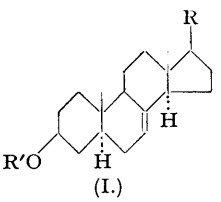
380. *The Application of the Method of Molecular Rotation Differences to Steroids. Part X.* "β-*Dihydroergosterol.*"

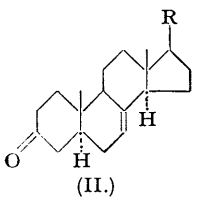
By D. H. R. Barton, J. D. Cox, and N. J. Holness.

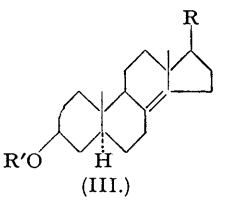
Isomerisation of "α"-dihydroergosteryl acetate gives an inseparable mixture, formerly designated "β-dihydroergosteryl acetate," of ergosta-8(14) : 22(23)-dien-3(β)-yl and ergosta-14(15) : 22(23)-dien-3(β)-yl acetate in equimolecular amounts. Hydrogenation of *iso*ergosterone in neutral solution affords a complex mixture of *ergost*-22(23)-*ene*, *coproergost*-22(23)-*en*-3-*one*, *ergost*-22(23)-*en*-3-*one*, and *ergosta*-4(5) : 22(23)-*dien*-3-*one* (*brassicastadienone*), from which further transformation products have been prepared. The changes in molecular rotation associated with the hydrogenation of a 22(23)-ethylenic linkage are discussed.

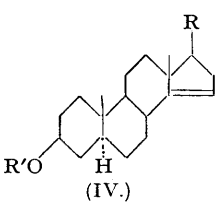
The preparation of "β-dihydroergosteryl acetate" by isomerisation of "α"-dihydroergosteryl acetate (I; R = C_9H_{17}, R' = Ac) with dry hydrogen chloride was described first by Heilbron, Johnstone, and Spring (*J.*, 1929, 2248). Another route to this substance was reported later by Dithmar and Achtermann (*Z. physiol. Chem.*, 1932, **205**, 55) who isomerised "α"-ergostadienone (II; R = C_9H_{17}) with hydrogen chloride and reduced the product, "β-ergostadienone," with sodium and alcohol to "β-dihydroergosterol." *

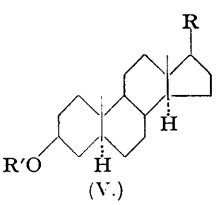
We have repeated the preparation of "β-dihydroergosterol" and its derivatives according to the directions of Heilbron, Johnstone, and Spring, and have obtained this substance with

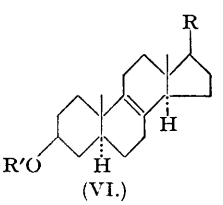

(I.)


(II.)


(III.)


(IV.)


(V.)


(VI.)

constants in fair agreement (Table I) with those recorded previously. We have carried out this preparation on several occasions and, provided that the "α"-dihydroergosteryl acetate used was entirely free from ergosteryl acetate, we have always been able to repeat exactly the physical constants we record in Table I (see also Experimental section).

* Dithmar and Achtermann actually called their product "dihydroergosterol III," but they considered it to be identical with the substance prepared earlier by Heilbron and his collaborators.

TABLE I.*

	Alcohol.		Acetate.		Benzoate.		Ketone.	
	m. p.	$[\alpha]_D$.	m. p.	$[\alpha]_D$.	m. p.	$[\alpha]_D$.	m. p.	$[\alpha]_D$.
Heilbron, Johnstone, and Spring (*loc. cit.*)	124°	− 7·0° †	108—109°	−25·2° †	—	—	125°	—
Dithmar and Achtermann (*loc. cit.*)	122	−16·7	108	−25·3	—	—	114	−5°
This paper	116	− 9	104·5	−17	116°	−8°	125—126	+4

* All rotations in this paper are for chloroform solutions and the Na_D line, unless specified to the contrary.

† For the 5461 A. line.

It is generally considered (Sobotka, " The Chemistry of the Sterids," Baillière, Tindall, and Cox, 1938) that " β-dihydroergosterol " is represented by the formula (IV; R = C_9H_{17}, R′ = H) and is, in fact, the 22 : 23-dehydro-derivative of " β "-ergostenol (IV; R = C_9H_{19}, R′ = H) (cf. W. Bergmann and Klacsmann, *J. Org. Chem.*, 1948, **13**, 21). Now in Part II of this series (*J.*, 1946, 512) we pointed out that this view was incompatible with both the chemical and the optical-rotation evidence and suggested that " β-dihydroergosterol " might be a mixture containing a proportion of the strongly lævorotatory compound ergosta-5(6) : 22(23)-dien-3(β)-ol (brassicasterol).

