[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

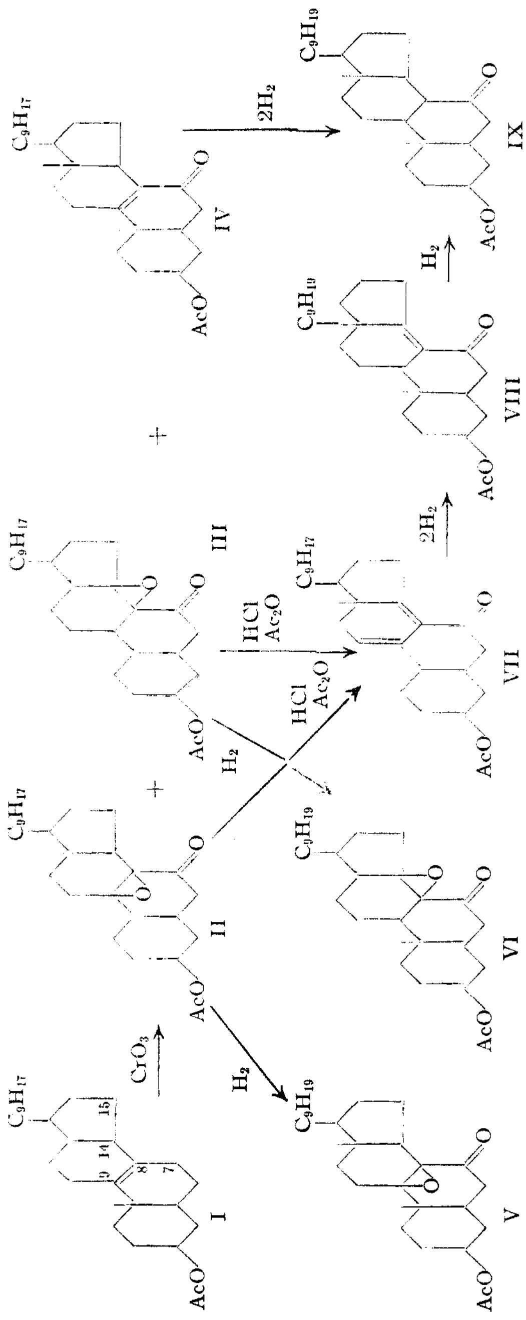
Steroids with Double Bonds between Quaternary Carbon Atoms. II. The Structure of α-Dihydroergosterol

BY HOMER E. STAVELY AND G. NORRIS BOLLENBACK

α-Dihydroergosterol is the product obtained when ergosterol is reduced with sodium and alcohol,[1] or catalytically with one mole of hydrogen.[2] Since 22-dihydroergosterol[3] and 7-dehydrocholesterol[4] both yield the $\gamma(\Delta^{7,8})$-stenol when reduced with sodium and alcohol the nuclear double bond in α-dihydroergosterol might be expected also to be in the 7,8 position. On the other hand, in all other known cases the 7,8 double bond is shifted in the presence of hydrogen and palladium or platinum catalyst to the 8,14 position without being reduced, and since α-dihydroergosterol can be prepared by catalytic hydrogenation it seems unlikely that the product would retain the 7,8 double bond. This dilemma has never been satisfactorily resolved, but it has been generally assumed that α-dihydroergosterol is $\Delta^{8,14;22,23}$-ergostadienol-3.

The problem is complicated by the fact that catalytic isomerization, the method usually used for distinguishing between the $\gamma(\Delta^{7,8})$ and the $\delta(\Delta^{8,9})$ double bonds on the one hand, and the $\alpha(\Delta^{8,14})$ on the other, cannot be applied because of the presence of a reducible double bond in the side chain. The reaction product will be α-ergostenol in any case.[5] As far as the available evidence goes, neither the 7,8 or 8,9 position for the nuclear double bond in α-dihydroergosterol is rigidly excluded. Since the mild oxidation of α-ergostenyl acetate[6] gave products characteristic for a steroid with an 8,14 double bond, a similar treatment of α-dihydroergosterol could be expected to indicate at least its presence or absence. The results obtained from the oxidation led us to the conclusion that the nuclear double bond in α-dihydroergosterol lies between the quaternary carbon atoms 8 and 9.

