271. *The Application of the Method of Molecular Rotation Differences to Steroids. Part V. Olefinic Unsaturation at the 7 : 8-Position.*

By D. H. R. Barton and J. D. Cox.

The formulæ previously suggested for α-dihydroergosterol and for α-spinasterol on optical-rotation grounds have been confirmed by chemical means. The assignment of 7 : 8-olefinic unsaturation in steroids which have rings A and B in the cholestane configuration has thereby been rendered unambiguous.

This is the first of a number of papers which will deal with the experimental proof of the more important formulæ suggested in Parts I, II, and III (*J.*, 1945, 813; 1946, 512, 1116) as a result of analysis of the literature. The experimental approach will be from both the chemical and the physical viewpoint and will at the same time provide further evidence as to the nature of " vicinal action " in steroids (see Part IV, this vol., p. 783).

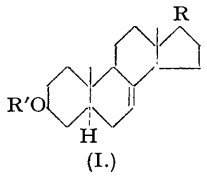
When ergosteryl acetate is hydrogenated in neutral solution with a platinum catalyst it affords γ-ergostenyl acetate (I; R = C_9H_{19}, R′ = Ac) the correct Δ values for which have been recorded in Part IV. Hydrogenation in acid solution gives α-ergostenyl acetate (II; R = C_9H_{19}, R′ = Ac) for which Δ values have similarly been reported. Hydrogenation in acid or (better) neutral solution until the hydrogen uptake corresponds to the saturation of one double bond leads to the isolation of α-dihydroergosteryl acetate for which the formula (II; R = C_9H_{17}, R′ = Ac) has been accepted until recently. The Δ values for α-dihydroergosterol are in exact agreement (Table I) with those required for the formula (I; R = C_9H_{17}, R′ = H), which was

Table I.*

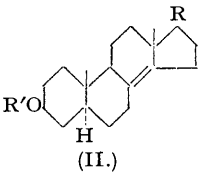
Substance.	Formula (R′ = H).	$[M]_D$. Alcohol.	Acetate.	Benzoate.	Ketone.	Δ_1.	Δ_2.	Δ_3.
γ-Ergostenol †.....	(I; R = C_9H_{19})	− 8	−18	+10	+ 88	−10	+18	+96
α-Dihydroergosterol	(I; R = C_9H_{17})	−76	−88	−50	+ 8	−12	+26	+84
α-Spinasterol	(I; R = $C_{10}H_{19}$)	−12	−23	+10	+ 82‡	−11	+22	+94
γ-Spinastenol	(I; R = $C_{10}H_{21}$)	+46	+36	+67	—	−10	+21	—
α-Ergostenol †.....	(II; R = C_9H_{19})	+44	+ 4	± 0	+119	−40	−44	+75
α-Spinastenol	(II; R = $C_{10}H_{21}$)	+95	+55	+52	—	−40	−43	—

* All rotations recorded in this paper are for chloroform solution and the Na_D line (see text).
† See Part IV, *loc. cit.* ‡ Simpson, *J.*, 1937, 730.

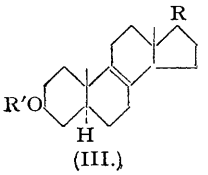
suggested in Part I on the basis of literature analysis. This formula is now confirmed by the fact (see Experimental) that, in agreement with Wieland and Benend (*Annalen*, 1943, **554**, 1), on catalytic hydrogenation in neutral solution with a platinum catalyst α-dihydroergosteryl acetate furnishes, in high yield, γ-ergostenyl acetate and not α-ergostenyl acetate. The oxidative evidence of Stavely and Bollenback (*J. Amer. Chem. Soc.*, 1943, **65**, 1290) that α-dihydroergosterol is (III; R = C_9H_{17}, R′ = H) has already been discounted in Part I and need not be discussed again here. If this formula had been correct, δ-ergostenyl acetate (III; R = C_9H_{19}, R′ = Ac) would have been obtained in the above experiment.



(I.)