Whereas " β "-ergostenol is hydrogenated to ergostanol (V; R = C_9H_{19}, R′ = H) in almost quantitative yield " β-dihydroergosteryl acetate " was said to afford " α "-ergostenyl acetate (III; R = C_9H_{19}, R′ = Ac) (Heilbron, Johnstone, and Spring, *loc. cit.*). In our hands catalytic hydrogenation of " β-dihydroergosteryl acetate " in acid solution gave a mixture of equal amounts of ergostanyl and " α "-ergostenyl acetate. Our previous suggestion with regard to a Δ^5-contaminant was based on the claims of Wieland and Benend (*Ber.*, 1942, **75**, 1708) that the nuclear olefinic linkages in zymosterol and zymostenol, now known to be (VI; R = C_8H_{15}, R′ = H) and (VI; R = C_8H_{17}, R′ = H), respectively (this vol., p. 214), could be in part rearranged to the Δ^5-position. These claims have now been withdrawn (Wieland and Gornhardt, *Annalen*, 1947, **557**, 248) and, indeed, oxidation of " β-dihydroergosterol " by the Oppenauer method, followed by chromatography, failed to reveal any trace of the Δ^4-3-one which might have been expected. The Δ_3 value (Table II) of +52°, for " β-ergostadienone " is in fair agreement with the normal one of +73° for *trans*-(A/B)-3(β)-stanols (*J.*, 1948, 783) whereas the value for the change to a Δ^4-3-one is very large (+511°). All attempts to show, either by fractional crystallisation of the acetate and benzoate or by chromatography of the acetate, that " β-dihydroergosterol " was a mixture, failed. Although there was slight evidence for heterogeneity in the latter experiments, it was quite impossible to obtain any clear-cut resolution.

At this stage of the investigation it was felt desirable to confirm that the 22(23)-olefinic linkage in the side-chain was not altered in any way by treatment with hydrogen chloride. This view was supported by the facts that ergosteryl B_1 and ergosteryl B_3 acetates, prepared by the hydrogen chloride induced isomerisation of ergosteryl acetate, gave (−)-methyl*iso*propylacetaldehyde on ozonolysis (Guiteras, Nakamiya, and Inhoffen, *Annalen*, 1932, **494**, 116) and that the ergosteryl acetate–maleic anhydride adduct was unchanged by treatment with hydrogen chloride (Inhoffen, *ibid.*, 1934, **508**, 81). On ozonolysis (see Experimental section) " β-dihydroergosteryl acetate " also gave (−)-methyl*iso*propylacetaldehyde. Furthermore stigmasteryl acetate, which has the same 22(23)-ethylenic linkage as ergosterol, was unchanged by treatment with hydrogen chloride. We conclude, therefore, that the side-chain of " α "-dihydroergosteryl acetate is not altered during the conversion into " β-dihydroergosteryl acetate."

The nature of " β-dihydroergosteryl acetate " was finally elucidated by a study of the product of its hydrogenation in neutral solution. In ethyl acetate as solvent and in presence of a not too active platinum catalyst " β-dihydroergosteryl acetate " afforded, in high yield, a substance, m. p. 93·5—94·5°, $[\alpha]_D$ +6°, whose constants were unchanged on recrystallisation. This material gave no depression in m. p. on admixture with an equimolecular mixture, m. p. 93·5—94·5°, $[\alpha]_D$ +6°, of " α "- and " β "-ergostenyl acetate prepared either by isomerisation of " α "-ergostenyl acetate by hydrogen chloride (Reindel, Walter, and Rauch, *ibid.*, 1927, **452**, 34; Reindel and Walter, *ibid.*, 1928, **460**, 212; cf. Hart, Speer, and Heyl, *J. Amer. Chem. Soc.*, 1930, **52**, 2016) or by acetylation of a mixture of equal parts of " α "- and " β "-ergostenol. In