A readily accessible sterol of this type would obviously be of interest, since such a substance might reasonably be expected to be susceptible to oxidation at carbon atom 11 to yield an 11-keto-steroid which might prove suitable as an intermediate for the partial synthesis of some of the hormones of the adrenal cortex. This expec-



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[1] Windaus and Brunken, *Ann.*, **460**, 225 (1928).

[2] Heilbron and Sexton, *J. Chem. Soc.*, 924 (1929).

[3] Windaus and Langer, *Ann.*, **508**, 105 (1934).

[4] Schenck, Buchholz and Weisse, *Ber.*, **69**, 2702 (1936).

[5] It has been demonstrated in this Laboratory that the isomerization of γ-cholestenol to α-cholestenol cannot be carried out in a nitrogen atmosphere. Therefore, no significance can be attached to the fact that α-dihydroergosterol is not changed when it is dissolved in ethyl acetate and shaken with palladium catalyst and nitrogen.

[6] Stavely and Bollenback, THIS JOURNAL, **65**, 1285 (1943).

tation seemed all the more likely since both carbon atoms in the α position to the double bond in α-ergostenol were found to be oxidizable to carbonyl groups.[6] However, no 11-ketones could be isolated from the mixture of oxidation products, although it cannot be stated with certainty that none were present. The reason for this negative result may lie in the relative lack of reactivity of a methylene group in the 11-position, which is known to be greatly hindered sterically.[7]

α-Dihydroergosteryl acetate was oxidized with chromic acid at room temperature, and the mixture of neutral products was fractionated by chromatographing on alumina. Among the products isolated was a ketone (IV), $C_{30}H_{46}O_3$, which had an absorption maximum at 252 mμ, ε 10,100 (see Fig. 1). According to the generalizations of Woodward[8] an α,β,β-substituted, α,β-unsaturated ketone whose carbon–carbon double bond is not exocyclic to any ring will have a $\lambda_{\text{max.}}$ value near 250 mμ. The nature of the steroid ring system makes possible only two representatives of this class, a $\Delta^{8,9}$-7-ketone and a $\Delta^{8,9}$-11-ketone. Some difficulty was encountered in separating the ketone IV because it was invariably adsorbed on the chromatographic column to about the same extent as one of the other oxidation products, and a better procedure for its isolation was devised. The total oxidation product was refluxed with hydrochloric acid in ethanol and then separated into ketonic and non-ketonic fractions with Girard ketone reagent T. Surprisingly, ketone IV was always found in the non-ketonic moiety. The unreactivity of the keto group led us to believe at first that the substance might be a $\Delta^{8,9}$-11-ketone.[7] This belief was strengthened by the observation that the ketone would not form a semicarbazone by the usual methods. Even on standing in ethanol with semicarbazide acetate for five days the reaction only proceeded to a negligible extent. However, after standing an hour in acidified ethanol with 2,4-dinitrophenylhydrazine a hydrazone separated, whereas the hydrazone of cholestenone, made under identical conditions, separated in less than a minute. When the ketone IV was catalytically hydrogenated with palladium in acetic acid[9] a saturated ketone was isolated which formed a semicarbazone and a 2,4-dinitrophenylhydrazone in a normal manner. Notwithstanding the anomalous behavior of the unsaturated ketone IV, the latter evidence definitely favored the presence of a 7-keto rather than an 11-keto group in IV.

