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

Steroids with Double Bonds between Quaternary Carbon Atoms. III. The Structure of α-Spinasterol

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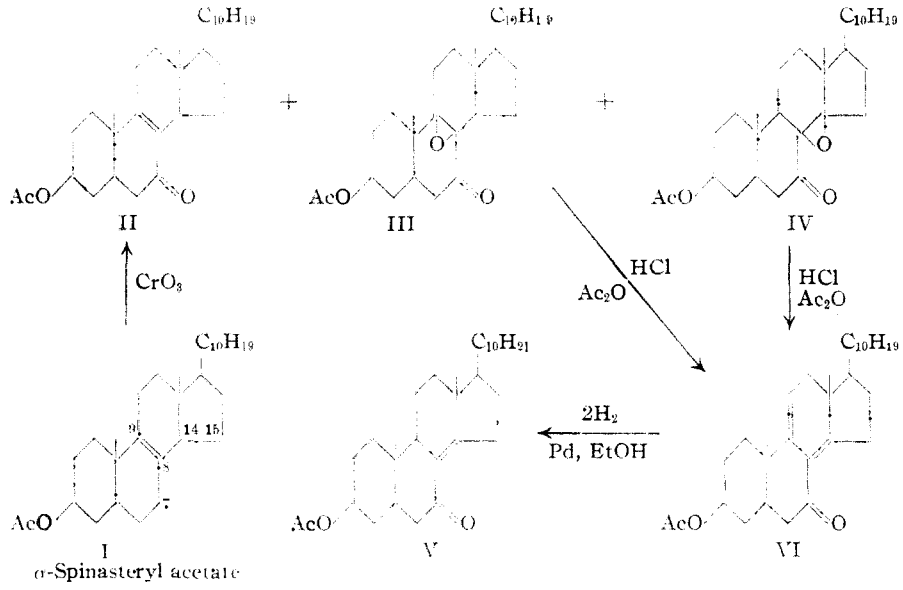
It has been firmly established that α-spinasterol contains twenty-nine carbon atoms,[1] that one of its two double bonds is located in the side-chain between carbon atoms 22 and 23,[2] and that the product of catalytic hydrogenation, α-spinastenol, is identical with $\Delta^{8,14}$-stigmastenol-3 (α-stigmastenol).[2] In consequence of the latter fact the nuclear double bond of α-spinasterol must lie in the 7,8, 8,9 or 8,14-position.



α-Spinasteryl acetate

It is well known that a sterol containing a γ-($\Delta^{7,8}$) or δ($\Delta^{8,9}$) double bond cannot be hydrogenated catalytically, but in the presence of hydrogen and platinum or palladium catalyst the double bond is shifted to the 8,14 position.[3] Originally this was always carried out by shaking the compound with hydrogen and catalyst, but more recently several investigators[2,4] have assumed that the double bond shift would occur in the absence of hydrogen, and have attempted to carry out the reaction under nitrogen. With but one exception the results have been negative under these conditions and the conclusion has been made that the double bond must have been already in the α-($\Delta^{8,14}$) position. The single positive result was obtained by Wieland, *et al.*,[4b] who claimed to have isomerized the $\Delta^{8,9}$ bond of the yeast sterol ascosterol to the 8,14 position in the presence of "finely divided platinum," nitrogen, and the solvent ethyl acetate.

We have investigated the isomerization of γ($\Delta^{7,8}$)-cholestenyl acetate[5] in ethyl acetate with platinum from Adams catalyst (platinum oxide), platinum black,[6] and palladium black. In no case was isomerization to α($\Delta^{8,14}$)-cholestenyl acetate (m. p. 78°) observed *unless the catalyst had been first saturated with hydrogen*. Each of the three catalysts used was able to isomerize the stenol in the presence of hydrogen, but not at the same rate. No significant change occurred in melting point or specific rotation when platinum or palladium black and γ-cholestenyl acetate dissolved in ethyl acetate were shaken under nitrogen. No change occurred when platinum black or platinum oxide was saturated with hydrogen, the hydrogen replaced with nitrogen and then shaken with γ-cholestenyl acetate. However, when palladium black in ethyl acetate was first saturated with hydrogen (20 cc. per 100 mg.) and then shaken with γ-cholestenyl acetate under nitrogen, complete isomerization took place. Thus palladium appeared to be the most efficacious of the three catalysts and hydrogen seemed to be an essential prerequisite for the isomerization.

