[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

STUDIES ON THE CONVERSION OF ERGOSTEROL TO ADRENAL CORTICAL HORMONES[1]

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In 1942 investigations were begun in this laboratory which aimed at a partial synthesis of adrenal cortical hormones from ergosterol (Ia). At that time this sterol appeared to be an attractive starting material because of the comparative ease with which it may be converted to derivatives like dehydroergosterol (IIa) (1) which possess unsaturation at C-11 and which might therefore lend themselves to the introduction of oxygen at this point. In addition the 22,23-double bond was expected to facilitate removal of the side chain to permit its replacement by one of the typical side chains of adrenal cortical hormones. Finally the 5,6-double bond, in case it could be retained during the contemplated series of reactions, should simplify the conversion of one of the final products to the 4,5-unsaturated ketone.

The present paper deals with some of the earlier exploratory steps taken towards the possible solution of the problem. The first concerned itself with the degradation of the side chain of ergosterol (Ia) under conditions which left intact the system of conjugated double bonds in ring B, which was to serve as a starting point for further alterations of the molecule. To protect this system, ergosteryl acetate (Ib) was converted into its maleic anhydride addition product by the method of Inhoffen (2). The adduct (III) was then subjected to various types of oxidation in an effort to bring about complete elimination of the side chain. When these attempts met with little tangible success, the action of ozone upon the adduct was investigated. Substantial attack of this reagent upon the 6,7-double bond of the adduct was not anticipated, since its relative inertness had already been demonstrated by Inhoffen (2), and since it was shown in this laboratory that even in the presence of a large excess of perbenzoic acid the adduct reacts to give only the 22,23-epoxide (IV).

Ozonization of the adduct (III) gave a satisfactory yield of solid material which at first was immediately oxidized to an acid which was isolated in the form of its methyl ester, m.p. 272°; $[\alpha]_D^{25}$ −10.8°. The observed analytical values for this derivative, and the fact that it gave off maleic anhydride upon heating, suggested its identity with the maleic anhydride adduct of methyl 3(β)-acetoxybisnor-5,7-choladienate (VI b). This was substantiated by the hydrolysis of the acid (VI a) to the tricarboxylic acid (VII a) and the conversion of the latter to the trimethyl ester (VII b), m.p. 184–185°; $[\alpha]_D^{25}$ −30°, and reconversion to the 3(β)-acetoxy-monocarboxylic acid (VI a), m.p. 260°. Heating of the methyl ester (VI b) *in*

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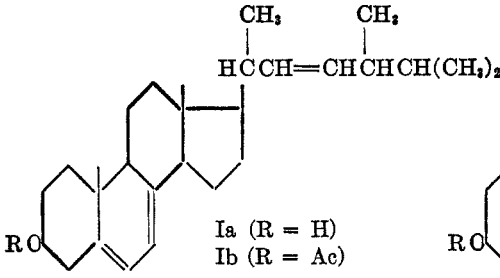
vacuo afforded maleic anhydride and an oil from which the desired methyl 3(β)-acetoxybisnor-5,7-choladienate (VIII b), m.p. 147°; $[\alpha]_D^{25}$ −83°, was eventually obtained in a yield of thirty per cent. The new ester shows the typical absorption spectrum of 5:6,7:8-unsaturated steroids, and its observed rotation agrees well within the limits of experimental error with the value calculated on the basis of Barton's method of comparing molecular rotation differences (3). Like the esters of other bisnoracids, the present ester is difficult to hydrolyze and reacts with alkali under moderate conditions only with hydrolysis of the 3(β)-acetoxy group to afford methyl 3(β)-hydroxybisnor-5,7-choladienate (VIII a), m.p. 163–165°; $[\alpha]_D^{25}$ −120°. Catalytic hydrogenation of the ester (VIII b) in a neutral medium gives a dihydro derivative, m.p. 136°; $[\alpha]_D^{25}$ −4.2°, which according to the work of Wieland (4) and Barton (3) is to be formulated as methyl 3(β)-acetoxybisnor-7-cholenate (IX).