(II.)



(III.)

Hitherto it has not been possible on the basis of purely chemical evidence to decide between the formulæ (I; R = $C_{10}H_{19}$, R′ = H), (II; R = $C_{10}H_{19}$, R′ = H), and (III; R = $C_{10}H_{19}$, R′ = H) for α-spinasterol. Through the courtesy of Dr. J. C. E. Simpson, to whom we are much indebted for the supply of a small quantity of this sterol, we have been able to confirm the conclusion reached in Part I that it is stigmasta-7 : 22-dien-3(β)-ol (I; R = $C_{10}H_{19}$, R′ = H). On hydrogenation in neutral solution with a platinum catalyst α-spinasteryl acetate furnished the monoethenoid *γ-spinastenyl acetate* (*γ-stigmastenyl acetate*), which showed Δ values in excellent agreement (Table I) with those required by the formula (I; R = $C_{10}H_{21}$, R′ = Ac). Similar hydrogenation in acid solution gave α-spinastenyl acetate (II; R = $C_{10}H_{21}$, R′ = Ac), whose Δ values (Table I) were also in excellent agreement with those required for $\Delta^{8(14)}$-unsaturation. As expected, γ-spinastenyl acetate appeared to be quantitatively rearranged to α-spinastenyl acetate under acid hydrogenating conditions, a reaction well known to be given by γ-ergostenyl acetate and γ-cholestenyl acetate. Again, the oxidation evidence of Stavely and Bollenback (*J. Amer. Chem. Soc.*, 1943, **65**, 1600) that α-spinasterol is (III; R = $C_{10}H_{19}$, R′ = H) is logically unsound and has already been dealt with in Part I.

It was pointed out in Part II that the Δ values on reduction of the various double-bond positions in the steroid nucleus are highly characteristic of those positions. This method of approach was used to support the correct formulæ for α-dihydroergosterol and α-spinasterol. It finds confirmation in the data recorded in Table II (taken from the experimental section in this Part), which should be inspected in conjunction with the figures given in Part II, Table I.

TABLE II.

Substance (unsaturated).	Derivative.	$[M]_D$. Unsaturated.	$[M]_D$. Saturated.	Δ.
γ-Ergostenol	Alcohol	− 8	+ 60	+68
γ-Spinastenol	Alcohol	+46	+104	+58
γ-Ergostenol	Acetate	−18	+ 27	+45
γ-Spinastenol	Acetate	+36	+ 73	+37
γ-Ergostenol	Benzoate	+10	+ 66	+56
γ-Spinastenol	Benzoate	+67	+109	+42

In view of the facile rearrangement of Δ^7-stenols to $\Delta^{8(14)}$-stenols, exemplified in this paper by γ- and α-spinastenols, it might be expected that α-dihydroergosteryl acetate (I; R = C_9H_{17}, R′ = Ac) would be similarly rearranged to (II; R = C_9H_{17}, R′ = Ac). Indeed, a rearrangement of this type has already been reported by Wieland, Rath, and Hesse (*Annalen*, 1941, **548**, 34; compare Stavely and Bollenback, *loc. cit.*, p. 1600; Wieland and Benend, *loc. cit.*), although the correctness of this claim has been strongly doubted in Part I. Wieland, Rath, and Hesse used a platinum catalyst suspended in ethyl acetate in an atmosphere of nitrogen to bring about their alleged rearrangement. We have carried out a number of experiments of this type (see Experimental) and conclude, in agreement with Stavely and Bollenback (*loc. cit.*), that the isomerisation of Δ^7- to $\Delta^{8(14)}$-bonds is only possible *provided hydrogen is adsorbed on the catalyst.* In the case of α-dihydroergosteryl acetate the adsorbed hydrogen is used for side-chain reduction preferentially to double-bond rearrangement. Clearly, this test for the nuclear position of a double bond can only be applied in steroids which contain no readily reducible grouping.

EXPERIMENTAL.

(M. p.s are uncorrected).