(1) Larsen, THIS JOURNAL, **60**, 2431 (1938).

(2) Fernholz and Ruigh, *ibid.*, **62**, 2341 (1940).

(3) Windaus, Linsert and Eckhardt, *Ann.*, **534**, 22 (1938); Schenck, Buchholz and Weisse, *Ber.*, **69**, 2696 (1936); Windaus and Zuhlsdorff, *Ann.*, **536**, 204 (1938).

(4) (a) Heath-Brown, Heilbron and Jones, *J. Chem. Soc.*, 1482 (1940); (b) Wieland, Rath and Hesse, *Ann.*, **543**, 34 (1940).

(5) The γ-cholestenyl acetate used was prepared from 3-acetoxy-cholestanol-7(8) by Wintersteiner and Moore, THIS JOURNAL, **65**, 1507 (1943). It had a m. p. of 104–111°. Although it contained small amounts of other double bond isomers, it was readily converted into α-cholestenyl acetate in good yield by catalytic isomerization in a hydrogen atmosphere.

(6) Feulgen, *Ber.*, **54**, 360 (1921).

TABLE I

	$\Delta^{8,9}$-7-Ketone A[a]	$\Delta^{8,9}$-7-Ketone B[b]	7-Keto-(8,9)-oxide A	7-Keto-(8,9)-oxide B	7-Keto(8,14)-oxide A	7-Keto(8,14)-oxide B	$\Delta^{9,11;8,14;22,23}$-7-Ketone A	$\Delta^{9,11;8,14;22,23}$-7-Ketone B	$\Delta^{8,14}$-7-Ketone A	$\Delta^{8,14}$-7-Ketone B
M. p., °C.	204	208	230	225	173	155	192	189	141	155
$\lambda_{max.}$, mμ	252	252	..	..	..	..	299	300	260	262
$[\alpha]^{\circ}D$	−36	−53	−32	−46	−77	−99	−24	−47	−53	−65
$\Delta[\alpha]^{\circ}D$[c]	−36	−33	−32	−26	−77	−79	−24	−27	−53	−45

[a] Derived from α-spinasteryl acetate, m. p. 183°, $[\alpha]D \pm 0$. [b] Derived from α-dihydroergosteryl acetate, m. p. 181°, $[\alpha]D$ −20°. [c] Difference in $[\alpha]D$ between derivative and its parent sterol acetate.

The single positive result obtained[4b] by the use of nitrogen is difficult to reconcile with these observations. Possibly a δ-($\Delta^{8,9}$)-stenol can be isomerized under nitrogen, even though the γ-($\Delta^{7,8}$)-stenol is unchanged under these conditions. Since the activity of catalysts sometimes varies considerably with the exact method of preparation it is possible that some procedures give catalysts which have isomerization activity under nitrogen and others do not. The German investigators may have prepared their "finely divided platinum" by the reduction *in situ* of platinum oxide, and therefore the catalyst had adsorbed enough hydrogen to bring about the isomerization. Experimental details in their publication on this point are lacking.

At any rate it is unsafe to conclude from a negative result obtained under nitrogen that a steroid already contains an α-(8,14) double linkage. The evidence of Fernholz and Ruigh[6] concerning the presence of an 8,14 double bond in α-spinasterol is based on this procedure and is therefore inconclusive. We have subjected α-spinasteryl acetate to the same oxidative procedure applied to the acetates of α-ergostenol[7] and α-dihydroergosterol[8] and the results favor the presence in α-spinasterol of an 8,9 rather than an 8,14 nuclear double bond.

Simpson[9] oxidized α-spinasteryl acetate with chromic acid at room temperature and crystallized two constant-melting substances from the neutral fraction. One of these had a melting point of 213° and analyzed approximately for an acetoxy monoketone. The other (m. p. 171°) contained two more oxygen atoms than the starting material. When the experimental conditions used by Simpson were duplicated, we isolated material with a constant melting point of 201°, $[\alpha]D$ −12°. The analytical figures for carbon and hydrogen approximated those for a substance with three oxygen atoms (monoketone). The product proved to be a mixture of α-spinasteryl acetate and an oxidation product with four oxygen atoms which could be separated easily by chromatographing on alumina. By increasing the concentration of chromic acid the amount of α-spinasteryl acetate recovered was greatly diminished.