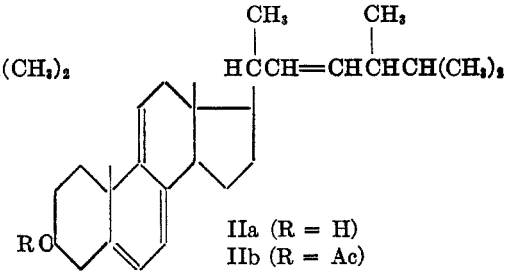
In subsequent studies it was observed that reduction of the ozonide of the adduct (III) afforded in a yield of seventy-five per cent the nicely crystalline aldehyde (V), m.p. 206–208°; $[\alpha]_D^{25}$ −16.4°. The identity of the aldehyde was established through the ready formation of a 2,4-dinitrophenylhydrazone, m.p. 246°. Later investigations, to be published elsewhere, have shown that by analogous methods other sterols, unsaturated in the side chain, may also be converted in good yields into the aldehydes, which should prove to be valuable intermediates in the partial synthesis of sterols with diverse side chains. Because of its greater reactivity, the aldehyde (V) is a more suitable intermediate for a further degradation of the side chain than the methyl ester (VIII b). It was at first attempted to convert the aldehyde (V) into the methyl ketone (XIII) by way of α-bromination, replacement of the bromine by hydroxyl, and oxidation of the ensuing hydroxy aldehyde. The first step in the contemplated series of reactions proved successful, and there was obtained a nicely crystalline monobromide (X), m.p. 180°. Difficulties, however, were encountered in completing the contemplated conversion. Further investigations along these lines were eventually temporarily discontinued in favor of a more promising approach based on the degradation of the enol acetate of the aldehyde. This derivative (XI or XII), m.p. 189–190°, is readily obtained by heating the aldehyde (V) with acetic anhydride in a sealed tube. It was expected that ozonolysis of this derivative would yield the desired methyl ketone (XIII). The actual reaction product, m.p. 287°, gave analytical data contra-indicating its identity with the expected product (XIII), and in agreement with those calculated for the 17-ketone (XIV). Unfortunately the splitting of the adduct, a prerequisite for the final proof of its structure has not yet given identifiable products, with the exception of some maleic anhydride. If subsequent studies should prove structure XIV to be that of the product of ozonolysis, it must be assumed that the acetylation of the aldehyde (V) is accompanied by a migration of a double bond to afford the diacetate (XII). An analogous sequence of reactions is now being studied in this laboratory on steroids, unsaturated in the side chain, which do not require protection by maleic anhydride.

The next exploratory step in the direction of a partial synthesis of adrenal cor-

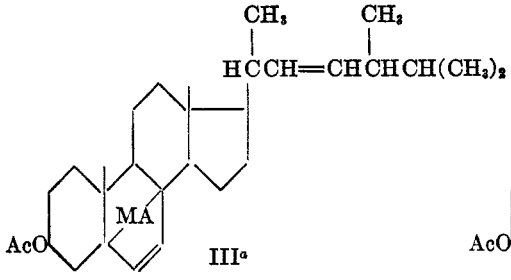
tical hormones from ergosterol dealt with the preferential addition of oxygen to the 9,11-double bond of dehydroergosteryl acetate (II b), and the degradation



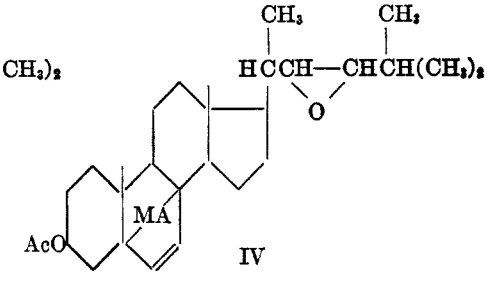
Ia (R = H)
Ib (R = Ac)



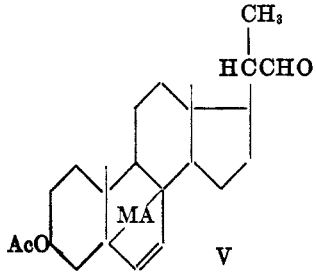
IIa (R = H)
IIb (R = Ac)



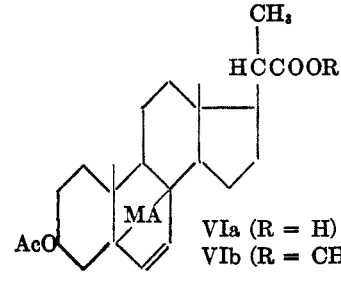
III[a]



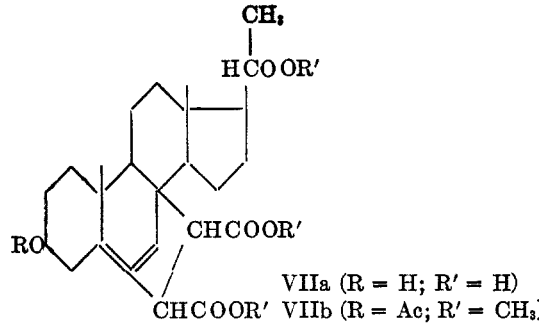
IV



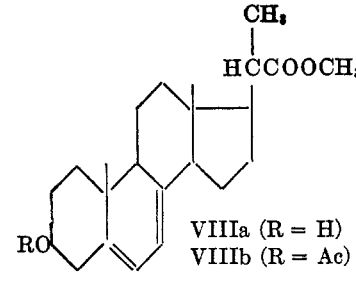
V



VIa (R = H)
VIb (R = CH₃)



