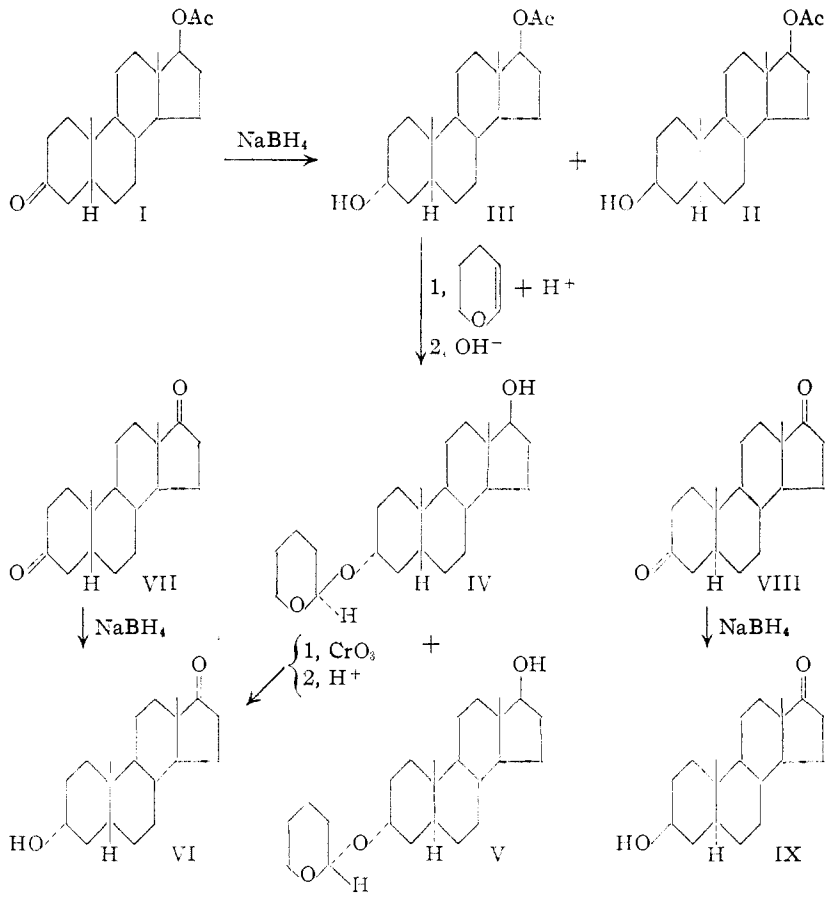
[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Preparation of 3α-Hydroxyetiocholane-17-one by a Differential Reduction with Sodium Borohydride[1a]

BY ELISE ELISBERG, HUBERT VANDERHAEGHE[1b] AND T. F. GALLAGHER

Partial reduction of etiocholane-3,17-dione with sodium borohydride in methanol gave 3α-hydroxyetiocholane-17-one in 70% yield together with 15% of 3β-hydroxyetiocholane-17-one. This reaction therefore affords a simple and efficient preparation of one of the principal urinary 17-ketosteroids. The same reaction applied to androstane-3,17-dione yielded isoandrosterone and a very small amount of androsterone. The rate of reaction of the C-3-ketone of the allo series was slower than for the normal series. 3α-Hydroxyetiocholane-17-one was also prepared from 17β-acetoxyetiocholane-3α-ol by formation of the tetrahydropyranyl ether followed by alkaline hydrolysis of the acetate, oxidation with chromium trioxide and hydrolysis to the desired product.

The availability of many steroid hormone metabolites through partial synthesis is limited by cumbersome methods, scarcity of suitable intermediates, or a very small yield of the desired product. This is particularly true of 3α-hydroxyetiocholane-17-one (VI), a urinary 17-ketosteroid for which a ready synthesis in a good yield from accessible starting materials has not been described. Since we required relatively large amounts of this substance for isotopic studies we have devised a procedure that permits the preparation of 3α-hydroxyetiocholane-17-one in a simple and efficient manner.



The method stems from the observation in these laboratories that 20-ketosteroids are reduced with difficulty to the corresponding alcohols by sodium borohydride. It seemed probable that a carbonyl group at C-17 would be similarly resistant to reduction by the reagent and this conjecture proved in fact to be true. A carbonyl group at C-3, however, is rapidly reduced and the stereochemical course of reaction is predominantly toward the desired product as might be expected from the theoretical considerations of Barton[2] and the experimental results of Shoppee and Summers[3] with lithium aluminum hydride. Thus, when etiocholane-3,17-dione was treated with a slight excess of sodium borohydride in dilute methanol solution, 3α-hydroxyetiocholane-17-one was obtained in 69% yield. The corresponding 3β-hydroxy compound was formed in 17% yield together with a minor amount of etiocholane-3α,17β-diol. The use of pyridine instead of methanol as the solvent resulted in a more sluggish reaction and a smaller yield of the same products. Since etiocholane-3,17-dione can readily be obtained by catalytic reduction of the easily available Δ^4-androstene-3,17-dione, the method affords a simple preparation in good yield of one of the principal urinary steroids.