The substances whose rotations are listed below were all purified as carefully as possible to constant m. p. and constant rotation. All specimens were dried in a vacuum at 20° below their m. p.s or at 120°, whichever was the lower temperature. All rotations are for the Na_D line and in chloroform solution. The measurements were made at room temperature which varied from 15° to 25°.

In order to improve the accuracy of the rotation measurements, most readings were taken in macro-tubes. Normally 1-dm. tubes were employed, but rotations marked with an asterisk were recorded from 2-dm. macro-tubes. Values obtained with a 1-dm. micro-tube have this fact indicated after each individual rotation. All values of $[\alpha]_D$ have been approximated to the nearest degree as in previous Parts of this series. Concentrations, *c*, are expressed in g. per 100 ml. of solution. For the calculations the specific rotations at *c*, 2·00, or at the nearest concentrations to this at which measurements were made, have been taken as the most suitable.

Acetylations were carried out by refluxing with acetic anhydride for 30 mins., benzoylations by the usual pyridine procedure, the mixture being left for 24 hours at room temperature to complete reaction. Alkaline hydrolyses were effected by several equivs. of potassium hydroxide by refluxing for 30 minutes in methanolic or dioxan–methanolic solution depending upon the solubility of the ester.

Oppenauer oxidations were performed under the usual conditions (Barton and Jones, *J.*, 1943, 599)

except that it was found advantageous to use only half the amount of acetone there specified and to carry out the refluxing for only 4 hours instead of 16 hours as normally considered necessary. It has also been found that the use of resublimed aluminium *tert.*-butoxide is not essential, and material crystallised from benzene has been employed instead.

With some substances it has proved advantageous to ensure purity by the use of the chromatographic method; the experimental technique of Barton and Jones (*ibid.*, p. 602) has been employed in such cases.

Ergostane Derivatives.—*α-Dihydroergosterol.* In spite of many experiments it has not been possible to find a method of preparation superior to that described below. 19 G. of ergosteryl acetate were dissolved in 150 ml. of chloroform, 200 mg. of platinum oxide catalyst added, and the solution hydrogenated until 1500 ml. of hydrogen (1·25 times that required for the saturation of one double bond) had been taken up. About 1½—2 hours were generally required. The catalyst was removed by filtration, and the solvent by evaporation in a vacuum. The residue was recrystallised from chloroform–methanol, to give α-dihydroergosteryl acetate, m. p. 178—180°; $[\alpha]_D$ − 19° (*c*, 2·05). The yield, on five separate occasions, was always about 6 g. (30—35%).

This method of preparation is better than all previous procedures, which are limited by the solubility of ergosteryl acetate in the solvents employed, for it enables large quantities of α-dihydroergosteryl acetate to be prepared at one time. As far as we are aware, the use of chloroform as a solvent for hydrogenation is novel. It cannot be replaced by carbon tetrachloride because of sulphur-containing impurities which inhibit the reaction.

A specimen of α-dihydroergosteryl acetate was specially purified for rotation measurements by treatment in acetone solution on the water-bath with a little potassium permanganate, followed by chromatography over alumina. Eight fractions were taken, and the last five, with m. p. 180—181°, were crystallised from acetone; m. p. 181°, $[\alpha]_D$ − 19° (*c*, 3·56), − 20° (*c*, 1·78), $[M]_D$ − 88°.

α-Dihydroergosterol [ergosta-7 : 22-dien-3(β)-ol]. Recrystallised from ethyl acetate–methanol; m. p. 176°, $[\alpha]_D$ − 19° (*c*, 1·80), − 20° (*c*, 0·72), $[M]_D$ − 76°.

α-Dihydroergosteryl benzoate. Recrystallised from dioxan–acetone, m. p. 200°, $[\alpha]_D$ − 10° (*c*, 1·47), $[M]_D$ − 50°.