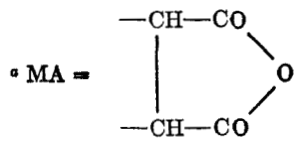
VIIa (R = H; R′ = H)
VIIb (R = Ac; R′ = CH₃)

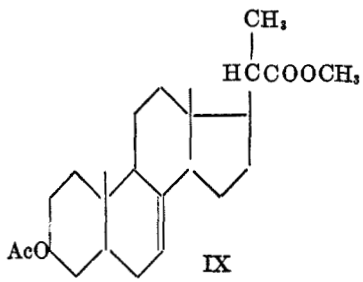


VIIIa (R = H)
VIIIb (R = Ac)

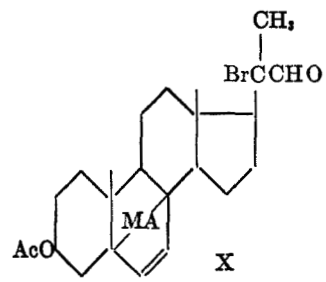
of the side chain of the resulting product. Dehydroergosteryl acetate (II b) was prepared from ergosteryl acetate (I B) by a modification of Windaus' method (1) which raised the yield to about fifty per cent. The reactive system in ring B was

then protected by the addition of maleic anhydride (5). Of the three double bonds of the adduct (XV), the one at 6,7 is as unreactive as the corresponding bond of the ergosterol adduct (III), and the one at 22,23 is more reactive than that at 9,11. In order to facilitate preferential addition of oxygen to the 9,11-

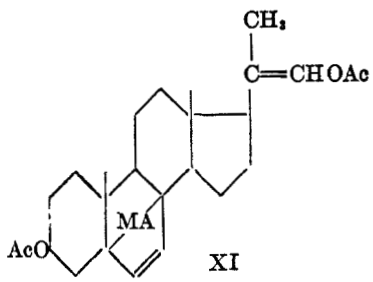




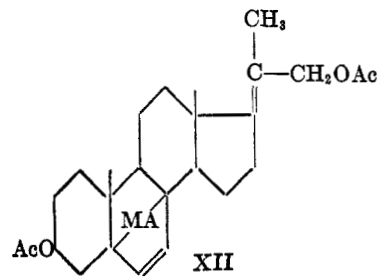
IX



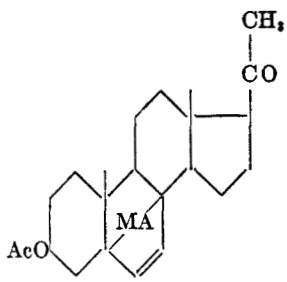
X



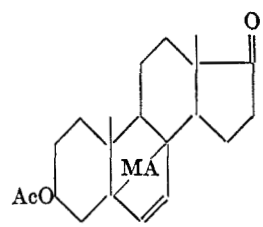
XI



XII



XIII

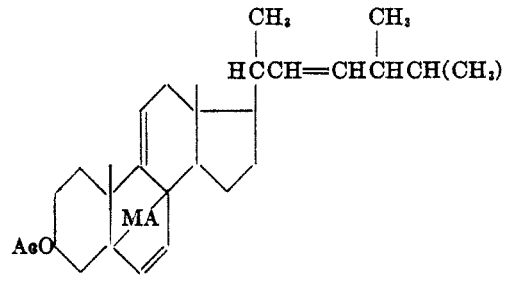


XIV

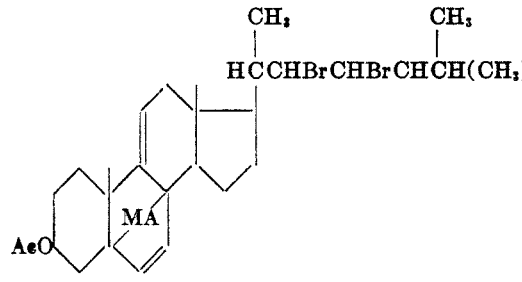
double bond, and to retain the 22,23-double bond for degradation of the side chain, the adduct was first converted to the known 22,23-dibromide (XVI) (5). Treatment of this bromide with perbenzoic acid readily afforded the 9,11-epoxide (XVII), m.p. 216–217°, which upon debromination with zinc in glacial acetic acid gave the desired maleic anhydride adduct of 9,11-oxidoergosteryl acetate, m.p. 220–221° (XVIII). Ozonolysis of this compound followed by oxi-

dation of the resulting aldehyde furnished the bisnor acid, which was isolated in the form of its methyl ester, m.p. 270–271° (XIX).

