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The Oxidative Degradation of Neoergosteryl Acetate*

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The oxidation of neoergosterol with ozone to form methylisopropylacetaldehyde was first reported by Inhoffen.[1] Remesov[2] later confirmed the formation of the aldehyde and reported also the production, in unspecified yield, of an acid melting at 210–212°. The crude acid was esterified with diazomethane to form a substance melting at 144–145° which Remesov inferred was methyl α-(3(β)-acetoxy-5,7,9-estratrien-17-yl)-propionate (IV). It was the aim of this investigation to repeat the oxidative degradation of neoergosterol in an effort to obtain the polycyclic moiety for further study with particular regard to its conversion to compounds which might serve for the preparation of steroid hormone types.

The first attempts were directed toward the ozonolysis of neoergosteryl acetate (I). This substance, which was prepared by a modification of the method of Windaus and Borgeaud,[3] was ozonized and from the non-crystalline oxidation product it was possible to obtain a crystalline hydroxy acid melting at 206.5–208.5°, presumably α-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-propionic acid (II). The yield of the acid was so low (6.5–9%) that it was decided to attempt its preparation by another procedure, namely, the per-iodic acid oxidation of the mixture of neoergostatriols (Ia) which might be expected to result from the hydroxylation of neoergosteryl acetate with osmium tetroxide.[4] As detailed in the experimental part, this method gave a greatly improved yield (55%) of an acid identical with that obtained by the ozonolysis procedure and its preparation by the two methods is strong evidence in support of the assigned structure II. Further confirmation of this was secured through the conversion of the acid to a methyl ester III and a methyl ester acetate IV. These esters reacted sluggishly with phenylmagnesium bromide to give low yields of 1,1-diphenyl-2-methyl-2-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-ethanol-1 (V) which was isolated by chromatographing the crude product on alumina. The utilization of methylmagnesium iodide in a similar reaction led to the formation of 1,1,2-trimethyl-2-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-ethanol-1 (VI) which was characterized as the 3-monoacetate VIa. In contrast with the diphenylethanol V, which on acetylation and subsequent dehydration loses water between the carbinol group and C_{20} of the neoergosterol side chain to form the expected acetoxy diphenylethylene VII, the trimethylethanol VI was converted to an olefinic compound VIII through dehydration involving the methyl groups. Some precedent for this behavior is found in the work of Whitmore[5] and his students who have shown that certain substituted

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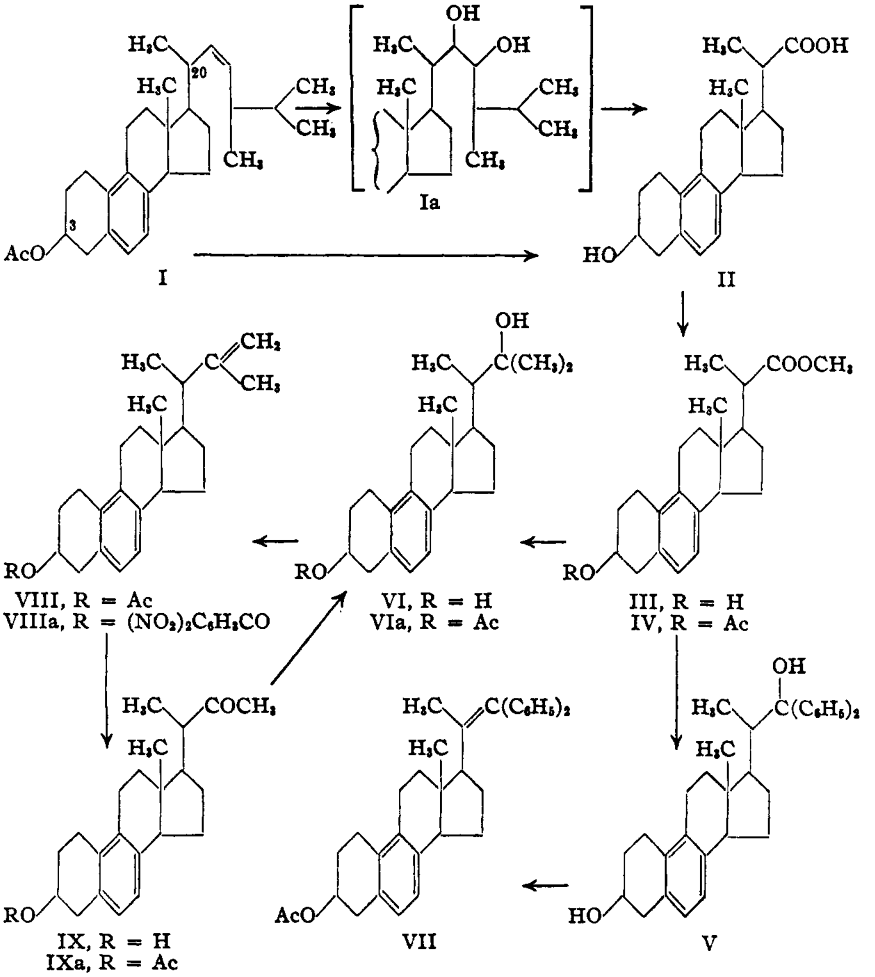
[1] Inhoffen, *Ann.*, **497**, 130 (1932).