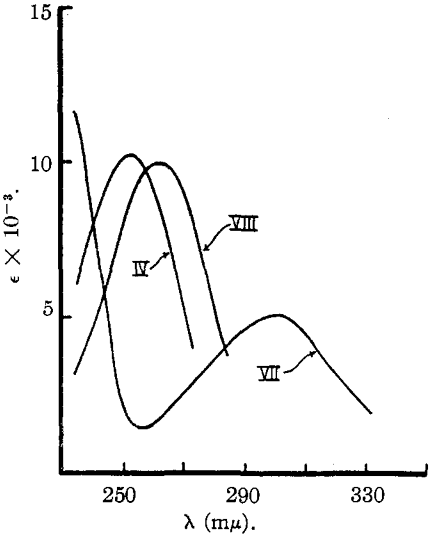

Fig. 1.—Absorption curves taken in ethanol of: IV, $\Delta^{8,9;22,23}$-ergostadienol-3-one-7 acetate; VIII, $\Delta^{8,14}$-ergostenol-3-one-7 acetate; VII, $\Delta^{9,11;8,14;22,23}$-ergostatrienol-3-one-7 acetate.

Two isomeric oxidation products, II and III, were isolated which had the formula $C_{30}H_{46}O_4$. Neither substance showed selective absorption in the ultraviolet. By catalytic reduction of the side chain double bond with palladium in ethanol two saturated substances, V and VI, were prepared from them. The reduction product of II (V) was different from either of the ketoxides derived from α-ergostenol.[6] The melting point (210°) was close to that of ergostanol-3-one-15-oxide-8,14 acetate[6] but was markedly depressed by admixture with the latter compound. Also, the specific rotations of the two substances differed by 32°. The reduction product of III (VI) had the same properties (melting point, mixture melting point, specific rotation) as ergostanol-3-one-7-oxide-8,14 acetate derived from α-ergostenyl acetate, and III is the only oxidation product of α-dihydroergosteryl acetate which has a nuclear structure in common with an oxidation product of α-ergostenyl acetate. Therefore, it must be a 7-keto-8,14-oxide, differing from the corresponding product from α-ergostenyl acetate only by the presence of a double bond in the side chain.

The conversion of II and III by acid hydrolysis to the same trienone VIII demonstrates that they

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(7) Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 817 (1937).

(8) Woodward, THIS JOURNAL, **64**, 76 (1942).

(9) See footnote 12, ref. 6.

are both α,β-ketoxides in which the keto group occupies the same (7) position.[10] The ultraviolet absorption curve of the trienone VII coincides with that of $\Delta^{9,11;8,14}$-ergostadienol-3-one-7 acetate[6] (Fig. 1), indicating that the two compounds differed only by the side chain double bond originally present in α-dihydroergosterol.

It is possible that II and III both contain 8,14 epoxide rings which differ in their orientation to the plane of ring C. Theoretically two *cis* epoxide rings (but no *trans* forms) are possible, but since in this case the epoxide ring is α,β to a keto group it is unlikely on steric grounds that both forms would be capable of existence. Indeed, only one 7-keto-8,14 oxide and one 15-keto-8,14-oxide could be found among the oxidation products of α-ergostenol acetate.[6] Moreover, $\Delta^{8,9;22,23}$-ergostadienol-3-one-7 acetate, a substance which would logically be the immediate precursor of a 7-keto-8,9-oxide, is present in the mixture of α-dihydroergosteryl acetate oxidation products. In view of these observations we believe that II is a 7-keto-8,9-oxide rather than a 7-keto-8,14-oxide epimeric to III. A compound with either structure would be expected to yield the trienone VII on acid hydrolysis.

In order to explain the presence of a 7-keto-8,14-oxide it must be assumed that the $\Delta^{8,9}$-7-ketone IV is the primary oxidation product, and that this substance is partially isomerized to a $\Delta^{8,14}$-7-ketone under the experimental conditions used. The latter ketone is then oxidized further to the ketoxide III, while only a part of the $\Delta^{8,9}$-7-ketone is oxidized to the ketoxide II. If the reverse change had occurred $\Delta^{8,9}$-ergostenol-3-one-7 acetate should have been found among the oxidation products of α-ergostenol acetate. Experimentally the 8,14 double bond in VIII showed no tendency to shift to the 8,9 position.