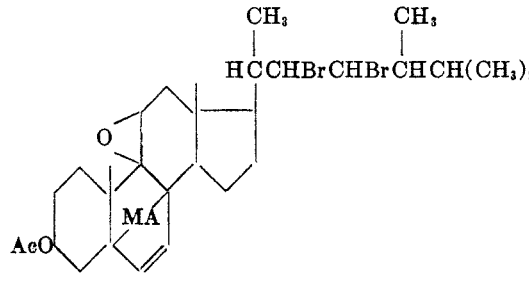
It was to be expected on the basis of the experience described above, that the pyrolytic elimination of maleic anhydride from (XIX) would give unsatisfactory results. In order to preserve the valuable supply of ester, the pyrolysis of the



XV



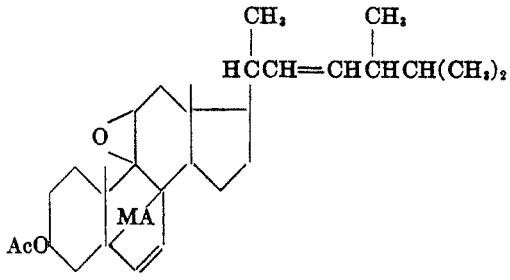
XVI



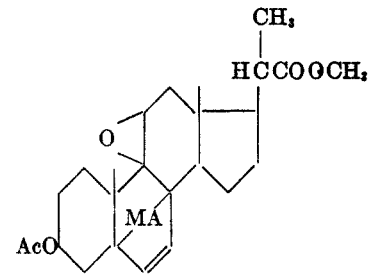
XVII

adduct of 9,11-oxidoergosteryl acetate (XVIII) was therefore first investigated. It afforded a volatile anhydride and an oil which after prolonged treatment with methanol eventually gave a crystalline product, m.p. 137–139°. The analytical values of this product indicated that the pyrolysis had been accompanied by a dehydrogenation to the extent of two atoms. The ease with which the product reacted with 2,4-dinitrophenylhydrazine suggested not only that the 9,11-epoxide has rearranged to an 11-ketone, but also that this ketone group is not sufficiently hindered to be unresponsive to the action of carbonyl reagents. The

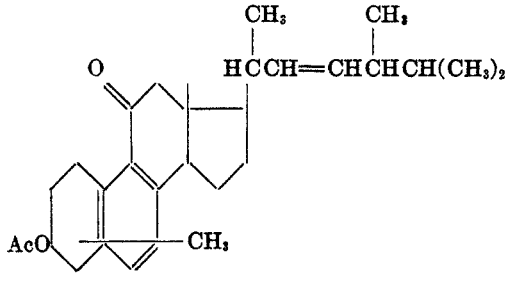
liberation of reactivity at C-11 makes it apparent that a radical structural change has taken place during the pyrolysis, such as is brought about for example by an aromatization of ring B. This view is supported by the absorption spectrum of the product, which with its maxima at mμ 257 (Log. E, 3.96) and mμ 305 (Log



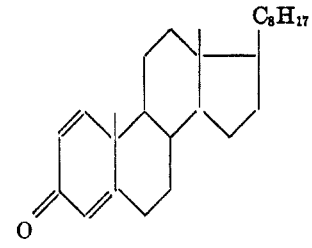
XVIII



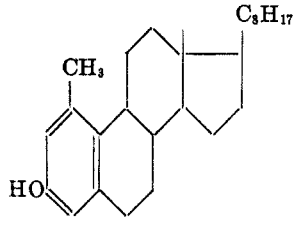
XIX



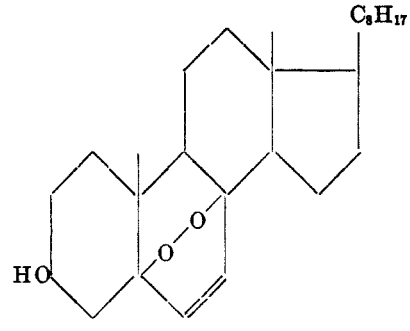
XX



XXI



XXII



XXIII

E, 3.37) shows resemblance to that of α-tetralone. The best known case in the steroid series of aromatization of ring B is the formation of neoergosterol from bisergostatrienol (6, 7). Here aromatization is brought about by the loss of the angular methyl group in form of methane. The present reaction, however, must take a different course, because the loss of a carbon atom is contra-indicated not only by the analytical values for the reaction product, but also by the fact that no evolution of methane took place during the pyrolysis. It appears at present

most likely that aromatization was brought about by dehydrogenation, accompanied by a migration of the angular methyl group to a position as yet unknown (XX). The occurrence in the steroid series of such migration of angular methyl groups has first been demonstrated by Inhoffen (8, 9) by the rearrangement of the ketone (XXI) to the phenol (XXII).