Similar but not identical results were obtained with the sodium borohydride reduction of androstane-3,17-dione. The principal product was isoandrosterone as expected, but there was a rather surprising difference in reactivity between 3-ketones of the androstane and of the normal series. Whereas under identical conditions the C-3 carbonyl group of etiocholane-3,17-dione was almost completely reduced to a carbinol, more than 30% of androstane-3,17-dione was recovered unchanged after treatment with sodium borohydride.

Earlier attempts in this Laboratory to prepare 3α-hydroxyetiocholane-17-one from readily access-

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(2) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(3) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 687 (1950).

ible steroids utilized another valuable property of sodium borohydride, namely, the reduction of a carbonyl group without simultaneous cleavage of an ester. Reduction of 17β-acetoxyetiocholane-3-one (I) gave an 80% yield of 17β-acetoxyetiocholane-3α-ol (III) together with 15% of the 3β-epimer II. The free alcohol group of III was then protected by conversion to the tetrahydropyranyl ether, a derivative resistant to alkaline hydrolysis.[4] The 17-acetoxy group was saponified with base, and two ethers, IV and V, were separated. The introduction of a new center of asymmetry in the pyran ring is responsible for the production of two compounds, remarkably similar in all properties including infrared spectra, but differing markedly in rotation (+96° and −22°). Greenhalgh, Henbest and Jones[5] have obtained a similar pair of epimers in the reaction of dehydroisoandrosterone with dihydropyran. The mixture of the two epimeric acetals IV and V was obtained in 94% yield and for preparative purposes separation was unnecessary. Chromium trioxide oxidation of the mixture of IV and V yielded the 17-ketone and removal of the tetrahydropyranyl ether by acid hydrolysis gave 3α-hydroxyetiocholane-17-one (VI). The over-all yield of VI from I through this sequence of reactions was 50%.

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Experimental[6]

Reduction of 17β-Acetoxyetiocholane-3-one (I) with Sodium Borohydride. 17β-Acetoxyetiocholane-3α-ol (III) and 17β-Acetoxyetiocholane-3β-ol (II).—A solution of 966 mg. (3.0 mmoles) of 17β-acetoxyetiocholane-3-one in 25 ml. of methanol was added to a solution of 115 mg. (3.0 mmoles) of sodium borohydride in 2 ml. of water and 10 ml. of methanol. The mixture warmed spontaneously and evolved gas. After standing at room temperature for 20 minutes, the solution was diluted with water and extracted with ether. The white solid residue obtained by the usual procedure was recrystallized from acetone–petroleum ether and yielded 828 mg. of white crystals and 151 mg. of mother liquors. The crystalline fraction was recrystallized from acetone–petroleum ether and 770 mg. of pure 17β-acetoxyetiocholane-3α-ol, m.p. 173–174°, was obtained together with 50 mg. of mother liquors. An additional 37 mg. of pure III was obtained by chromatography of the combined mother liquors; the yield of pure product was 807 mg. or 80% of theoretical. The purest sample melted at 175–176°; $[\alpha]^{27}D$ +17° (chloroform).

From the chromatogram of the mother liquors 149 mg. (15%) of 17β-acetoxyetiocholane-3β-ol (II) was obtained and was recrystallized from ethyl acetate as prisms, m.p. 140.5–142.5°; $[\alpha]^{24}D$ +9° (chloroform). The compound crystallized from acetone–petroleum ether as a solvate, m.p. 102–104°, and heating *in vacuo* was required for conversion to the unsolvated form.

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.37; H, 10.26. Found: C, 75.52; H, 10.02.

17β-Hydroxyetiocholane-3α-yl-(2¹-tetrahydropyranyl) Ether (IV and V).—To a solution of 5.73 g. (17 mmoles) of 17β-acetoxyetiocholane-3α-ol in 135 ml. of dihydropyran, 2 ml. of concentrated hydrochloric acid was added dropwise. The mixture was allowed to stand overnight and the product was isolated in the usual manner. The pale yellow, oily residue was distilled with steam until 8 liters of distillate were collected. The non-crystalline residue was dissolved in 300 ml. of methanol, 45 ml. of 1.7 *N* potassium hydroxide and 20 ml. of water was added and the solution was heated on the steam-bath for 20 minutes. The product, isolated in the usual manner, was an oil weighing 6.66 g. Half of the oil was chromatographed on alumina and 3.008 g. of IV and V was obtained together with 215 mg. of etiocholane-3α,17β-diol. The first eluates from the chromatogram, on recrystallization from petroleum ether, formed needles, m.p. 140–144°; $[\alpha]^{24}D$ −22° (chloroform).