[2] Remesov, *Rec. trav. chim.*, **55**, 797 (1936); **56**, 1093 (1937).

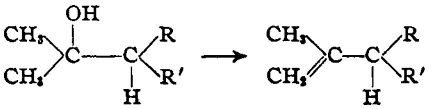
[3] Windaus and Borgeaud, *Ann.*, **460**, 235 (1928).

[4] Methods using osmium tetroxide in the hydroxylation of olefins have been described by (a) Criegee (*Ann.*, **522**, 75 (1936); **550**, 99 (1942); *Angew. Chem.*, **51**, 519 (1938)) and (b) Milas and Sussman (THIS JOURNAL, **58**, 1302 (1936); **59**, 2345 (1937)).

[5] Paper XIX of this series, by Whitmore, Rowland, Wrenn and Kilmer, THIS JOURNAL, **64**, 2970 (1942).



trimethyl carbinols of the type A undergo dehydration mainly from the unsubstituted methyl



groups with the formation of olefins of the type B as the principal products. The trimethylethanol VI, in which R = CH_3 and R′ = acetoxyestratrienyl, thus appears to undergo dehydration in a manner analogous to some of the carbinols (R and R′ = CH_3; R = $(CH_3)_3C$, R′ = H) studied by these investigators. The validity of the formulation of the olefinic dehydration product as 2-methyl-3-(3(β)-acetoxy-5,7,9-estratrien-17-yl)-butene-1 (VIII) was demonstrated by its conversion through hydroxylation and subsequent oxidation with per-iodic acid to the hydroxy ketone IX with the loss of only one skeletal carbon atom, and by the reaction of the latter compound with methylmagnesium iodide to form the original trimethylethanol VI; this series of reactions thus serves as definite proof of the structure of both the methylbutene VIII and its oxidation product.

The author wishes to express his thanks to Dr. Arthur T. Ness of this Laboratory for making the microanalytical determinations.

Experimental[6]

The Preparation of Neoergosteryl Acetate.—A suspension of 57.7 g. of crude biergostatrienol[7] in 1500 cc. of *n*-amyl alcohol was refluxed under an atmosphere of nitrogen. The solid dissolved gradually during two hours of heating; after another hour the amyl alcohol was removed by steam distillation and the semi-crystalline residue was taken up in ether and washed with alkali and water. When the dried ether solution was concentrated to a small volume and diluted with warm methanol, it deposited 17.7 g. of neoergosterol, m. p. 150.5–152°. An additional 3.0 g. of material was obtained by saponification of the 3,5-dinitrobenzoate (4.5 g.) which was formed by removing the solvent from the mother liquor and treating the residue with 3,5-dinitrobenzoyl chloride in pyridine. A small sample of neoergosterol which was further purified by recrystallization from methanol melted at 152.5–154°; $[\alpha]^{19}_D$ −10° (*c* = 1.19 in chloroform). Neoergosteryl acetate, m. p. 118–119°, was obtained in nearly quantitative yield by acetylating the material, m. p. 150.5–152°, with acetic anhydride in pyridine and crystallizing the product from methanol–acetone. Windaus and Borgeaud[3] record melting points of 151–152° and 116–117°, respectively, for neoergosterol and its acetate.