The results of the exploratory studies outlined above demonstrate the feasibility of a side chain degradation of ergosterol and the introduction of oxygen at C-11 of this sterol. They are as yet, however, of little practical value. The principal disadvantage of the present method rests on the fact that the elimination of maleic anhydride from the oxidation and degradation products entails most serious losses. In the case of esters, the yield of desired material rarely exceeds thirty per cent, and in the case of carbonyl compounds it becomes almost inconsequential. Attempts to bring about fission of the adducts by less vigorous methods, such as heating them with large excesses of butadiene or cyclopentadiene have so far been unsuccessful. Substitution of the maleic anhydride adducts by the transannular peroxides of ergosterol (XXIII) (10) and dehydroergosterol (1) in the oxidation and degradation experiments has also been investigated. Because of the relatively high reactivity of the 6,7-double bond of these peroxides towards oxidizing agents and ozone, these studies have so far met with little success.

EXPERIMENTAL[3]

All melting points are corrected. All rotations were taken in chloroform at concentrations of about one per cent.

Maleic anhydride adduct of 22,23-oxidoergosteryl acetate (IV). A solution of ergosteryl acetate maleic anhydride adduct (III) in chloroform was treated with four equivalents of perbenzoic acid dissolved in chloroform. After forty-eight hours the solution was extracted with an aqueous sodium bicarbonate solution, washed with water, dried over sodium sulfate and evaporated to dryness. After several recrystallizations from ethyl acetate and methanol the oxide gave diamond-like crystals of m.p. 229–231°.

Anal. Calc'd for $C_{32}H_{48}O_6$: C, 73.9; H, 8.75.
Found: C, 73.8; H, 9.0.

Maleic anhydride adduct of 3(β)-acetoxybisnor-5,7-choladien-22-al. (V). A stream of 9% ozone was passed through a vigorously stirred suspension of 5.36 g. of finely ground ergosteryl acetate-maleic anhydride adduct, (III) in 120 cc. of glacial acetic acid until a clear solution had been obtained. During the reaction the temperature was kept at 20°. After five additional minutes the ozonization was discontinued, and the temperature of the solution was reduced to 15°. With stirring, 8 g. of zinc dust was then gradually added, and after 15 minutes 1 cc. of a 1% solution of silver nitrate. The stirring was continued until the solution gave a negative test with starch-iodide paper, which generally required about one hour. The unreacted zinc dust was then filtered by suction and washed with 100 cc. of glacial acetic acid. The combined filtrate and washing were poured into one liter of water, and the amorphous, white precipitate which immediately formed was filtered, washed with water and dried *in vacuo* at 2 mm. over potassium hydroxide. Addition of petroleum ether to a solution of the aldehyde in ethyl acetate afforded a nicely crystalline precipitate, which after two recrystalizations from the same combination of solvents melted at 206–208°, $[\alpha]_D^{25}$ −15.4°; yield 75%.

[3] In cooperation with J. A. Klacsmann.

Anal. Calc'd for $C_{28}H_{36}O_6$: C, 71.8; H, 7.75.
Found: C, 71.8; H, 8.0.

2,4-Dinitrophenylhydrazone of (*V*). An excess of a 1% solution of 2,4-dinitrophenylhydrazine in ethanol containing 1% of hydrochloric acid was added to a 1% solution of the aldehyde (V) in ethyl acetate. The hydrazone, which formed instantaneously, was recrystallized several times from ethyl acetate; m.p. 246°.

Anal. Calc'd for $C_{34}H_{40}N_4O_9$: C, 63.0; H, 6.2.
Found: C, 63.0; H, 6.3.