Anal. Calcd. for $C_{24}H_{40}O_3$: C, 76.59; H, 10.71. Found: C, 77.03; H, 10.66.

The later eluates upon recrystallization from petroleum ether formed prisms, m.p. 140–143°; $[\alpha]^{26}D$ +96° (chloroform).

Anal. Calcd. for $C_{24}H_{40}O_3$: C, 76.59; H, 10.71. Found: C, 76.76; H, 10.50.

The two materials showed a depression of the melting point and differed only slightly in their infrared spectrum in the "finger print" region (1200 to 850 cm.$^{-1}$) in carbon disulfide solution. The combined yield of the two diastereoisomers of 17β-hydroxyetiocholane-3α-yl-(2¹-tetrahydropyranyl) ether was 94%.

3α-Hydroxyetiocholane-17-one (VI).—To a solution of 0.799 g. (2.1 mmoles) of 17β-hydroxyetiocholane-3α-yl-(2¹-tetrahydropyranyl) ether (mixture of IV and V) in 5 ml. of glacial acetic acid was added, at room temperature, a solution of 12 ml. of 0.18 *M* chromium trioxide in 90% acetic acid. The solution was allowed to stand for 2 hours and after dilution with water and extraction with ether, the product was isolated in the usual manner. The residual oil was dissolved in 75 ml. of ethanol, 25 ml. of water and 2 ml. of concentrated hydrochloric acid and was refluxed for 1.5 hours. The solution was extracted with ether and purification by the usual procedure yielded 0.584 g. of oil which was dissolved in benzene–petroleum ether. A flocculent material, 29 mg., was removed and identified as etiocholane-3α,17β-diol, m.p. 215–228°. The filtrate was concentrated and 343 mg. of VI, m.p. 140–146°, was collected. The melting point of a mixture with authentic etiocholanolone showed no depression. A second crop of 76 mg., m.p. 136–141°, was obtained. Chromatography of the mother liquors yielded 26 mg. identified by infrared spectrum as VI and 30 mg. of etiocholane-3,17-dione. The yield of 3α-hydroxyetiocholane-17-one was 445 mg. (71%).

3α- and 3β-Hydroxyetiocholane-17-one by Reduction of Etiocholane-3,17-dione (VII) with Sodium Borohydride in Methanol.—A solution of 1.068 g. (3.7 mmoles) of etiocholane-3,17-dione in 25 ml. of methanol was added 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 and ether were added and the product was isolated from the ether solution in the usual manner. The residual white solid, 1.088 g., was separated by chromatography on alumina and recrystallization. The products obtained were: (a) 686 mg. of analytically pure 3α-hydroxyetiocholane-17-one, m. p. 151–152°, together with 58 mg. of a slightly less pure product (69% yield); (b) 121 mg. of analytically pure 3β-hydroxyetiocholane-17-one, m.p. 151–153°(17% yield); (c) 41 mg. of etiocholane-3α,17β-diol.

Reduction of Etiocholane-3,17-dione with Sodium Borohydride in Pyridine.— A solution of 288 mg. (1 mmoles) of etiocholane-3,17-dione in 5 ml. of dry pyridine was added to a solution of 14 mg. (1.25 meq.) of sodium borohydride in 15 ml. of pyridine. The mixture was allowed to stand at room temperature for 15 minutes, the water and ether were added and the product was isolated in the usual manner. The white crystalline residue was separated by chromatography on alumina and recrystallization, yielding 25% of 3α-hydroxyetiocholane-17-one and 42% of unchanged starting material.

Reduction of Androstane-3,17-dione with Sodium Borohydride in Methanol.—A solution of 1.068 g. (3.7 mmoles)

(4) G. F. Woods and D. N. Kramer, THIS JOURNAL, **69**, 2246 (1947).

(5) C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 1190 (1951).

(6) All melting points were taken in capillary tubes and are corrected. The phrase "in the usual way" means the organic solvent was washed with either acid or base or both, as appropriate, followed by washing with water and drying the solution over sodium sulfate before distillation of the solvent.

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]

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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).

(3) A. Ruff and T. Reichstein, *Helv. Chim. Acta*, **34**, 70 (1951).

(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).