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The Anthrasteroid Rearrangement. VI. The Preparation of an Analog of the Androgens and Estrogens[1]

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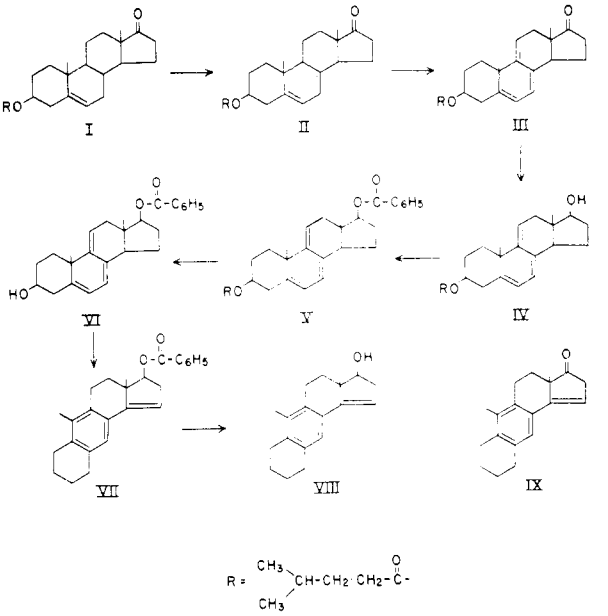
The conversion of dehydroepiandrosterone to 5,7,9,14-anthrastatetraen-17β-ol is described.

In a previous paper[2] in this series the anthrasteroid rearrangement of 5,7,9(11)-androstatrien-3β-ol-17-one isocaproate (III) was reported. Contrary to our experience with analogous trienes bearing side chains related to ergosterol, cholesterol, 23,24-bisnorcholanic acid and progesterone,[1-3] the rearrangement did not proceed either in the expected yield nor did it afford a product (IX) with the expected physical properties. Instead, two compounds were isolated in very small amounts. One of these was a chloro-14,15-dihydro derivative of IX. The other was a ketone with the empirical formula of IX, but the ultraviolet and infrared spectra were anomalous. While the structure of this material is uncertain, it is possible that it actually was IX and that the presence of the carbonyl group disturbed the normal spectral pattern as a result of conformational changes in ring D. This would also explain why the chemical properties of IX or its precursors were sufficiently different from other anthrasteroids to allow the formation of a chloro derivative under the conditions of the rearrangement.

Our interest in the possible biological significance[1,3] of anthrasteroids prompted us to reinvestigate the rearrangement of steroids lacking a carbon side chain in the hope of preparing an anthrasteroid related to the androgens and estrogens to which a definite structure could be assigned.

On the assumption that a conformational change is induced in the molecule by the 17-keto group, we decided to carry out the rearrangement on the triene bearing a 17β-hydroxyl group. Dehydroepiandrosterone isocaproate (I) was converted to the corresponding 5,7,9(11)-triene (III) as already described[2] and III was reduced with sodium borohydride to the corresponding 17β-alcohol, 5,7,9(11)-androstatriene-3β,17β-diol 3-isocaproate (IV). The selective reduction took place in a nearly quantitative yield. Compound IV, however, failed to give the expected anthrasteroid VIII when submitted to acid catalysis. The corresponding 3β,17β-diol was equally anomalous in its behavior. In view of the well established pathway of the conversion of 5,7,9(11)-trienes such as IV to anthrasteroids,[4] we felt that the failure of the compounds with a 17β-hydroxyl group to proceed normally through the acid-catalyzed transformations was caused by dehydration in ring D leading to undesirable polyenes. Since it has been shown that esters dehydrate more slowly than alcohols under the conditions of the rearrangement,[5] we prepared the mixed ester V which was selectively hydrolyzed to the half-ester VI bearing a benzoylated hydroxyl group at C-17 and a free hydroxyl group at C-3. When VI was submitted to the conditions of the rearrangement, the reaction proceeded normally to give the anthrasteroid, 5,7,9,14-anthrastatetraen-17β-ol benzoate (VII),[6] in a yield of *ca.* 50%. Its isolation was more difficult than we had experienced with other anthrasteroids, but by following chromatograms of the reaction products spectrophotometrically and rechromatographing the appropriate fractions, we obtained VII crystalline in a 10% over-all yield from VI.[4] Saponification readily gave the alcohol VIII. The spectroscopic properties of both VII and VIII were entirely as expected. A strong absorption band was present near 12.3 μ. This band has been found in the spectrum of all other anthrasteroids[7,1] possessing a conjugated double bond with the sole exception of the "anthrasteroid" bearing a 17-keto group.[2] The ultraviolet spectrum of VIII (λ_{max} 221, 227, 266, 296 and 308 mμ) was qualitatively and quantitatively correct and the ultraviolet spectrum of the benzoate VII was likewise that expected for a compound with the absorption of a



