoate ion at carbon 20 is thus facilitated and subsequent separation of a benzoate ion leaving an electron deficient oxygen atom on carbon 20 would lead to elimination of ethyl acetate as indicated. The same mechanism is applicable to the cleavage of the Δ^{20}-enol ether in which case formaldehyde is eliminated. This mechanism accounts satisfactorily for the stability of the corresponding enol acetate epoxides toward perbenzoic acid because the resonance hybrid of the acetoxy group would oppose opening of the oxide by a proton, as shown in scheme 2B, since identical charges would result on two adjacent atoms. The attack of the perbenzoate ion would be repelled and the epoxide structure would be greatly favored.

(11) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

(12) H. Vanderhaeghe and T. F. Gallagher, THIS JOURNAL, **74**, 2810 (1952); Moffett and Weisblat, *ibid.*, **74**, 2183 (1952).

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(14) H. Fernholz, *Chem. Ber.*, **84**, 110 (1951).

Experimental[15]

Preparation of Enol-20-ethyl Ether of 3α-Hydroxypregnane-11,20-dione.—A solution of 5 g. of 3α-acetoxypregnane-11,20-dione (I) in 50 ml. of ethyl orthoformate, 45 ml. of absolute ethanol and one drop of concd. sulfuric acid was heated under reflux for 3 hours. The product, isolated in the usual manner was an oil which was dissolved in 400 ml. of xylene. The solvent was slowly distilled at atmospheric pressure over a period of 3 hours and the remaining xylene was removed *in vacuo*. The residual oil was chromatographed on 150 g. of alumina but resisted crystallization. A middle fraction was examined for infrared spectrum. It exhibited an absorption band at 1709 cm.$^{-1}$ in carbon disulfide suggestive of the presence of an 11-ketone group and in the "fingerprint" region (1180 to 750 cm.$^{-1}$) absorption bands differing from the starting material. All fractions gave positive tetranitromethane tests. This material is referred to as "enol-20-ether" (II).

3α,11β-Dihydroxypregnane-20-one from "Enol-20-ether."—The oily "enol-20-ether" was dissolved in ether and added to a solution of 5 g. of lithium aluminum hydride in 500 ml. of anhydrous ether. The mixture was heated under reflux for 3 hours and the excess reagent was destroyed with ethyl acetate. The ethereal phase yielded an oil which was used as such. A solution of 250 mg. of the oil in 10 ml. of ethanol and one drop of concd. hydrochloric acid was stored 1 hour at room temperature. The solution was diluted with ether and the product, after isolation in the usual manner, was chromatographed on 7.5 g. of alumina to yield 115 mg. of crystalline material melting at 195–205°. Three recrystallizations from ethyl acetate afforded 68 mg. of prisms of 3α,11β-dihydroxypregnane-20-one m.p. 221–223°. The acetate crystallized from ether-petroleum ether as prisms, m.p. 180–182°, $[\alpha]^{25}D$ +149° (acetone); the infrared spectrum of the acetate was identical with that of the compound prepared by von Euw, Lardon and Reichstein (reported[16] for 3α,11β-dihydroxypregnane-20-one m.p. 222–225°; 3-monoacetate, m.p. 182°, $[\alpha]^{25}D$ +148°).

3α,11β-Dihydroxyetiocholane-17-one (VII) and Ethyl 3α,11β-Dihydroxyetianate (VI) from "Enol-20-ether" II.—Approximately 4.0 g. of oily "enol-20-ether" was reduced with lithium aluminum hydride as in the preceding experiment. The reduction product was treated with 100 ml. of 1.5 *M* perbenzoic acid in benzene and after one hour the solution was diluted with ether and the product was isolated in the usual manner. Chromatography on alumina yielded 1.23 g. of material m.p. 185–195° (Fraction A) and 258 mg. m.p. 231–237° (Fraction B). Recrystallization of Fraction B from ethyl acetate yielded needles of 3α,11β-dihydroxyetiocholane-17-one (VII), m.p. 237–239°; $[\alpha]^{17}D$ +96° (chloroform), reported by Sarett[8] m.p. 237–239°.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.20; H, 9.13.

The acetate crystallized from acetone as prisms, m.p. 237–239°; $[\alpha]^{25}D$ +107° (chloroform) and was identical in all respects with an authentic sample of 3α-acetoxy-11β-hydroxyetiocholane-17-one (reported[8] m.p. 237–238°). Fraction A was recrystallized from ethyl acetate as fine needles of ethyl 3α,11β-dihydroxyetianate (VI), m.p. 201–202°, $[\alpha]^{25}D$ +75° (chloroform).

Anal. Calcd. for $C_{22}H_{36}O_4$: C, 72.53; H, 9.89. Found: C, 72.32; H, 9.86.

Acetylation of the ethyl ester and crystallization from acetone yielded ethyl 3α-acetoxy-11β-hydroxyetianate (VIa) as needles m.p. 190–191°; $[\alpha]^{28}D$ +86° (chloroform).

Anal. Calcd. for $C_{24}H_{38}O_5$: C, 70.93; H, 9.35. Found: C, 71.10; H, 9.14.

(15) All melting points are corrected. The phrase "in the usual manner" means that the organic solvent was washed with either dilute acid or dilute base or both, as appropriate, the solution was then dried over sodium sulfate and the solvent was removed by distillation.

(16) von Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944).