view of the origin of this complex, which it is impossible to resolve by crystallisation, we propose for it the name " αβ-ergostenyl acetate." The acetate from the hydrogenation experiment was converted into the mixed benzoates which by fractional crystallisation afforded both " α "- and " β "-ergostenyl benzoate (cf. Heilbron and Wilkinson, *J.*, 1932, 1708). All this evidence is conclusive that the " β-dihydroergosteryl acetate " hydrogenation product must be " αβ-ergostenyl acetate." In so far as the previous studies in this series have established that hydrogenation in neutral solution using a *platinum* catalyst does not lead to rearrangement, the experiments are proof that the so-called " β-dihydroergosterol " is an inseparable equimolecular mixture of ergosta-8(14) : 22(23)-dien-3(β)-ol (III; R = C_9H_{17}, R′ = H) and ergosta-14(15) : 22(23)-dien-3(β)-ol (IV; R = C_9H_{17}, R′ = H). This view is confirmed by consideration of the optical-rotation evidence discussed in detail below, and is in agreement with the behaviour of " β-dihydroergosteryl acetate " on hydrogenation in acid solution (see above).

In Part II of this series (*J.*, 1946, 512) the changes in molecular rotation associated with the reduction of a 22(23)-ethylenic linkage were surveyed and a mean value of +61° was found. This value, which is derived from nuclear substituted substances where " vicinal action " (*J.*, 1948, 783) might be expected and which relates to varying substituents at the 24-position where again " vicinal action " is possible, received apparent confirmation from the rotations recorded for " α "-dihydroergosterol and for " γ "-ergostenol (Table II) where the mean Δ value is +67°. When the latter value is used, the calculated molecular rotations (Table II) for " β-dihydroergosterol," on the basis of the composition proved above, are still not in good agreement with those observed, though the discrepancies are now only *ca.* 8° in specific rotation compared with *ca.* 18° for the previously recorded data (compare Part II, *J.*, 1946, 512). In order to resolve this discrepancy, which is outside the limits of experimental error, the preparation of compounds of the ergostane series containing a saturated nucleus and a single ethylenic linkage at the 22(23)-position, was undertaken. These substances should, according to our previous studies (*J.*, 1948, 783), be entirely free from any suspicion of " vicinal action " and thus should give the required Δ values without ambiguity.

TABLE II.

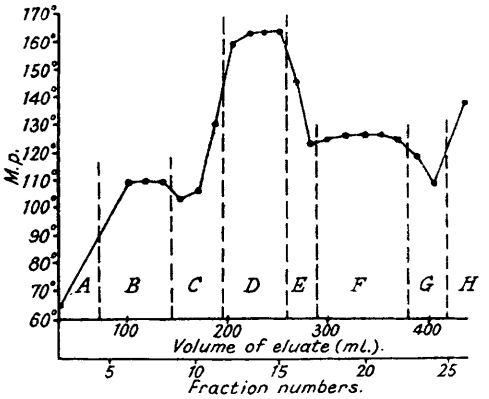
Substance.	$[M]_D$. Alcohol.	Acetate.	Benzoate.	Ketone.	Ref.
" α "-Dihydroergosterol	−76°	−88°	− 50°	+ 8°	1
" γ "-Ergostenol	− 8	−18	+ 10	+ 88	2
(From above : mean Δ = + 67°)	+68	+60	+ 60	+ 80	—
" α "-Ergostenol	+44	+ 4	± 0	+119	2
" β "-Ergostenol	+88	+58	+116	+159	2
Mean	+66	+31	+ 58	+139	—
" β "-Dihydroergosterol : Calculated using Δ = + 67°	− 1	−36	− 9	+ 72	—
Calculated using Δ = + 103° *	−37	−72	− 45	+ 36	Exptl.
" β "-Dihydroergosterol : Observed rotations	−36	−75	− 40	+ 16	Exptl.

* See Table IV. The mean Δ value includes the data for ergost-22(23)-ene (Exptl.) but excludes the value derived from coproergostan-3-one. References : 1, *J.*, 1948, 1354; 2, *J.*, 1948, 783.

When ergosterone (VII; R = C_9H_{17}) is treated with hydrogen chloride it gives *iso*-ergosterone (VIII; R = C_9H_{17}) from which the required compounds were readily prepared. Partial hydrogenation of *iso*ergosterone in neutral solution until the uptake of hydrogen corresponded to 1·3—1·4 equivalents furnished a complex mixture of products which, on chromatography over alumina, afforded the results shown in the figure.