α-(3(β)-Hydroxy-5,7,9-estratrien-17-yl)-propionic Acid (II). (a) **By Ozonolysis.**—In several experiments neoergosteryl acetate was dissolved in a 2:1 acetic acid–carbon tetrachloride mixture and treated at 0° with one to five times the calculated quantity of ozone. In each case the resulting solution was shaken with cold water and then

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(6) All melting points are corrected.

(7) Jacobsen, THIS JOURNAL, **62**, 2612 (1940).

either treated with steam or boiled for thirty minutes; the non-crystalline product was taken up in ether and the acidic fraction was separated and saponified. The crude, solid acid was crystallized from aqueous acetone and formed flattened needles, m. p. 203.5–206°; the yield was uniformly 6.5–9%.

(b) **By Hydroxylation and Per-iodic Acid Oxidation.**—A solution of 3.0 g. of neoergosteryl acetate and 2.0 g. of osmium tetroxide in 150 cc. of dry ether was allowed to stand for two and one-half days at room temperature. The ether was removed under diminished pressure and the residue was subjected to reductive hydrolysis with aqueous alcoholic sodium sulfite according to the method of Criegee.[4a] The black solid which remained after removal of most of the alcohol was collected, dried under diminished pressure over anhydrous calcium chloride, and then exhaustively extracted with ether. The dark-colored ether solution was concentrated to about 70 cc. and oxidized at 15° with 5.0 g. of per-iodic acid in 50 cc. of ether containing a small amount of methanol. After one hour the reaction mixture was washed with water and the acidic fraction was extracted with alkali and saponified; the neutral material (0.5 g.) remaining in the ether was treated as described below. The yield of crystalline acid, purified as in (a), was 55%. On recrystallization, the product prepared by either method melted at 206.5–208.5°; $[\alpha]^{19}\text{D}$ −7° (c = 0.636 *in acetone*). No depression was noted on mixing material obtained by the two methods. The analysis of the acid indicated that it contained water of crystallization which was not removed completely without decomposition.

Anal. Calcd. for $C_{21}H_{28}O_3 \cdot {}^1/_2H_2O$: C, 74.74; H, 8.66. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: (dried one hour, 110°, 15 mm.) C, 74.44; H, 8.42: (five hours, 110°, 1 mm.) C, 76.33; H, 8.54: (four hours, 138°, 1 mm.) C, 77.29; H, 8.68.

The proportion of acidic and neutral products obtained in the reaction with per-iodic acid seemed to depend on the amount of colloidal osmium compounds in the ether extract. In another experiment, when the reductive hydrolysis was carried out for a longer period than usual, the extract was not as dark colored and the amount of acid formed was smaller. In this experiment particularly, it seemed advisable to oxidize the neutral fraction further in order to convert any estratrienyl-propionaldehyde to the corresponding acid. For this purpose, the ether was removed by distillation, the residual oil was reacetylated, and the non-crystalline product was oxidized at 15° with chromic anhydride in acetic acid; any additional solid acid obtained by this operation was combined with the main fraction.

The **methyl ester III** was prepared by the reaction of a suspension of 0.27 g. of the hydroxy acid in 25 cc. of cold ether with an excess of diazomethane; the mixture was warmed gradually and diluted with 50 cc. of ether to complete the esterification. The ester, which crystallized in elongated hexagonal plates from the concentrated ether solution upon the addition of hexane, melted at 173–175°, and the melting point was raised to 174–176.5° by recrystallization from ether and drying at 110° *in vacuo*.

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 77.15; H, 8.83. Found: C, 77.16; H, 8.85.

Methyl α-(3(β)-Acetoxy-5,7,9-estratrien-17-yl)-propionate (IV).—A solution of 0.85 g. of the hydroxy acid in 5 cc. of pyridine and 3 cc. of acetic anhydride was warmed to 60°, allowed to stand overnight at room temperature, and then heated on the steam-bath for thirty minutes; the solid material which was formed by treating the acetylation mixture with water melted at 206–211°. The esterification of this product with diazomethane yielded 0.80 g. of crystalline ester melting at 154–157.5° and repeated crystallization of this material from ether formed thin hexagonal plates melting at 159.5–161.5°. Neither this compound nor the methyl ester III corresponded in properties with the substance, m. p. 144–145°, reported by Remesov.[2]

Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 75.09; H, 8.35. The values calculated from the data given by Remesov[2] are C, 73.91; H, 8.92.