(1) Part V, W. R. Nes, J. A. Steele and E. Mosettig, THIS JOURNAL, **80**, 5230 (1958).

(2) W. R. Nes, R. B. Kostic and E. Mosettig, *ibid.*, **78**, 436, 6423 (1956).

(3) W. R. Nes and E. Mosettig, *ibid.*, **76**, 3182 (1954).

(4) Five steps are involved. For an outline and references see Part V[1] of this series.

(5) W. R. Nes, THIS JOURNAL, **78**, 193, 6421 (1956).

(6) See Parts I[2] and V[1] for a discussion of nomenclature and structure.

(7) I. Scheer, W. R. Nes and P. Smeltzer, THIS JOURNAL, **77**, 3300 (1955).

benzoyl chromophore superimposed on the normal anthrasteroid absorption.

The selective hydrolysis of the mixed ester V was carried out by a modification of the procedure used by Ruzicka, Wettstein and Kägi[8] in the partial hydrolysis of 5-androstene-3β,17β-diol 3-acetate 17-benzoate. These authors carried out the reaction in methanolic alkali. Following their suggestion that the saponification takes place faster in ethanol, we found that in 0.061 *M* ethanolic potassium hydroxide hydrolysis of the isocaproate took place almost to completion in three hours with little attack on the benzoyl moiety at C-17. A few per cent. of starting material was separated chromatographically from the pure half-ester. This procedure also was employed successfully with the corresponding Δ^5- and $\Delta^{5,7}$-steroids. In all instances the appropriate half-ester of the 3β,17β-diol was prepared by reduction of the corresponding 17-keto compound with sodium borohydride.

5,7-Androstadiene-3β,17β-diol and 5,7,9(11)-androstatriene-3β,17β-diol were obtained by reduction of the corresponding 17-keto-3-isocaproates with lithium aluminum hydride and, in the case of the triene, by hydrolysis of IV.[2,9] They were examined for hormonal action by the Endocrinology Branch of the National Cancer Institute under the direction of Dr. Roy Hertz. The $\Delta^{5,7,9(11)}$-compound was found to be less than 10% as effective an androgen in the castrate adult rat as testosterone. It was also found to have no anti-androgenic potency when tested against a basal dose of testosterone at a ratio of 5 parts to 1. The $\Delta^{5,7}$-compound, on the other hand, was found to be about 20% as active as testosterone in the castrate adult rat test. Its effects on prostate, seminal vesicle and *vas deferens* were qualitatively identical with that of testosterone. Biological examination of the anthrasteroid VIII is under way.

Experimental[10]

5,7-Androstadien-3β-ol-17-one Isocaproate (II from I).—Allylic bromination of 5-androsten-3β-ol-17-one isocaproate (I) was carried out by the agency of N-bromosuccinimide under irradiation using 2 General Electric RSP 2 photospot lamps, and the intermediate 7-bromo derivative was dehydrobrominated with collidine essentially according to the procedure previously described from this Laboratory.[2] The yield of material melting at 74–77° was 35%; lit.[2] m.p. 75–77°.

Reduction of II with lithium aluminum hydride in ether gave **5,7-androstadiene-3β,17β-diol**, m.p. 205–207°, lit. m.p. 210–212°[9a,b] and 223–226°.[9c]

5,7,9(11)-Androstatrien-3β-ol-17-one Isocaproate (III from II).—Dehydrogenation of the $\Delta^{5,7}$-compound II was accomplished with mercuric acetate as already has been reported.[2] In this instance, however, the product was chromatographed, which greatly increased the amount of pure material obtainable. The yield of product melting at 135–136° was 25%; lit.[2] m.p. 134–135°.