Maleic anhydride adduct of methyl 3(β)-acetoxybisnor-5,7-choladienate (*VI b*). To a solution of 4.4 g. of the crude, amorphous aldehyde (V) in 50 cc. of glacial acetic acid was added dropwise over a period of forty-five minutes a solution of 2 g. of chromic acid anhydride in the minimum amount of water and 5 cc. of glacial acetic acid. During the reaction the temperature was kept below 20°. After four hours the excess reagent was reduced by sodium bisulfite, and the mixture poured into 1 *N* sulfuric acid. The amorphous precipitate was filtered, washed with water, and dried *in vacuo;* yield 85–88%. The crude acid (VI a), 4.1 g., was dissolved in 200 ml. of ether and some insoluble, greenish material removed by centrifugation. To the clear solution was then added diazomethane, dissolved in ether, until a yellow color persisted for at least ten minutes after the last addition. During the reaction the sparingly soluble ester began to crystallize. It was recrystallized several times from glacial acetic acid; m.p. 272°; $[\alpha]_D^{25}$ −10.8°; yield 85%.

Anal. Calc'd for $C_{29}H_{38}O_7$: C, 69.8; H, 7.7; CH_3O, 6.2; CH_3CO, 8.6.
Found: C, 69.2; H, 7.5; CH_3O, 6.7; CH_3CO, 8.1.

Maleic anhydride adduct of 3(β)-hydroxybisnor-5,7-choladienic acid (*VII a*). An ether solution of the crude monocarboxylic acid described above was thoroughly shaken with 4 *N* sodium hydroxide. This treatment brings about an opening of the anhydride ring and hydrolysis of the 3-acetoxy group. Acidification of the alkaline layer afforded the tricarboxylic acid (VII a), which after several recrystallizations from dilute acetic acid melted with decomposition at 277–281°. Upon drying *in vacuo* at 100° the acid forms the anhydride.

Anal. Calc'd for $C_{26}H_{34}O_6$: C, 70.6; H, 7.7.
Found: C, 70.4; H, 7.7.

Trimethyl ester of the maleic acid adduct of 3(β)-acetoxybisnor-5,7-choladienic acid (*VII b*). An excess of diazomethane dissolved in ether was added to a methanol solution of the tricarboxylic acid (VII a) described above. After several hours the solvent and excess diazomethane were removed *in vacuo* and the residual oil was refluxed with acetic anhydride for forty minutes. Upon cooling, the ester separated in nice, hexagonal prisms. After two recrystallizations from acetic anhydride it melted at 184–185°; $[\alpha]_D^{25}$ −30.0°; yield 50%.

Anal. Calc'd for $C_{31}H_{44}O_8$: C, 68.4; H, 8.1; CH_3O, 17.1.
Found: C, 67.6; H, 8.3; CH_3O, 16.2.

Maleic anhydride adduct of 3(β)-acetoxybisnor-5,7-choladienic acid (*VI a*). The crude tricarboxylic acid (VII a) described above was refluxed for twenty minutes with acetic anhydride, and the crystalline material which separated upon cooling was recrystallized from the same solvent; m.p. 260°.

Anal. Calc'd for $C_{28}H_{36}O_7$: C, 69.4; H, 7.5.
Found: C, 69.2; H, 7.7.

Methyl 3(β)-acetoxybisnor-5,7-choladienate (*VIII b*). One-gram samples of the adduct (VI b) were heated in a small retort at 20–30 mm. and 280° for twenty-five minutes. The maleic anhydride which had distilled over was then removed, and the heating of the retort continued at 230–250° and 0.001 mm. There was obtained an oily distillate which upon digestion with methanol became crystalline. Numerous recrystallizations from methanol eventually gave the ester in the form of nice plates, m.p. 147°; $[\alpha]_D^{25}$ −83°; yield 30%.

Anal. Calc'd for $C_{25}H_{36}O_4$: C, 74.8; H, 9.6; CH_3O, 7.7.
Found: C, 74.7; H, 8.9; CH_3O, 7.8.

Methyl 3(β)-hydroxybisnor-5,7-choladienate (*VIII a*). To a warm methanol solution of

the methyl ester (VIII b) was added 6 cc. of a 1% solution of potassium hydroxide in methanol. The mixture was kept in the dark for twelve hours, and the long, spike-like crystals, which had separated were filtered, and recrystallized from methanol; m.p. 163–165°; $[\alpha]_D^{25}$ −120°; yield 72%.

Anal. Calc'd for $C_{23}H_{34}O_3 \cdot H_2O$. C, 73.4; H, 9.5.
Found: C, 73.4; H, 9.1.

Methyl 3(β)-acetoxybisnor-7-cholenate (IX). A 5% solution of the acetoxy ester (VIII b) in neutral ethyl acetate was shaken with hydrogen and a platinum black catalyst at room temperature. Hydrogen uptake ceased after slightly more than one equivalent of hydrogen had been consumed. The filtered solution was evaporated to dryness, and the residue recrystallized several times from methanol; m.p. 136°; $[\alpha]_D^{25}$ −4.2°; yield 70%.