The total neutral oxidation product was chromatographed on alumina and two isomeric substances, III and IV were isolated. Both compounds lost the elements of water when heated with hydrochloric acid in ethanol and yielded the same trienone V. A third oxidation product, the α,β-unsaturated ketone II could only be obtained from the crude oxidation mixture in a pure state after treatment with hydrochloric acid and separation with Girard's ketone reagent T. The new ketone was invariably found in the non-ketonic fraction. The three oxidation products II, III, and IV and the derived products V and VI obtained from α-spinasteryl acetate are entirely analogous to the products from α-dihydroergosteryl acetate[8] as shown in Table I.

It is evident that the two series of derivatives are homologous, differing only by one carbon atom in the side chain. The reasoning which led us to assign structures corresponding to II, III, IV, V and VI to the products obtained from α-dihydroergosterol apply, of course, with equal force to the new compounds described here.

The ketoxides III and IV differ greatly in stability toward acid. Treatment of a crude oxidation mixture containing both with Girard's ketone reagent T in 10% acetic acid–90% ethanol results in almost complete conversion of IV to the trienone VI, whereas III is unaffected. The epoxide ring in III is stable to boiling acetic acid, and this ketoxide is invariably found in the non-ketonic fraction. Neither III or IV will form a semicarbazone under ordinary conditions. Since the trienone VI reacts with ketone reagents in a normal fashion the unreactivity of the keto group in III and IV is probably due to steric hindrance afforded by the epoxide ring.

Acknowledgment.—The authors wish to thank Dr. O. Wintersteiner for his interest and advice,

(7) Stavely and Bollenback, THIS JOURNAL, **65**, 1285 (1943).
(8) Stavely and Bollenback, *ibid.*, **65**, 1290 (1943).
(9) Simpson, *J. Chem. Soc.*, 730 (1937).

Dr. N. H. Coy of the Squibb Vitamin Research Laboratory for the spectrographic measurements, Mr. J. F. Alicino of this Laboratory for the microanalyses, and Miss Margaretta Taylor for technical assistance.

Experimental

Isomerization of γ-Cholestenyl Acetate in the Presence of Hydrogen.—Palladium black (25 mg.) in ethyl acetate was saturated with hydrogen (5 cc.). γ-Cholestenyl acetate (25 mg.), m. p. 104–111°, $[\alpha]_D$ 2°, dissolved in ethyl acetate was added, and the mixture was shaken with hydrogen for two hours. The catalyst was removed by filtration and the solvent evaporated. The residue had a m. p. of 70–75°; $[\alpha]^{23}D$ 13 ± 1° (0.54% in chloroform). In another experiment palladium catalyst and γ-cholestenyl acetate in ethyl acetate was shaken under nitrogen for two hours after preliminary saturation of the catalyst with hydrogen. The product had a m. p. of 69–75°, $[\alpha]^{24}D$ 13 ± 2° (0.57% in chloroform).

The experiment was repeated using platinum oxide (30 mg.). After shaking with hydrogen for five hours, the total product had a m. p. of 85–90°; $[\alpha]^{23}D$ 18 ± 2° (0.40% in chloroform). When platinum catalyst made by saturating platinum oxide with hydrogen was shaken under nitrogen with γ-cholestenyl acetate no isomerization took place.

The experiment was repeated using platinum black (30 mg.) and 80 mg. of γ-cholestenyl acetate. After shaking for five hours with hydrogen, the product had a m. p. of 85–95°; $[\alpha]^{23}D$ 8 ± 1° (0.44% in chloroform). When platinum black was shaken under nitrogen with γ-cholestenyl acetate in ethyl acetate after preliminary saturation of the catalyst with hydrogen no isomerization took place.

Attempted Isomerization of γ-Cholestenyl Acetate in the Presence of Nitrogen.—Palladium black (25 mg.) and γ-cholestenyl acetate (25 mg.) in ethyl acetate were shaken with nitrogen for four hours. The catalyst was filtered and the solvent evaporated. The residue had a m. p. of 103–111°, $[\alpha]^{23}D$ 3 ± 1° (0.37% in chloroform). In another experiment shaking was continued for twenty-four hours without significant change in the m. p. or specific rotation of the γ-cholestenyl acetate.

The experiment was repeated using platinum black (25 mg.). The residue obtained on evaporation of the solvent had a m. p. of 104–111°; $[\alpha]^{23}D$ 1 ± 2° (0.40% in chloroform).