1,1-Diphenyl-2-methyl-2-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-ethanol-1 (V).—A solution of 0.80 g. of the methyl ester acetate IV in 70 cc. of toluene was added to an ether solution of the Grignard reagent prepared from one gram of magnesium and 4.3 cc. of bromobenzene. Part of the ether was removed by distillation and the remaining solution was refluxed for four hours. After treatment with ice and dilute hydrochloric acid, the ether–toluene layer was washed with water and subjected to steam distillation to remove the solvents. The residue was stirred with successive small portions of petroleum ether to yield an amorphous solid which was dissolved in carbon tetrachloride and chromatographed on a 1.1 × 8.5 cm. column of alumina. When the column was eluted with carbon tetrachloride and carbon tetrachloride–ether mixtures, some of the latter fractions yielded crystalline material; in all, there was obtained 0.13 g. of product which crystallized in fine needles from moist carbon tetrachloride–hexane and melted with effervescence at 112–120°.

Anal. Calcd. for $C_{33}H_{38}O_2 \cdot {}^1/_2H_2O$: C, 83.33; H, 8.27. Found: C, 83.66; H, 8.27.

In subsequent experiments it was possible to induce crystallization in the amorphous, petroleum ether-insoluble material by seeding with the above hydrate; the yield of the diphenylethanol was not increased.

1,1-Diphenyl-2-methyl-2-(3(β)-acetoxy-5,7,9-estratrien-17-yl)-ethylene (VII).—The acetylation of 0.20 g. of the diphenylethanol hydrate with acetic anhydride in pyridine yielded a non-crystalline product which was refluxed for one hour in 4 cc. of acetic acid containing 0.1 cc. of acetic anhydride. Upon the addition of water to the warm acetic acid solution, a solid (0.20 g.) separated in leaves melting at 189–193°. The recrystallization of this material from aqueous acetic acid and from ether raised the melting point to 197–201°; $[\alpha]^{19}\text{D}$ +171° (c = 1.66 in chloroform).

Anal. Calcd. for $C_{35}H_{38}O_2$: C, 85.67; H, 7.81. Found: C, 85.66; H, 7.78.

In an effort to improve the yield of the acetoxy diphenylethylene from the esters III and IV, the crude amorphous product from the Grignard reaction was acetylated and subjected to dehydration; no significant increase in the yield (16%) of VII was attained.

1,1,2-Trimethyl-2-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-ethanol-1 (VI).—A solution of one gram of the methyl ester III in 75 cc. of warm toluene was added to the Grignard reagent prepared from 2.0 g. of magnesium and 5.3 cc. of methyl iodide in 50 cc. of ether. After part of the ether had been distilled the remaining solution was refluxed for five hours and then treated with ice and acid; the product, which was obtained from this solution as a white granular solid by the steam distillation of the ether and toluene, separated from ether as a micro-crystalline powder (0.80 g.) melting at 179–183°; $[\alpha]^{19}_{\rm D}$ −27° (c = 1.41 in chloroform).

Anal. Calcd for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.78; H, 9.94.

The **3(β)-monoacetate VIa** was formed by warming (60°) a solution of 0.80 g of the trimethylethanol in 8 cc. of pyridine and 5 cc. of acetic anhydride and then allowing it to stand for several hours at room temperature. The product, m. p. 122–126°, which was obtained by diluting the reaction mixture with water was recrystallized from aqueous methanol and formed leaves which melted at 127–130°

Anal. Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.06; H, 9.11.

2-Methyl-3-(3(β)-acetoxy-5,7,9-estratrien-17-yl)-butene-1 (VIII).—Two hundred milligrams of the monoacetate VIa in 3 cc. of acetic acid containing 0.1 cc. of acetic anhydride was heated in a sealed tube at 150–155° for one and one-half hours. When the warm solution was diluted with water there precipitated 0.18 g. of solid material which crystallized from methanol in leaves m. p. 133.5–136°; the melting point was raised by recrystallization and drying to 135–136°; $[\alpha]^{19}_{\rm D}$ −14° (c = 1.23 in chloroform).