Catalytic reduction of the trienone VII with palladium in ethanol gave $\Delta^{8,14}$-ergostenol-3-one-7 acetate (VIII), previously obtained from $\Delta^{9,11;8,14}$-ergostadienol-3-one-7 acetate.[6] When the hydrogenation was carried out in acetic acid[9] three moles of hydrogen were utilized and the product was identical with the saturated ketone obtained by hydrogenating the ketone IV. Thus the possibility that IV is an 11-ketone is definitely eliminated and the position of the keto group in IV is definitely fixed at carbon atom 7.

It is interesting that the 7-keto group is capable of activating the 8,14 (or 8,9) double bond so that it can be catalytically hydrogenated, whereas this bond is ordinarily completely unreactive toward hydrogen. Certainly the rule that olefinic bonds conjugated with a keto group are reduced more slowly than isolated double bonds[11] does not apply to the diquaternary cyclic double bonds in the steroid nucleus. The two added hydrogen atoms are probably *trans*, since no inversion occurs when the saturated ketone so formed is heated with alkali.[12]

A consideration of the oxidation products of α-dihydroergosteryl acetate as compared with the products obtained from α-ergostenyl acetate leads to the conclusion that the two substances almost certainly do not contain the same nuclear double bond. This is demonstrated most clearly by the fact that three of the six oxidation products of α-ergostenyl acetate have keto groups in the 15 position, whereas the carbonyls present in the three oxidation products of α-dihydroergosteryl acetate are all in position 7. We believe that α-dihydroergosterol is $\Delta^{8,9;22,23}$-ergostadienol-3.

Acknowledgment.—The authors wish to thank Dr. O. Wintersteiner for his interest and advice, Dr. N. H. Coy of the Squibb Vitamin Research Laboratory for the spectrographic measurements, and Mr. J. F. Alicino of this Laboratory for the microanalyses.

Experimental

Oxidation of Dihydroergosteryl Acetate.—α-Dihydroergosteryl acetate (10 g.) was dissolved in 600 cc. of glacial acetic acid and 300 cc. of benzene. A solution of 8.0 g. of chromic trioxide in 100 cc. of 90% acetic acid was added dropwise with stirring. After standing for twenty-two hours at room temperature 50 cc. of ethanol was added and the solution concentrated to a small volume *in vacuo*. Water was added and the mixture thoroughly extracted with ether. Acid oxidation products and acetic acid were removed by washing the ether with water and 2 *N* sodium hydroxide. After washing out excess alkali with water and drying over sodium sulfate, the ether was distilled off, yielding 8.45 g. of neutral oxidation product.

Chromatographic Separation of Neutral Oxidation Products.—The mixture of oxidation products was dissolved in hot ethanol and from the solution two crops of crystals were obtained. The mother liquor was evaporated to dryness. The two crops of crystals and the mother liquor residue were each dissolved in hexane and chromatographed separately on columns of alumina 25 to 30 × 2.1 cm. Each column was eluted with successive

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[10] The possibility that II and III might be β,γ- or γ,δ-unsaturated, α-tertiary hydroxy ketones is not excluded. If so an α,β-ketoxide must have been the immediate precursor.

[11] Gilman, "Organic Chemistry," second edition, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 802.

[12] See Linstead, Whetstone and Levine, THIS JOURNAL, **64**, 2014 (1942).

portions of hexane, 20% benzene in hexane, 50% benzene in hexane, benzene, 20% ether in benzene, ether and finally with 10% ethanol in ether. After passing through the column the eluant was collected in portions of about 100 cc. Each chromatogram yielded from 30 to 50 fractions. The residue from each fraction was examined and combined with residues of similar m. p. If the pooled residues had a narrow (5–10°) m. p. range they were recrystallized to a constant m. p. and pure compounds were obtained from them. If the melting point range was wide the pooled residues were rechromatographed on a new column. By these methods only two pure products (II and III) could be obtained.