Oxidation of α-Spinasteryl Acetate.—α-Spinasteryl acetate (2 g.) was dissolved in 600 cc. of glacial acetic acid and a solution of 1.1 g. of chromic trioxide dissolved in 40 cc. of 90% acetic acid was added dropwise with stirring. After standing for twenty-two hours at room temperature, 10 cc. of ethanol was added and the solution was concentrated to a small volume *in vacuo*. Water was added and the mixture was extracted with ether. Acid oxidation products and acetic acid were removed by washing with 2 *N* sodium hydroxide. After washing out excess alkali with water and drying over sodium sulfate the ether was removed by distillation, yielding 1.8 g. of neutral oxidation product. After recrystallization from ethanol four times, the m. p. was constant at 201°; $[\alpha]_D$ −12 ± 1° (0.96% in chloroform). The material was chromatographed in a column of alumina 1.5 × 15 cm. and separated into two fractions. The hexane eluant yielded α-spinasteryl acetate (60%) and the benzene eluant gave a substance (III), m. p. 230°,[10] which is described in detail below. The mother liquor residues were chromatographed and yielded only one pure substance (IV), m. p. 173°. When the oxidation was repeated using 0.8 g. of chromic trioxide per gram of α-spinasteryl acetate in a smaller volume of solvent (acetic acid–benzene) very little α-spinasteryl acetate remained unoxidized. Careful chromatographing procedures failed to yield any pure products except III and IV.

Δ^{22}-Stigmastenol-3-one-7-oxide-(8,9) Acetate (III).—Recrystallized from ethanol, the m. p. was 229–230°; $[\alpha]_D$ −32 ± 1.5° (0.70% in chloroform).

Anal. Calcd. for $C_{31}H_{48}O_4$: C, 76.81; H, 9.98. Found: C, 76.61; H, 10.05.

The compound showed no selective absorption in the ultraviolet above 230 mμ.

Δ^{22}-Stigmastenol-3-one-7-oxide-(8,14) Acetate (IV).—Recrystallized from 80% ethanol or acetone, the m. p. was 171–173°; $[\alpha]^{24}D$ −77 ± 3° (0.58% in chloroform).

Anal. Calcd. for $C_{31}H_{48}O_4$: C, 76.81; H, 9.98. Found: C, 76.68; H, 10.10.

The compound showed no selective absorption above 230 mμ.

Treatment of Neutral Oxidation Products with Girard Reagent.—The neutral residue from an α-spinasteryl acetate oxidation as described above (4.3 g.) was heated with 100 cc. of 10% acetic acid–90% ethanol and 6 g. of Girard ketone reagent T for one and one-half hours. In the usual manner 2.2 g. of non-ketonic and 1.7 g. of ketonic material were isolated. Each fraction was chromatographed separately on alumina. From the non-ketonic fraction was isolated 1.1 g. of the ketoxide III, 0.05 g. of the ketoxide IV and 0.5 g. of rather impure $\Delta^{8,9;\,22,23}$-stigmastadienol-3-one-7 acetate (II). From the ketonic fraction was obtained 0.8 g. of the trienone VI which could be prepared directly from III and IV as described below.

$\Delta^{8,9;\,22,23}$-Stigmastadienol-3-one-7 Acetate (II).—The total neutral oxidation product from an α-spinasteryl acetate oxidation (4.1 g.) was refluxed with 200 cc. of ethanol and 10 cc. of concd. hydrochloric acid for two hours. The mixture was poured into 400 cc. of water and extracted with ether, the ether was washed with water, dried over sodium sulfate and the ether removed by distillation. The residue was treated with Girard ketone reagent T and separated into ketonic and non-ketonic fractions. Both fractions were acetylated in pyridine-acetic anhydride overnight. The acetylated non-ketonic material was chromatographed and the benzene eluant yielded the ketone II, recrystallized from methanol, m. p. 202–204°; $[\alpha]^{23}D$ −36 ± 2° (0.60% in chloroform); yield 3%.

Anal. Calcd. for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32. Found: C, 79.60; H, 10.13.

The substance had an absorption maximum at 252

(10) All melting points are uncorrected.

mμ, ε 8300.[11] The ketone was unreactive toward semicarbazide acetate.

$\Delta^{9,11;\ 8,14;\ 22,23}$-Stigmastatrienol-3-one-7 Acetate (VI).—The acetylated ketonic fraction of the preceding section was chromatographed and 1.2 g. of the trienone VI was isolated. It was recrystallized from aqueous acetone, m. p. 190–192°, $[\alpha]^{23}D$ −24 ± 2° (0.82% in chloroform).