Anal. Calc'd for $C_{25}H_{36}O_4$: C, 75.0; H, 9.1.
Found C, 74.8; H, 9.2.

Maleic anhydride adduct of 3(β)-acetoxy-20-bromobisnor-5,7-choladien-22-al (X). A 1% solution of bromine in glacial acetic acid was gradually added to a solution of 0.52 g. of the aldehyde (V) in 6 cc. of glacial acetic acid. In the presence of sunlight, decolorization took place rapidly under formation of hydrogen bromide until about one equivalent of bromine had been consumed. The solution was then evaporated to dryness *in vacuo*, and the residual oil dissolved in ethyl acetate. Upon addition of petroleum ether a nicely crystalline precipitate was formed, which after recrystallization from ethyl acetate-petroleum ether melted at 180°.

Anal. Calc'd for $C_{28}H_{35}BrO_6$: C, 61.4; H, 6.4; Br, 14.6.
Found: C, 61.9; H, 6.6; Br, 14.9.

Enol acetate of 3(β)-acetoxybisnor-5,7-choladien-22-al (XI) or (XII). A mixture of 0.51 g. of the aldehyde (V), 0.2 g. of freshly fused sodium acetate and 10 cc. of acetic anhydride was heated in a nitrogen-filled, sealed tube for five hours at 175°. The content of the tube was then washed with acetic anhydride into a distilling flask and evaporated to dryness *in vacuo*. The residue was then thoroughly extracted with ether, the extract evaporated, and the product dissolved in ethyl acetate. Upon addition of petroleum ether, the enol acetate crystallized in needle-like prisms. It was recrystallized from a mixture of ethyl acetate and petroleum ether; m.p. 189–190°; yield 60%.

Anal. Calc'd for $C_{30}H_{38}O_7$: C, 70.6; H, 7.5.
Found: C, 70.6; H, 7.5.

Ozonization of the enol acetate. A stream of ozone was passed through a vigorously stirred suspension of 0.26 g. of finely ground enol acetate in 3.5 cc. of glacial acetic acid at 20°. The ozonization was discontinued five minutes after all material had become dissolved, and zinc dust and a drop of silver nitrate were added. The stirring was continued until the mixture no longer gave a positive test with starch-iodide paper. The filtered solution was then poured into water and the precipitate collected, washed, and dried (0.23 g.). It was dissolved in 7 cc. of acetic acid, and the solution treated with 0.1 g. of chromic acid anhydride. After three hours the excess reagent was reduced with methanol, and the reaction product precipitated with water. It was dissolved in ether, and the solution washed with dilute sodium carbonate. Evaporation of the ether gave a neutral residue which after recrystallization from acetic acid gave clusters of rectangular plates, m.p. 287°.

Anal. Calc'd for $C_{25}H_{30}O_6$: C, 70.4; H, 7.0.
Found: C, 70.3; H, 7.0.

Dehydroergosteryl acetate (II b). A solution of 325 g. of mercuric acetate in 5.2 liters of glacial acetic acid was added to 200 g. of ergosteryl acetate dissolved in 2.8 liters of chloroform, and the mixture shaken for eighteen hours. The mercurous acetate was then removed and washed with ether, and the combined filtrate and washings were concentrated *in vacuo* at a temperature not exceeding 45°. The crystalline precipitate which formed was collected and washed with acetic acid and methanol. Concentration of the mother liquor gave a second fraction. The fractions were combined, dissolved in ether, and the solution was filtered and concentrated until crystals began to separate. One liter of ethanol was then

added and the mixture heated until most of the ether had evaporated. The crystalline material which separated upon cooling weighed 86.3 g., m.p. 143–146°; yield 43%. Concentration of the mother liquor gave an additional crop of 9 g. of somewhat less pure material.

Dehydroergosteryl acetate-maleic anhydride adduct (XV). A solution of 86.3 g. of dehydroergosteryl acetate and 65.5 g. of maleic anhydride in 225 cc. of benzene was refluxed for four hours. The benzene and excess maleic anhydride were then removed *in vacuo* at a temperature not exceeding 100°. The residue was digested with a small amount of ether and dried; yield 82.2 g. or 78%. After recrystallization from acetic acid the adduct melted at 228–229°. The m.p. reported by Honigmann (5) is 220–240°.