3α,11β-Dihydroxyetianic Acid.—When 170 mg. of ethyl 3α,11β-dihydroxyetianate was heated under reflux for 4 hours with 0.5 *N* potassium hydroxide in 80% ethanol an acid was obtained upon crystallization from ethyl acetate with a constant melting point of 254–255°; $[\alpha]^{25}D$ +51° (acetone). From the analysis it appeared that some dehydration had occurred and recrystallization had not yielded a pure compound.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Calcd. for $C_{20}H_{30}O_3$: C, 75.47; H, 9.43. Found: C, 74.24, 74.04; H, 9.96, 10.80.

Esterification of 15 mg. of crude acid with ethereal diazoethane followed by crystallization from ethyl acetate gave 4 mg. of ethyl 3α,11β-dihydroxyetianate, m.p. 200–202°, no depression on admixture with the sample previously obtained. The infrared spectra of the two samples were likewise identical.

Ethyl 3α-Acetoxy-11-ketoetianate.—Thirty mg. of VI was dissolved in 1 ml. of acetic acid and 1 ml. of a 2% chromic acid solution in 95% acetic acid added. After 2 hours at room temperature, the solution was poured into water and the oxidation product was isolated in the usual manner. Crystallization from methanol yielded ethyl 3α-acetoxy-11-ketoetianate, m.p. 120–120.5°; $[\alpha]^{26}D$ +88° (chloroform).

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.30; H, 8.91. Found: C, 71.61; H, 9.15.

3α-Hydroxyetiocholane-11,17-dione (V) and Ethyl 3α-Acetoxy-11-ketoetianate (IV) from "Enol-20-ether."—A solution of 3.7 g. of "enol-20-ether" in 15 ml. of benzene was added to 100 ml. of 1.5 *M* perbenzoic acid in benzene with intermittent ice cooling. After 1 hour the solution was diluted with a large volume of ether and the product was isolated in the usual manner. The residue was dissolved in 50 ml. of ethanol, 25 ml. of 0.5 *N* hydrochloric acid was added and after 1 hour at room temperature the solution was poured into ether. Purification in the usual manner gave an oily residue that was chromatographed on 60 g. of alumina. The first eluates contained 945 mg. of oil (fraction A), followed by 440 mg. of crystals, m.p. 182–186° (fraction B). Recrystallization of fraction B from ethyl acetate gave needles m.p. 186–187° (reported[11] m.p. 187–188°) identical in all respects with authentic 3α-hydroxyetiocholane-11,17-dione (V). After acetylation of a small portion of Fraction A followed by crystallization from methanol long needles of ethyl 3α-acetoxy-11-ketoetianate were obtained, m.p. 119–120°, identical in all respects including infrared spectrum with the product previously obtained from the oxidation of VIa.

3α-Hydroxy-11-ketoetianic Acid.—A solution of 854 mg. of amorphous ethyl 3α-hydroxy-11-ketoetianate (Fraction A from preceding section) was heated under reflux for 4 hours with 40 ml. of 0.5 *N* potassium hydroxide in 80% ethanol. The alkaline solution was diluted with water and extracted once with ether. After acidification and extraction with ethyl acetate, purification in the usual manner followed by crystallization from ethanol yielded 564 mg., m.p. 286–289°. After two recrystallizations from ethanol 3α-hydroxy-11-ketoetianic acid, m.p. 290–292°; $[\alpha]^{25}D$ +80° (ethanol), was obtained (reported[17] m.p. 289–293°).

Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.85; H, 9.05. Found: C, 71.76; H, 9.18.

The acetate methyl ester crystallized as prisms from methanol, m.p. 149–151°, and was identical by melting point of a mixture and in its infrared spectrum with an authentic sample (reported[18] m.p. 147–149°).

Isoandrosterone (X) and Ethyl 3β-Hydroxyalloetianate (IX).—The "enol-20-ether" prepared from 3 g. of 3β-hydroxyallopregnane-20-one as previously described was treated with an excess of 1.5 *M* perbenzoic acid in benzene followed by hydrolysis with alcoholic hydrochloric acid. The resulting oil weighed 3 g. and was chromatographed on 90 g. of alumina. Three fractions were collected: the first weighed 141 mg. and consisted of essentially pure ethyl 3β-hydroxyalloetianate; the second fraction consisted of 1.20 g. of an oily mixture and the third fraction was 274 mg. of essentially pure isoandrosterone, m.p. 172–173° after recrystalization from ethyl acetate. The second fraction when extracted with petroleum ether deposited 640 mg. of isoandrosterone. From the filtrate, 561 mg. of ethyl 3β-hydroxyalloetianate was obtained. Several recrystallizations of the latter from petroleum ether afforded very fine needles, m.p. 132–133°; $[\alpha]^{25}D$ +44° (chloroform).

Anal. Calcd. for $C_{22}H_{36}O_3$: C, 75.86; H, 10.34. Found: C, 75.51; H, 10.48.

The yields of ester and ketone were 23 and 28%, respectively.

3β-Hydroxyalloetianic Acid.—Saponification of the preceding ethyl 3β-hydroxyalloetianate was accomplished by heating under reflux with ethanolic potassium hydroxide for 4 hours. The acid obtained in 70% yield crystallized from ethanol in the form of needles, m.p. 249–250°, $[\alpha]^{25}D$ +39° (ethanol) (reported[19] m.p. 251–253°). It caused no depression of the melting point of an authentic sample. The methyl ester acetate prepared in the usual manner, crystallized from methanol as leaflets, m.p. 149–150°; $[\alpha]^{25}D$ +40° (chloroform); reported[20] 149–151°, $[\alpha]D$ +36° (chloroform). The infrared spectrum was identical with an authentic sample of methyl 3β-acetoxyalloetianate.

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