5,7,9(11)-Androstatriene-3β,17β-diol 3-Isocaproate (IV from III).—To a warm (45°) solution of 2.16 g. of 5,7,9(11)-androstatrien-3β-ol-17-one isocaproate (III) in 74 ml. of methanol was added 378 mg. of sodium borohydride. After gas evolution had ceased the solution was cooled to 0° and diluted with water. The precipitated product was washed well with water and dried yielding 2.07 g. (96%) of colorless needles, m.p. 115–116°. Chromatography failed to alter the melting point appreciably. The analytical sample melted at 116–118° and was composed of needles and prisms, $[\alpha]_D$ +212°; λ_{max} 310, 324 and 339 mμ (ε 10300, 11600 and 7200).

Anal. Calcd. for $C_{25}H_{36}O_3$ (384.5): C, 78.08; H, 9.44. Found: C, 78.04; H, 9.40.

Saponification of IV in ethanolic potassium hydroxide yielded **5,7,9(11)-androstatriene-3β,17β-diol** which had an identical infrared spectrum with material obtained by reduction (lithium aluminum hydride) of III, although in both cases the melting point (m.p. 175°) was lower than that (m.p. 190°) previously obtained.[2,9c]

By the same procedure 5-androsten-3β-ol-17-one isocaproate was reduced with sodium borohydride to **5-androstene-3β,17β-diol 3-isocaproate** which after crystallization from methanol–water melted at 122–123°.

Anal. Calcd. for $C_{25}H_{40}O_3$ (388.6): C, 77.27; H, 10.38. Found: C, 77.36; H, 10.71.

Likewise, 5,7-androstadien-3β-ol-17-one isocaproate was reduced to **5,7-androstadiene-3β,17β-diol 3-isocaproate** which after crystallization from methanol–water gave needles, m.p. 105–108°.

Anal. Calcd. for $C_{25}H_{38}O_3 \cdot H_2O$ (404.6): C, 74.21; H, 9.47. Found: C, 74.53; H, 9.98.

5,7,9(11)-Androstatriene-3β,17β-diol 3-Isocaproate 17-Benzoate (V from IV).—Benzoylation of the monoester IV in the usual way with benzoyl chloride in pyridine and recrystallization of the product from ethanol gave V as colorless, flat needles, m.p. 161–163°, $[\alpha]_D$ +198°; λ_{max} 227, 310, 324 and 339 mμ (ε 17100, 11600, 12100 and 8200).

Anal. Calcd. for $C_{32}H_{40}O_4$ (488.6): C, 78.65; H, 8.25. Found: C, 78.56; H, 8.25.

Similarly, **5-androstene-3β,17β-diol 3-isocaproate 17-benzoate** was obtained from the corresponding monoester. It was crystallized from ethanol–water and melted at 127–128°.

Anal. Calcd. for $C_{32}H_{44}O_4$ (492.7): C, 78.01; H, 9.00. Found: C, 78.12; H, 8.92.

In the same manner the $\Delta^{5,7}$-isocaproate yielded **5,7-androstadiene-3β,17β-diol 3-isocaproate 17-benzoate**, which after crystallization from ethanol formed colorless needles, m.p. 162–165°; λ_{max} 229, 271, 281 and 293 mμ (ε 14800, 12100, 12300 and 6500); $[\alpha]_D$ −37°.

Anal. Calcd. for $C_{32}H_{42}O_4$ (490.7): C, 78.33; H, 8.63. Found: C, 78.16; H, 8.62.