α-Ergostadienone. Prepared by Oppenauer oxidation of α-dihydroergosterol, purified by chromatography, and recrystallised from ethyl acetate–methanol; m. p. 184·5°, $[\alpha]_D$ + 2° (*c*, 1·54; micro-tube), + 2° (*c*, 0·53), $[M]_D$ + 8°.

Hydrogenation of α-Dihydroergosteryl Acetate to γ-Ergostenyl Acetate.—800 Mg. of pure α-dihydroergosteryl acetate (see above) were dissolved in 100 ml. of ethyl acetate and hydrogenated by means of a platinum oxide catalyst (about 100 mg.) until the uptake of hydrogen ceased (about 4 hours were required). The catalyst was removed by filtration, and the solvent by evaporation in a vacuum. The crystalline residue had m. p. 145—155° and afforded, after one recrystallisation from chloroform–methanol, 550 mg. (70%) of γ-ergostenyl acetate, identical in all respects with that prepared by the direct hydrogenation of ergosteryl acetate (see Part IV, *loc. cit.*).

Attempted Rearrangement of α-Dihydroergosteryl Acetate to Ergosta-8(14) : 22-dien-3(β)-yl Acetate.—(i) *Using palladium catalysts.* Attempts to rearrange the acetate by shaking with a freshly reduced palladium catalyst (8% $Pd/BaSO_4$) in ethyl acetate solution, either in a vacuum or in an atmosphere of nitrogen, were unsuccessful and only led to partial hydrogenation. An inseparable mixture, m. p. ~ 125° (indefinite), $[\alpha]_D \pm 0°$, resulted. Sufficient hydrogen must have been adsorbed on the catalyst for partial reduction of the side chain.

(ii) *Using a platinum catalyst.* α-Dihydroergosteryl acetate was recovered unchanged after many hours' shaking in ether–acetic acid solution with a freshly reduced platinum oxide catalyst in an atmosphere of nitrogen.

Ergostan-3(β)-yl Acetate.—The preparation of this ester will be described in a further communication. Recrystallised from chloroform–methanol, it had m. p. 144—145°, $[\alpha]_D$ + 6° (*c*, 1·80; micro-tube), + 6° (*c*, 1·30; micro-tube), $[M]_D$ + 27°.

Ergostan-3(β)-ol.—Recrystallised from aqueous methanol; m. p. 141°, $[\alpha]_D$ + 15° (*c*, 1·15; micro-tube), $[M]_D$ + 60°.

Ergostan-3(β)-yl Benzoate.—Recrystallised from chloroform–methanol, this *ester* had m. p. 161°, $[\alpha]_D$ + 13° (*c*, 2·23; micro-tube), $[M]_D$ + 66° (Found: C, 82·0; H, 11·2. $C_{35}H_{54}O_2$ requires C, 83·0; H, 10·7%).

Stigmastane Derivatives.—*α-Spinasterol* [*Stigmasta-7 : 22-dien-3(β)-ol*]. Recrystallised from acetone–methanol; m. p. 167—168°, $[\alpha]_D$ − 3° (*c*, 3·00), − 3° (*c*, 1·50), $[M]_D$ − 12°.

α-Spinasteryl acetate. Recrystallised from ethyl acetate–methanol; m. p. 185°, $[\alpha]_D$ − 5° (*c*, 2·24), − 5° (*c*, 1·12), $[M]_D$ − 23°.

α-Spinasteryl benzoate. Recrystallised from ethyl acetate–methanol; m. p. 201°, $[\alpha]_D$ + 2° (*c*, 3·12), + 2° (*c*, 1·56), $[M]_D$ + 10°.

γ-Spinastenol [*Stigmast-7-en-3(β)-ol*]. 180 Mg. of α-spinasteryl acetate (see above) were dissolved in 100 ml. of ether, 200 mg. of platinum oxide catalyst were added, and the suspension was shaken in an atmosphere of hydrogen for 6 hours. The catalyst was removed by filtration and the solvent by evaporation in a vacuum. After three recrystallisations of the residue from chloroform–methanol, 40 mg. (22%) of γ-spinastenyl acetate, m. p. 156—157°, $[\alpha]_D$ + 9° (*c*, 1·17; micro-tube), were obtained. On hydrolysis in the usual manner there resulted *γ-spinastenol*, m. p. 144—145°, $[\alpha]_D$ + 11° (*c*, 1·45; micro-tube), $[M]_D$ + 46° (Found: C, 84·3; H, 12·0. $C_{29}H_{50}O$ requires C, 84·1; H, 12·1%).