Δ^{22}-Ergostenol-3-one-7-oxide-8,9 Acetate (II).—Recrystallized from 80% ethanol the m. p. was 223–225°[13]; $[\alpha]^{24}\text{D}$ −46 ± 2° (0.75% in chloroform); yield 16%.

Anal. Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.40; H, 9.88.

The substance showed no absorption in the ultraviolet region above 230 mμ. It was recovered unchanged when treated with semicarbazide acetate in pyridine–ethanol for three days at room temperature.[14] On treatment with 2,4-dinitrophenylhydrazine an orange 2,4-dinitrophenylhydrazone precipitated, m. p. 209° (dec.).

Δ^{22}-Ergostenol-3-one-7-oxide-8,14 Acetate (III).—Recrystallized from 80% ethanol the m. p. was 155°; $[\alpha]^{24}\text{D}$ −99 ± 3° (1.03% in chloroform); yield 18%.

Anal. Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.65; H, 9.88.

The substance showed no absorption in the ultraviolet region above 230 mμ. It was recovered unchanged when treated with semicarbazide acetate in pyridine–ethanol for three days at room temperature. With 2,4-dinitrophenylhydrazine, an orange dinitrophenylhydrazone was precipitated from acidified ethanol, m. p. 218° (dec.).

Isolation of $\Delta^{8,9;22,23}$-Ergostadienol-3-one-7 Acetate (IV).—All of the neutral oxidation product except the pure compounds II and III was dissolved in 100 cc. ethanol and 5 cc. concd. hydrochloric acid was added and the mixture refluxed for two hours. It was poured into 400 cc. water and extracted with ether. The excess acid was washed out with water, the ether was dried with sodium sulfate and evaporated. The residue was refluxed for one hour with 5 g. of Girard ketone reagent T in 45 cc. of ethanol and 5 cc. glacial acetic acid, and separated into ketonic and non-ketonic fractions in the usual manner. Each fraction was acetylated in pyridine–acetic anhydride overnight and chromatographed on a column of alumina 25 × 2.1 cm.

Only one pure crystalline substance could be isolated from the chromatogram of the ketonic fraction, m. p. 188°. The same compound was prepared from each of the two ketoxides II and III, as described below.

When the non-ketonic fraction was chromatographed the starting material, α-dihydroergosteryl acetate (200 mg.), was found in the hexane eluant. Another compound, ketone IV, was present in the benzene eluant, recrystallized from methanol, m. p. 206–208°; $[\alpha]^{24}\text{D}$ −53 ± 2.0° (0.66% in chloroform); yield 4%.

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20. Found: C, 79.31; H, 10.14.

The substance showed maximum absorption at 252 mμ, ε 10,100.[15] When treated with semicarbazide acetate in pyridine–ethanol for three days at room temperature, the ketone was recovered unchanged. In another experiment the ketone stood with an excess of semicarbazide acetate in ethanol for five days. The product was washed with water and ether, m. p. 195–204°. An analysis showed a nitrogen content of 2.4% (calculated for a semicarbazone, 8%). On standing with 2,4-dinitrophenylhydrazine in acidic ethanol, an orange 2,4-dinitrophenylhydrazone separated only after an hour. In a control experiment carried out under identical conditions the 2,4-dinitrophenylhydrazone of cholestenone precipitated in less than a minute.

Ergostanol-3-one-7-oxide-8,9 Acetate (V).—Palladium catalyst (20 mg.) in ethanol was saturated with hydrogen, and 40 mg. of the ketoxide II dissolved in ethanol was added. Within thirty minutes 2.4 cc. of hydrogen (1.1 moles) was utilized. The product was recrystallized from 80% ethanol, m. p. 211°; $[\alpha]^{23}\text{D}$ −38 ± 3° (0.885% in chloroform).

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.05; H, 10.32.

The m. p. of a mixture with ergostanol-3-one-15-oxide-8,14 acetate[6] (m. p. 210°) was 180–195°.