22,23-Dibromodehydroergosteryl acetate-maleic anhydride adduct (XVI) (5). A cooled solution of 11.5 g. of bromine in 25 cc. of chloroform was slowly added to an ice-cold solution of 38.4 g. of dehydroergosteryl acetate-maleic anhydride adduct in 120 cc. of chloroform. The solvent was then removed *in vacuo* and the residue recrystallized from a mixture of acetone and methanol; m.p. 235–236°, $[\alpha]_D^{25}$ +68.8°; yield 36.4 g. or 73%.

Anal. Calc'd for $C_{34}H_{46}Br_2O_6$: Br, 23.0. Found: Br, 23.0.

22,23-Dibromo-9,11-oxidodehydroergosteryl acetate-maleic anhydride adduct (XVII). A solution of 9.54 g. of the dibromide (XVI) in 100 cc. of chloroform was treated with 73 cc. of a 0.252 *N* solution of perbenzoic acid in chloroform. The solution was kept at 4° for six days, and was then washed with 2% sodium hydroxide and water, dried over potassium carbonate and evaporated to dryness. The residue was dissolved in acetone and the solution freed from some insoluble material by centrifugation. It was concentrated *in vacuo* until crystallization began, which was brought to completion by addition of methanol. The oxide was then recrystallized from a mixture of acetone and methanol; m.p. 216–217°; yield 7.6 g. or 80%.

Anal. Calc'd for $C_{34}H_{46}Br_2O_6$: Br, 22.5. Found: Br, 22.5.

9,11-Oxidodehydroergosteryl acetate-maleic anhydride adduct (XVIII). A total of 112 g. of zinc dust was added in small portions to a vigorously stirred solution of 5.6 g. of the dibromo oxide (XVII) in 400 cc. of warm glacial acetic acid. The reaction temperature was kept at 105–110°. After five hours, the hot solution was filtered and the zinc dust was washed thoroughly with warm acetic acid. The combined filtrate and washings were poured into water, and the precipitate was filtered and recrystallized several times from acetic acid; m.p. 220–221°; yield 2.9 g. or 70%.

Anal. Calc'd for $C_{34}H_{46}O_6$: C, 74.2; H, 8.4.
Found: C, 74.5; H, 8.7.

Maleic anhydride adduct of methyl 3(β)-acetoxy-9,11-oxidobisnor-5,7-choladienate (XIX). A rapid stream of ozone (9%) was passed through a vigorously stirred suspension 1.1 g. of the 9,11-oxide (XVIII) in 25 cc. of acetic acid. After about twenty minutes, when all material had gone into solution, ozonization was discontinued and zinc dust and a drop of silver nitrate solution were added with stirring. When the solution no longer gave a positive test with starch-iodide paper it was filtered, and the filtrate poured into water. The amorphous material was collected, washed with water and dried. It readily reacted with 2,4-dinitrophenylhydrazine. The product was dissolved in 20 cc. of glacial acetic acid and the solution treated with 0.04 g. of chromic acid anhydride over a period of three hours. The excess reagent was then reduced by the addition of sodium bisulfite, and the solution poured into water. The precipitate was collected, washed with water, dried, and dissolved in ether. The filtered solution was then treated with an excess of diazomethane. The ester crystallized at once in form of glittering prisms. It was recrystallized from acetic acid; m.p. 270–271°; yield 42%.

Anal. Calc'd for $C_{29}H_{38}O_8$: C, 67.7; H, 7.4; CH_3O, 6.0.
Found: C, 67.6; H, 7.2; CH_3O, 6.4.

Pyrolysis of the maleic anhydride adduct of 9,11-oxidoergosteryl acetate (XVIII). Small portions of the adduct were heated in a retort at 10 mm. pressure to 275–285° for from fifteen to twenty minutes until the anhydride had ceased to come over. The retort was then cooled

to 200° and the pressure reduced to 0.001 mm. An oily distillate was obtained, which after prolonged digestion with small amounts of methanol began to crystallize. After repeated recrystallizations from methanol the substance was obtained in form of well defined prisms; m.p. 137–139°; yield 15–30%.

Anal. Calc'd for $C_{30}H_{42}O_3$: C, 80.0; H, 9.4.
$C_{30}H_{44}O_3$: C, 79.6; H, 9.8.
Found: C, 79.9; H, 9.3.

The compound readily reacts with 2,4-dinitrophenylhydrazine. Absorption spectrum: maxima at 257 mμ (Log. E, 3.96) and 305 mμ (Log. E, 3.37).

SUMMARY

The degradation of ergosterol to derivatives of 3(β)-hydroxybisnor-5,7-choladienic acid, 3(β)-hydroxybisnor-5,7-choladiene-22-al and 3(β)-hydroxy-9,11-oxidobisnor-5,7-choladienic acid has been described.

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