γ-Spinastenyl benzoate. Prepared by benzoylation of the alcohol in the usual manner (see above), and recrystallised from chloroform–methanol, this *ester* had m. p. 180·5°, $[\alpha]_D$ + 13° (*c*, 1·47; micro-tube), $[M]_D$ + 67° (Found: C, 83·1; H, 10·3. $C_{36}H_{54}O_2$ requires C, 83·4; H, 10·4%).

γ-Spinastenyl acetate. This *ester* was prepared by acetylation of the alcohol in the usual manner (see above) and recrystallised from chloroform–methanol; m. p. 156—157°, $[\alpha]_D$ + 8° (*c*, 0·82; micro-tube), $[M]_D$ + 36° (Found: C, 81·0; H, 11·1. $C_{31}H_{52}O_2$ requires C, 81·5; H, 11·4%).

The mother-liquors from the crystallisation of γ-spinastenyl acetate (see above) were combined and evaporated in a vacuum. The residue was dissolved in 50 ml. of 1 : 1 ether–acetic acid and hydrogenated

for 4 hours by means of a platinum catalyst. The catalyst was filtered off and the solvent partly removed by evaporation in a vacuum. Ether and water were added, and the ethereal layer was separated and washed with sodium carbonate solution. After removal of the ether, the residue was recrystallised from aqueous methanol to give α-spinastenyl acetate, m. p. 116—117°, $[\alpha]_D$ + 12° (*c*, 2·18; micro-tube), $[M]_D$ + 55°.

α-Spinastenol [*Stigmast*-8(14)-*en*-3(β)-*ol*]. Recrystallised from aqueous methanol; m. p. 112—113° after drying at 80° in a vacuum, $[\alpha]_D$ + 23° (*c*, 1·36; micro-tube), $[M]_D$ + 95°.

α-Spinastenyl benzoate. Recrystallised with difficulty from acetone–methanol; m. p. 87—89°, $[\alpha]_D$ + 10° (*c*, 3·11; micro-tube), $[M]_D$ + 52°.

Rearrangement of γ-Spinastenyl Acetate to α-Spinastenyl Acetate.—23 Mg. of purest γ-spinastenyl acetate (see above) were dissolved in 50 ml. of 1 : 1-ether–acetic acid and shaken in an atmosphere of hydrogen for 6 hours, a platinum catalyst being used. After working up in the customary manner, 18 mg. (80%) of pure α-spinastenyl acetate were isolated.

Stigmastan-3(β)-*yl Acetate.*—Prepared by catalytic hydrogenation of stigmasteryl acetate (for which we are much indebted to C.I.B.A.; see Part IV, *loc. cit.*) in ether–acetic acid solution by means of a platinum catalyst, purified by treatment according to the general method of Anderson and Nabenhauer (*J. Amer. Chem. Soc.*, 1924, **46**, 1957), and recrystallised from ethyl acetate–methanol; m. p. 130°, $[\alpha]_D$ + 16° (*c*, 1·32), + 16° (*c*, 0·66), $[M]_D$ + 73°.

Stigmastan-3(β)-*ol.*—Recrystallised from ethyl acetate–methanol; m. p. 136°, $[\alpha]_D$ + 25° (*c*, 4·17; micro-tube), $[M]_D$ + 104°.

Stigmastan-3(β)-*yl Benzoate.*—Recrystallised from ethyl acetate–methanol; m. p. 136°, $[\alpha]_D$ + 21° (*c*, 1·67; micro-tube), $[M]_D$ + 109°.

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