Ergostanol-3-one-7-oxide-8,14 Acetate (VI).—With palladium catalyst in ethanol one mole of hydrogen was utilized in 30 minutes. The product was recrystallized from 80% ethanol, m. p. 132–133°; $[\alpha]^{23}\text{D}$ −77 ± 4°. The m. p. of a mixture of the product with ergostanol-3-one-7-oxide-8,14 acetate (m. p. 134°) prepared from α-ergostenol[6] was 132–134°.

Preparation of the Trienone (VII).—The ketoxide III (200 mg.) was dissolved in 20 cc. ethanol, 1 cc. of concd. hydrochloric acid was added and the mixture refluxed for two hours. It was poured into 100 cc. of water and extracted with ether. The ether was washed free from acid with water, dried over sodium sulfate and evaporated. The residue was reacetylated with pyridine–acetic anhydride and the product recrystallized from methanol, m. p. 187–189°; $[\alpha]^{24}\text{D}$ −47 ± 2.5° (0.975% in chloroform).

Anal. Calcd. for $C_{30}H_{44}O_3$: C, 79.60; H, 9.80. Found: C, 79.38; H, 9.92.

The substance exhibited maximum absorption at 300 mμ, ε 5000. It formed an orange 2,4-dinitrophenylhydrazone, m. p. 222° (dec.).

A product with the same m. p., specific rotation, absorption spectrum, and analysis was obtained from the ketoxide II by the same procedure.

$\Delta^{8,14}$-Ergostenol-3-one-7-Acetate (VIII).—Palladium catalyst (100 mg.) in ethanol was saturated with hydrogen, and a solution of the trienone VII (300 mg.) in ethanol was added. In seventy minutes 33 cc. of hydrogen (2 mols) was utilized. The product was recrystallized from 80% ethanol, m. p. 155°; $[\alpha]^{24}\text{D}$ −65 ± 2° (0.92% in chloroform).

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.60. Found: C, 79.19; H, 10.60.

The substance was identical with $\Delta^{8,14}$-ergostenol-3-one-

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(13) All melting points are uncorrected.

(14) Dutcher and Wintersteiner, THIS JOURNAL, **61**, 1992 (1939).

(15) All spectrographic measurements were made in absolute ethanol.

7-acetate obtained previously[6] as shown by the m. p., specific rotation, and absorption spectrum.

Ergostanol-3-one-7 Acetate (IX).—Palladium catalyst (80 mg.) in acetic acid was saturated with hydrogen, and 180 mg. of the trienone VII dissolved in acetic acid was added. In three hours, 33.6 cc. hydrogen (3.5 mols) was utilized. The product was chromatographed on a column of alumina 1 × 18 cm. By elution with 20% benzene in hexane 95 mg. of α-ergostenyl acetate, m. p. 108°, was isolated. By elution with benzene and evaporation of the solvent 75 mg. of residue was obtained which was recrystallized from 80% ethanol, m. p. 183–184°; $[\alpha]^{23}\mathrm{D}$ $-36 \pm 1.5°$ (0.89% in chloroform).

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.42; H, 10.90.

The same substance was obtained in 45% yield from $\Delta^{8,9;22,23}$-ergostadienol-3-one-7-acetate (IV) by hydrogenation in acetic acid with palladium. The remainder of the product was α-ergostenol acetate.

On standing with semicarbazide acetate in pyridine–ethanol for three days, a semicarbazone was formed, recrystallized from ethanol, m. p. 225–228°.

Anal. Calcd. for $C_{31}H_{53}O_3N_3$: C, 72.21; H, 10.36; N, 8.15. Found: C, 71.92; H, 10.31; N, 8.18.

The ketone formed a yellow 2,4-dinitrophenylhydrazone, m. p. 216° (decomp.).

Ergostanol-3-one-7.—The acetate IX (30 mg.) was refluxed with 5% potassium hydroxide in methanol for two hours. The product was recrystallized from 80% ethanol, m. p. 154°.