5,7,9(11)-Androstatriene-3β,17β-diol 17-Benzoate (VI from V).—A mixture of 2.25 g. of the diester V and 450 ml. of 0.061 *M* ethanolic potassium hydroxide solution was allowed to stand at 26° for 3 hours with efficient stirring. The steroid completely dissolved within about 30 min. The clear, nearly colorless solution was exactly neutralized with hydrochloric acid, diluted with 1.2 liters of water, and extracted six times with 200-ml. portions of chloroform. The residue from the chloroform solution was chromatographed on 60 g. of alumina ("almost neutral"). Elution with ether yielded 49 mg. of starting material (m.p. 160–162°). Elution with chloroform yielded 1.7 g. (96%) of the monoester VI in 8 fractions. The first and last fractions were crystallized from ethanol–water and yielded needles, m.p. 133–135° and 131–132°, respectively. The analytical sample obtained in a previous experiment melted at 130–132°, $[\alpha]_D$ +106°; λ_{max} 227 and 323 mμ (ε 16900 and 10700), λ_{infl} 311 and 339 mμ (ε 10300 and 7400).

Anal. Calcd. for $C_{26}H_{30}O_3 \cdot {}^1/_2H_2O$ (399.5): C, 78.16; H, 7.82. Found: C, 78.12; H, 7.89.

By the same procedure **5,7-androstadiene-3β,17β-diol 17-benzoate** was obtained from the corresponding diester. It melted at 195–197°, lit.[9c] m.p. 201–205°. From 5-androstene-3β,17β-diol 3-isocaproate, 17-benzoate there also was obtained **5-androstene-3β,17β-diol 17-benzoate**, m.p. 218–219°; λ_{max} 229, 273 and 280 mμ (ε 14000, 890 and 710); lit.[8] m.p. 220–222°.

5,7,9,14-Anthrastatetraen-17β-ol (VIII).—A solution of 1.27 g. of 5,7,9(11)-androstatriene-3β,17β-diol 17-benzoate (VI) in 60 ml. of 0.14 *M* hydrogen chloride in chloroform

(8) L. Ruzicka, A. Wettstein and H. Kägi, *Helv. Chim. Acta*, **18**, 1478 (1935).

(9) These alcohols have been prepared by several routes; see (a) A. Butenandt, E. Hausmann and J. Paland, *Ber.*, **71**, 1316 (1938); (b) F. Neumann, G. Rosenkranz, J. Romo and C. Djerassi, THIS JOURNAL, **73**, 5478 (1951); (c) R. Antonucci, S. Bernstein, D. Giancola and K. J. Sax, *J. Org. Chem.*, **16**, 1126, 1159 (1951).

(10) For general procedures, see footnote 18 in Part V of this series.

was allowed to remain at 26° for 1.8 hours during which time the solution turned green. The ultraviolet absorption (λ_{max} 227 and 266 mμ, ε 31000 and 10000) indicated a *ca.* 50% conversion to VII. The chloroform was removed at reduced pressure and the tanish yellow residue was dissolved in 4 ml. of carbon tetrachloride and adsorbed on 100 g. of alumina ("almost neutral"). Elution with a 1/1 mixture of ether and carbon tetrachloride afforded very little material. This was followed by elution with pure ether in 7-ml. fractions. This removed 474 mg. of crude VII with a weight distribution among 15 fractions. Four of these fractions (291 mg.) under the principal part of the elution band were combined and rechromatographed on 20 g. of alumina ("almost neutral") in a similar fashion. The three fractions under the principal part of the elution band from the second chromatogram yielded crystalline material when cooled to −10° in acetone–methanol. The product melting at 98–100° weighed 119 mg. (10%). Recrystallization from acetone–methanol readily gave **5,7,9,14-anthrastatetraen-17β-ol benzoate (VII)** as colorless needles, m.p. 103–105°; λ_{max} 227, 266, 296 and 308 mμ (ε 39300, 19000, 2500 and 2100); λ_{min} 245 mμ (ε 9300), λ_{max} 12.33 μ.

Anal. Calcd. for $C_{26}H_{28}O_2$ (372.48): C, 83.83; H, 7.58. Found: C, 84.03; H, 7.77.

The alcohol VIII was obtained by hydrolysis of the benzoate VII in 5% ethanolic potassium hydroxide (30 min. at reflux). From ethanol–water it formed colorless needles, m.p. 136–138°; λ_{max} 221, 227, 266, 297 and 308 mμ (ε 24400, 25600, 17200, 2500 and 2100); λ_{min} 242 mμ (ε 5150), λ_{infl} 233 mμ (ε 16600), λ_{max} 12.30 μ.

Anal. Calcd. for $C_{19}H_{24}O$ (268.4): C, 85.03; H, 9.01. Found: C, 84.91; H, 9.27.