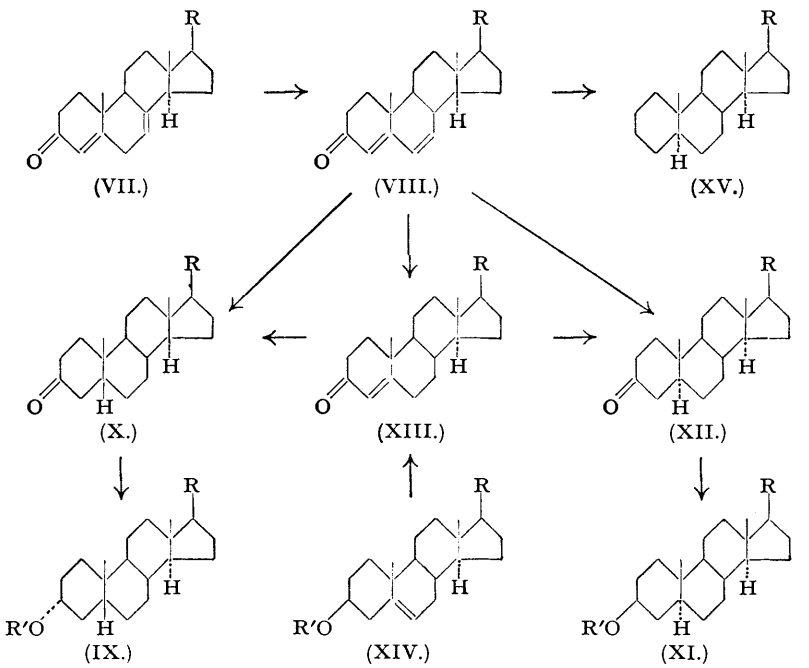
The first fraction (*A*), which was very easily eluted from the column, is discussed further below. Fraction (*B*) gave a pure ketone, m. p. 110·5°, which analysed for $C_{28}H_{46}O$ and showed no absorption in the ultra-violet. On complete hydrogenation in neutral solution this ketone furnished coproergostan-3(α)-ol (IX; R = C_9H_{19}, R′ = H), with only a trace of the digitonin-precipitable 3(β)-isomer. These observations show that ketone (*B*) must be a derivative of coproergostane and thus must be either coproergost-6(7)-en-3-one or coproergost-22(23)-en-3-one. Now the 22(23)-ethylenic linkage is reduced only slowly by hydrogenation in neutral solution,

whereas the hydrogenation of *iso*ergosterone proceeds with great rapidity (see Experimental section). We formulate ketone (*B*) therefore as *coproergost*-22(23)-*en*-3-*one* (X; R = C_9H_{17}).



Fraction numbers.	Elutriant.	Fraction numbers.	Elutriant.	
1— 4	Benzene	A	16—17	Benzene	E
5— 8	Benzene	B	18—22	90 Benzene : 10 Ether	F
9—11	Benzene	C	23—24	70 Benzene : 30 Ether	G
12—15	Benzene	D	25—26	Ether	H

Reduction with sodium and *n*-propanol gave *coproergost*-22(23)-*en*-3(α)-*ol* (IX; R = C_9H_{17}: R′ = H) further characterised as the *acetate*, *acetate dibromide*, and *benzoate*. Fraction (*D*) on



recrystallisation furnished a pure ketone, m. p. 165·5°, also $C_{28}H_{46}O$, which showed no absorption in the ultra-violet. On complete hydrogenation in neutral solution this ketone afforded ergostan-3(β)-ol (XI; R = C_9H_{19}, R′ = H) with only a trace of the non-digitonin-precipitable isomer. Ketone (*D*) must therefore be a derivative of ergostane and accordingly is to be formulated as *ergost*-22(23)-*en*-3-*one* (XII; R = C_9H_{17}). Reduction with sodium and *n*-propanol gave *ergost*-22(23)-*en*-3(β)-*ol* (XI; R = C_9H_{17}, R′ = H) further characterised as the *acetate*, *acetate dibromide*, and *benzoate*. Ketone (*D*) bears the same relation to ketone (*B*) as

cholestanone does to coprostanone; this is borne out by the comparative data shown in Table III.

TABLE III.