Anal. Calcd. for $C_{25}H_{34}O_2$: C, 81.92; H, 9.35. Found: C, 81.94; H, 9.27.

The dehydration of the monoacetate in boiling acetic acid containing acetic anhydride, fused zinc chloride, or fused oxalic acid did not proceed as satisfactorily as by the above method. The acetoxy methylbutene formed in these preliminary experiments was saponified and isolated as a sparingly soluble **3,5-dinitrobenzoate VIIIa** which crystallized from methanol–chloroform in small, bright-orange leaves, m. p. 252–255° dec. The sample for analysis was dried at 136° *in vacuo*.

Anal. Calcd. for $C_{30}H_{34}O_6N_2$: C, 69.48; H, 6.61. Found: C, 69.41; H, 6.63.

3-(3(β)-Hydroxy-5,7,9-estratrien-17-yl)-butanone-2 (IX).—A solution of 1.12 g. of the acetoxy methylbutene and 1 g. of osmium tetroxide in 50 cc. of dry ether was allowed to stand at room temperature for three days. The residue remaining after removal of the ether was refluxed with aqueous alcoholic sodium sulfite according to the usual procedure[4a] and the resulting solid was dried and extracted thoroughly with a 5:1 ether–methanol mixture. The extract was concentrated and oxidized with per-iodic acid as in the preparation of the hydroxy acid II. The ether solution was washed and concentrated whereupon the product crystallized in leaves, m. p. 174–178°; a second crop from ether–hexane melted at 172–177°. The combined material (0.65 g.) was saponified and crystallized repeatedly from aqueous acetone to yield 0.56 g. of rectangular plates, m. p. 177–181°; $[\alpha]^{19}_{\rm D}$ −22° (c = 1.44 in chloroform).

Anal. Calcd. for $C_{22}H_{30}O_2$: C, 80.94; H, 9.26. Found: C, 81.08, 80.97; H, 9.33, 9.38.

The **acetate IXa** was formed by warming a solution of 0.10 g. of the hydroxy ketone in 2 cc. of pyridine and 1 cc. of acetic anhydride. The product crystallized from aqueous methanol in leaves, m. p. 148–152°.

Anal. Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75. Found: C, 78.00; H, 8.71.

Conversion of the Hydroxy Ketone IX to the Trimethyl Ethanol VI.—A solution of 0.56 g. of the hydroxy ketone in 40 cc. of toluene was added to an excess of methylmagnesium iodide in ether. The intermediate addition compound separated rapidly and complete solution was not achieved by adding 150 cc. of ether and 75 cc. of toluene and refluxing the suspension for six hours. The reaction mixture was manipulated in the usual way and the solid product (0.55 g.) was recrystallized from ether to yield material which possessed the same rotation and analysis as the trimethylethanol VI and which showed no depression in melting point when mixed with an authentic sample of that substance. The material from the Grignard reaction, when acetylated and subjected to dehydration as already described, formed a product, m. p. 132.5–136°, which was identical with the acetoxy methylbutene VIII as indicated by analysis and mixed melting point. Moreover, the above product (m. p. 132.5–136°) was oxidized with osmium tetroxide and per-iodic acid to regenerate the original hydroxy ketone IX as shown by mixed melting point determinations with both the oxidation product and its acetate.

Summary

The per-iodic acid oxidation of the intermediate glycols obtained by the osmium tetroxide hydroxylation of neoergosteryl acetate has been shown to form α-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-propionic acid in a much higher yield (55%) than that (8%) obtained when neoergosteryl acetate is oxidized by the ozonolysis procedure. The methyl ester acetate of the acid, on treatment with phenylmagnesium bromide, formed 1,1-diphenyl-2-methyl-2-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-ethanol-1 which was dehydrated to the expected diphenylethylene. The corresponding 1,1,2-trimethylethanol, however, was dehydrated to yield a methylbutene through loss of water involving the methyl groups. The validity of the structure assigned to the methylbutene has been demonstrated through its oxidation to 3-(3(β)-hydroxy-5,7,9-estratrien-17-yl)-butanone-2 and by the conversion of the latter compound to the parent trimethylethanol and its derivatives. The preparation and properties of these and other new compounds are described.

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