Anal. Calcd. for $C_{28}H_{48}O_2 \cdot H_2O$: C, 77.36; H, 11.59. Found: C, 77.63; H, 11.27.

Treatment of $\Delta^{8,14}$-Ergostenol-3-one-7 Acetate (VIII) with Hydrochloric Acid.—The ketone VIII (40 mg.) was dissolved in 10 cc. ethanol, 0.5 cc. concd. hydrochloric acid was added, and the mixture refluxed for two and one-half hours. The product was reacetylated in pyridine–acetic anhydride and recrystallized from ethanol, m. p. 153°; $[\alpha]^{24}\mathrm{D}$ $-59 \pm 2°$ (0.46% in chloroform). No double bond isomerization had occurred.

Summary

Mild oxidation of α-dihydroergosteryl acetate with chromic acid yielded only 3 pure products as compared with the six obtained from α-ergostenyl acetate by an identical isolation procedure. Two of these compounds, II and III, are α,β-ketoxides and the third is an α,β-unsaturated monoketone (IV). Only one of the three compounds (III) has a nuclear structure in common with one of the oxidation products of α-ergostenol.

The α,β-unsaturated ketone IV shows selective absorption at 252 mμ, a value in accordance with the generalization of Woodward[8] concerning the most probable $\lambda_{\max.}$ value for an α,β,β-substituted, α,β-unsaturated ketone whose carbon–carbon double bond is not exocyclic to any ring. It has been assigned the structure of $\Delta^{8,9;22,23}$-ergostadienol-3-one-7-acetate.

A comparison of the neutral oxidation products obtained from the acetates of α-dihydroergosterol and α-ergostenol leads to the conclusion that α-dihydroergosterol is $\Delta^{8,9;22,23}$-ergostadienol-3.

NEW BRUNSWICK, N. J. RECEIVED MARCH 18, 1943

[CONTRIBUTION FROM THE NEW YORK STATE COLLEGE OF FORESTRY AND DEPARTMENT OF RADIOLOGY, SYRACUSE UNIVERSITY]

X-Ray Studies of Reactions of Cellulose in Non-Aqueous Systems. II. Interaction of Cellulose and Primary Amines[1]

BY W. E. DAVIS,[2] A. J. BARRY,[3] F. C. PETERSON[3] AND A. J. KING

Introduction

The work to be described herein is a natural extension of the earlier investigation of the action of liquid ammonia on cellulose,[4] in which the ammonia-cellulose formed at atmospheric pressure was characterized by the following interplanar spacings: $d_{101} = 8.86$ Å., $d_{10\bar{1}} = 4.47$ Å., $d_{002} = 4.05$ Å. A second ammonia-cellulose derivative was later found[5] to be produced when cellulose is sealed in a glass tube with liquid ammonia at room temperature. The pressure developed in this system is of the order of 10 atmospheres, and under these conditions the derivative has $d_{101} = 10.6$ Å. This same derivative was also reported by Hess and Gundermann,[6] who found for d_{101} the

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(1) Presented before the Cellulose Division of the American Chemical Society at the Buffalo meeting, September 10, 1942.

(2) Present address, Hercules Powder Co., Wilmington, Del. This paper is taken in part from a thesis submitted by W. E. Davis to the Faculty of the New York State College of Forestry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1941.

(3) Present address, Dow Chemical Co., Midland, Mich.

(4) A. J. Barry, F. C. Peterson and A. J. King, THIS JOURNAL, **58**, 333 (1936). The structure proposed in this paper for ammonia-cellulose was based on the cellulose unit cell of H. Mark and Kurt H. Meyer, *Z. physik. Chem.*, **B2**, 115 (1929).

(5) A. J. Barry, A. J. King and F. C. Peterson, informal report before the Cellulose Division of the American Chemical Society at the Chapel Hill, N. C. meeting, April 5–9, 1937.

(6) K. Hess and J. Gundermann, *Ber.*, **70B**, 1788 (1937).