	$C_{28}H_{46}O$.		$C_{27}H_{46}O$.	
	(i) Ketone (*B*).	(ii) Ketone (*D*).	(i) Coprostanone.	(ii) Cholestanone.
$[M]_D$	−4°	+24°	+146°	+162°
(i) − (ii)	−28°		−16°	
M. p.	110·5°	165·5°	64°	128°
(i) − (ii)	−55°		−64°	
Main product of Na–*n*-PrOH reduction...	3(α)-ol	3(β)-ol	3(α)-ol	3(β)-ol
Chromatographic behaviour	(i) Eluted before (ii)		(i) Eluted before (ii)	

Fraction (*F*) gave a pure ketone, m. p. 127—128°, which analysed as $C_{28}H_{44}O$ and showed a strong band at 242 mμ, ε 18,200. From these facts and its marked dextrorotation it must be *ergosta*-4(5) : 22(23)-*dien*-3-*one* (XIII; R = C_9H_{17}). Now the investigations of Fernholz and Stavely (*J. Amer. Chem. Soc.*, 1939, **61**, 142; 1940, **62**, 1875) have shown that the naturally occurring brassicasterol is best represented as ergosta-5(6) : 22(23)-dien-3(β)-ol (XIV; R = C_9H_{17}, R′ = H), so that it should give ketone (*F*) on oxidation by the Oppenauer method. Through the courtesy of Dr. H. E. Stavely (Squibb Institute for Medical Research) who kindly supplied us with a specimen of brassicasterol we were able to show that this is indeed the case. Partial hydrogenation of ketone (*F*) in neutral solution afforded, on chromatography, ketones (*B*) and (*D*) and thus confirmed unambiguously the formulæ ascribed above to the latter ketones.

Fractions (*C*) and (*E*) were combined and again chromatographed over alumina to give ketones (*B*), (*D*), and (*F*), but no trace of any other ketone. Fractions (*G*) melted unsharply even after repeated crystallisation, and were probably mixtures of ketone (*F*) with unchanged *iso*ergosterone. Chromatography of a specimen of *iso*ergosterone which had been hydrogenated to less than one equivalent of hydrogen uptake confirmed that unchanged *iso*ergosterone separated in the chromatogram after ketone (*F*).

Fraction (*A*) consisted of an oily mixture of hydrocarbons which melted unsharply. On treatment with bromine it afforded a crystalline *dibromide* from which, by debromination, a homogeneous mono-olefinic hydrocarbon, $C_{28}H_{48}$, was prepared. The latter product is formulated as *ergost*-22(23)-*ene* (XV; R = C_9H_{17}) since it gave ergostane (XV; R = C_9H_{19}) on hydrogenation in neutral solution and had almost the same molecular rotation as ergost-22(23)-en-3(β)-ol (XI; R = C_9H_{17}, R′ = H) (see Barton and Klyne, *Chem. and Ind.*, 1948, 755T).

Fraction (*H*), which melted unsharply, was resolved by the digitonin method into impure coproergost-22(23)-en-3(α)-ol (IX; R = C_9H_{17}, R′ = H) and ergost-22(23)-en-3(β)-ol (XI; R = C_9H_{17}, R′ = H).

The molecular rotations and Δ values for the compounds prepared above are summarised in Table IV and compared with the appropriate standard values. It will be seen that an isolated

TABLE IV.

	$[M]_D$.							
Substance.	Alcohol.	Acetate.	Benzoate.	Ketone.	Δ_1.	Δ_2.	Δ_3.	Refs.
Ergostan-3(β)-ol	+ 60°	+ 27°	+ 66°	+132°	−33°	+ 6°	+72°	1, Exptl.
Ergost-22(23)-en-3(β)-ol	− 40	− 75	− 40	+ 28	−35	± 0	+68	Exptl.
Δ (from above)	+100	+102	+106	+104	—	—	—	—
Coproergostan-3(α)-ol	+ 93 *	+173	—	+115 *	+80	—	+22	2, 3, Exptl.
Coproergost-22(23)-en-3(α)-ol	− 16	+ 71	+ 45	− 4	+87	+61	+12	Exptl.
Δ (from above)	+109	+102	—	+119	—	—	—	—

* Calculated from the corresponding coprostan-3(α)-ol derivatives by subtraction of 31 units—the standard difference in $[M]_D$ between cholestanol and ergostanol derivatives.