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Kinetics and Mechanism of Solvolysis of Steroid Hydrogen Sulfates[1]

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A kinetic study of the solvolysis of steroid hydrogen sulfates in homogeneous phase in various organic solvents has been presented. The solvolysis was found to proceed by first-order kinetics in a variety of organic solvents of low polarity and was especially fast in ethers. The effect of acid concentration and of steroid sulfate structure has been studied. Activation energies have been determined for several media. Based on the results of these studies, a mechanism has been suggested which is consistent with the observation that increasing the polarity of the medium greatly retards the rate. A transition state complex which involves the undissociated hydrogen sulfate or the dipolar ion $ROSO_3^{\ominus}$ has been proposed. $^{\oplus}H$

Introduction

This paper presents a kinetic study of the solvolysis of steroid hydrogen sulfates in organic solvents in the homogeneous phase. Unlike *most* solvolytic reactions in organic media which are accelerated by increasing the polarity of the environment, the type of solvolysis described in this paper is unusual in that it is greatly retarded by polar media.

The hydrolysis of alkyl hydrogen sulfates has been the subject of numerous studies because of their industrial and biological importance. The relative stability of the simple alkyl hydrogen sulfates toward dilute aqueous alkali and acid is well known.[2,3] In acid solution, prolonged boiling is sometimes necessary to achieve complete hydrolysis and even more drastic conditions are required in alkaline media.[4] Because of the drastic conditions employed, several products in addition to the alcohol are formed and therefore a study of the mechanism of the hydrolysis of alkyl hydrogen sulfates has been difficult. Stereochemical evidence has been presented which indicates that the alkyl hydrogen sulfates are hydrolyzed by a fission of the S–O bond. Sulfates of asymmetric alcohols are hydrolyzed[4,5] without inversion and thus an attack on the central sulfur atom seems possible.

Since steroidal metabolites, as well as many other classes of substances, are excreted in the urine as sulfates, mild methods of hydrolysis which do not result in side reactions (dehydration, rearrangement, displacement, etc.) are desirable. One such method which has found wide use involves the continuous extraction with ether of appropriately acidified solutions of the sulfates kept at room temperature. With this method, a quantitative hydrolysis of C_{19}-ketosteroid sulfates[5,6] has been achieved. In another study[7] on the mechanism of hydrolysis by the continuous ether method it has been demonstrated that this hydrolysis occurs not in the aqueous phase but in the *ether phase*, and is thus related to the procedures of Grant and Beall[8] and Cohen and Oneson[9] who found that steroid sulfates can be quantitatively hydrolyzed in dioxane. The extreme ease with which this reaction occurs in dioxane or ether as compared to hydrolysis in

(1) Supported by a research grant (No. PHS A-1083) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Department of Health, Education and Welfare.

(2) C. M. Suter, "The Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 1–94.

(3) For kinetic data in aqueous acid see, for example: G. A. Linhart, *Am. J. Sci.*, **184**, 289 (1912); P. N. Evans and J. M. Albertson, THIS JOURNAL, **39**, 456 (1917); K. H. Bauer and W. Poethke, *J. prakt. Chem.*, [2] **126**, 296 (1930).

(4) R. L. Burwell, THIS JOURNAL, **74**, 1462 (1952); G. M. Calhoun and R. L. Burwell, *ibid.*, **77**, 6441 (1955); G. H. Green and J. Kenyon, *J. Chem. Soc.*, 1389 (1950).

(5) S. Lieberman, L. B. Hariton and D. K. Fukushima, THIS JOURNAL, **70**, 1427 (1948).

(6) S. Lieberman and K. Dobriner, *Recent Prog. in Hormone Res.*, **3**, 71 (1948); S. Lieberman, B. Mond and E. Smyles, *ibid.*, **9**, 113 (1954).

(7) S. Burstein and S. Lieberman, *J. Biol. Chem.*, **233**, 331 (1958).

(8) G. A. Grant and D. Beall, *Recent Prog. Hormone Res.*, **5**, 307 (1950).

(9) S. L. Cohen and I. B. Oneson, *J. Biol. Chem.*, **204**, 215 (1953).