Anal. Calcd. for $C_{31}H_{46}O_3$: C, 79.78; H, 9.95. Found: C, 79.92; H, 10.10.

The substance had an absorption maximum at 299 mμ, ε 5300.

The same product (VI) was obtained from either of the pure ketoxides III or IV by refluxing them with hydrochloric acid in ethanol followed by acetylation in pyridine–acetic anhydride.

$\Delta^{8,14}$-Stigmastenol-3-one-7 Acetate (V).—Palladium black (50 mg.) in ethanol was saturated with hydrogen, and 100 mg. of the trienone VI dissolved in ethanol was added. In one hour 11 cc. (2.1 moles) of hydrogen had been adsorbed. The product was recrystallized from 80% ethanol, m. p. 140–141°; $[\alpha]D$ −53 ± 1.5° (0.87% in chloroform).

Anal. Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.03; H, 10.48.

The substance had an absorption maximum at 260 mμ, ε 7800.

When the trienone V was hydrogenated with palladium black or platinum oxide in acetic acid, the sole product was α-spinastenyl acetate, m. p. 117°; $[\alpha]D$ 13 ± 1° (0.61% in chloroform).

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.28; H, 11.40.

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

Summary

Mild oxidation of α-spinasteryl acetate yielded three pure products, the α,β-unsaturated ketone II, and two isomeric α,β-ketoxides III and IV. When heated with hydrochloric acid in ethanol both ketoxides were converted into the same trienone VI, which was reduced catalytically to the $\Delta^{8,14}$-7-ketone V. The compounds II, III, IV, V and VI are entirely analogous to the corresponding derivatives obtained by the same procedures from α-dihydroergosteryl acetate. The two series are homologous and differ only by one carbon atom in the side chain.

Comparison of these two series with the compounds obtained by the same procedures from $\Delta^{8,14}$-ergostenol-3(α-ergostenol) leads to the conclusion that the nuclear double bond of α-spinasterol, like that of α-dihydroergosterol, lies between carbon atoms 8 and 9, rather than 8 and 14, as proposed by Fernholz and Ruigh.[6] Therefore α-spinasterol is $\Delta^{8,9;\ 22,23}$-stigmastadienol-3.

A $\Delta^{7,8}$ or $\Delta^{8,9}$ steroid double bond will not invariably shift to the 8,14 position when a $\Delta^{7,8}$- or $\Delta^{8,9}$-steroid is shaken with a hydrogenating catalyst and nitrogen. It is unsafe to conclude from a negative result obtained in the absence of hydrogen that the double bond occupies the 8,14 position.

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The Isomeric *p*-Dibutylbenzenes[1]

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Introduction

Investigations[2,3,4] conducted in this Laboratory on the alkylation of benzene and alkylbenzenes with alcohols and boron fluoride, alone or with a co-condensing agent such as phosphorus pentoxide, sulfuric acid, or benzenesulfonic acid, have led to the conclusions (a) that the dialkylbenzenes so obtained are almost exclusively of the *para* constitution and (b) that isomerization of *n*- and isobutyl groups occurs, thus introducing *s*- and *t*-butyl groups, respectively. The present investigation was undertaken in order to make available for comparison purposes all the possible *p*-di-butylbenzenes. By means of these data we hope to extend our alkylation studies, especially in connection with isomerizations and rearrangements. Authentic reference compounds are needed since no other means are available for determining the structure of a butyl group in a complex hydrocarbon.

Only two of the ten possible *p*-dibutylbenzenes are well known. The di-tertiary isomer has been reported many times,[2,3,5] while the di-secondary

(1) Paper XXVIII on organic reactions with boron fluoride; previous paper THIS JOURNAL, **64**, 2751 (1942).

(2) McKenna and Sowa, *ibid.*, **59**, 470 (1937).

(3) Toussaint and Hennion, *ibid.*, **62**, 1145 (1940).

(4) Welsh and Hennion, *ibid.*, **63**, 2603 (1941).

(5) (a) Goldschmidt, *Ber.*, **15**, 1067 (1882); (b) Meyer and Bernhauer, *Monatsh.*, **53-54**, 721 (1929); (c) Ipatieff and Pines, THIS JOURNAL, **58**, 1056 (1936); (d) Simons and Archer, *ibid.*, **60**, 986, 2952, 2953 (1938); (e) Potts and Dodson, *ibid.*, **61**, 2553 (1939).