References: 1, *J.*, 1948, 1354; 2, *J.*, 1949, 337; 3, *J.*, 1946, 1116.

ethylenic linkage in the side-chain does not exert "vicinal action" at the 3-position. This is in agreement with our previous study of the subject (Barton and Cox, *J.*, 1948, 783). The data do, however, reveal a marked difference between the Δ values for side-chain reduction of the 22(23)-ethylenic linkage in "α"-dihydroergosterol (Table II),* as compared with the

* This effect is also shown by other compounds having 7(8)- and 22(23)-ethylenic linkages. See this vol., p. 337.

nuclearly saturated compounds described in this paper. Such " vicinal action," transmitted through four saturated carbon atoms, would not have been expected from our previous studies (Barton and Cox, *loc. cit.*) although these have not hitherto included diethylenic derivatives. The case is noteworthy because in " β-dihydroergosterol " (see above) it appears that the ethylenic linkages at positions 8(14) and 14(15), which are separated by three saturated carbon atoms from position 22, do not affect the Δ value for the reduction of the 22(23)-bond. It should also be pointed out that the Δ_2 value for " β-dihydroergosterol " is in exact agreement with that expected for a mixture of the composition found.

Experimental.

The substances whose rotations are listed below 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, unless specified to the contrary. 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 using 1-dm. macro-tubes. Values obtained using 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. 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. In no case, however, was there any marked variation of specific rotation with concentration.

Acetylations were carried out, unless specified to the contrary, by heating the substance under reflux with acetic anhydride for 30 minutes, and benzoylations by the usual pyridine procedure, the reactants being left for 24 hours at room temperature to complete reaction. Alkaline hydrolyses were effected by using several equivs. of potassium hydroxide and heating the reactants under reflux for 30 minutes in methanolic or dioxan–methanolic solution depending upon the solubility of the ester. Oppenauer oxidations were performed under the normal 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 heat the mixtures under reflux for only 4 hours instead of the usual 16 hours. It was found that the use of resublimed aluminium *tert.*-butoxide is not essential, and material crystallised from benzene was employed. When the chromatographic technique was used, the usual precautions were taken (see Barton and Jones, *loc. cit.*). M. p.s are uncorrected.

" *β-Dihydroergosteryl Acetate.*"—6-G. portions of carefully purified " α "-dihydroergosteryl acetate (for prep. see *J.*, 1948, 1354) were dissolved in 100 ml. of dry chloroform and treated with dry hydrogen chloride at 0° for 1 hour. The reaction product was worked up according to the directions of Heilbron, Johnstone, and Spring (*J.*, 1929, 2248) and on recrystallisation from ethanol gave " β-dihydroergosteryl acetate," m. p. 104·5°, $[\alpha]_D$ −17° (*c*, 3·74), −17° (*c*, 2·85), −17° (*c*, 0·70), $[M]_D$ −75°, in the form of long needles. Repeated crystallisation did not change the m. p. or rotation. There was no absorption in the ultra-violet.

On one occasion a specimen of " α "-dihydroergosteryl acetate containing, from the absorption spectrum, 8% of ergosteryl acetate, was used for the preparation. On being worked up as above it was not possible by fractional crystallisation to obtain reproducible constants. The m. p. rose very slowly from 106° to 107°, whilst the rotation eventually attained $[\alpha]_D$ −34° (*c*, 1·67; micro-tube). The absorption spectrum of this material showed a weak maximum at 248 mμ, $E^{1\%}_{1\,cm.}$ 40, corresponding to about 15% of a conjugated diene with the ethylenic linkages distributed between two rings (probably ergosteryl B_3 acetate), and confirmed that a contaminant was concentrated by recrystallisation. This material was dissolved in xylene and treated at the b. p. for 6 hours with an excess of maleic anhydride. After the mixture had been worked up in the customary manner " β-dihydroergosteryl acetate," $[\alpha]_D$ −18° (*c*, 0·67; micro-tube), was isolated, identical with the material described above. These experiments illustrate the importance of the careful purification of the " α "-dihydroergosteryl acetate before isomerisation.

" *β-Dihydroergosterol.*"—This was prepared from the above acetate by alkaline hydrolysis in the usual way. Recrystallised from methanol it had m. p. 116°, $[\alpha]_D$ −9° (*c*, 3·38), $[M]_D$ −36°. There was no absorption in the ultra-violet.

" *β-Dihydroergosteryl Benzoate.*"—The above alcohol was benzoylated, and the benzoate recrystallised from ethyl acetate–methanol; this *compound* had m. p. 116°, $[\alpha]_D$ −9° (*c*, 1·78; micro-tube), −8° (*c*, 1·64; micro-tube), $[M]_D$ −40°, unchanged on repeated recrystallisation (Found: C, 83·8; H, 9·9. $C_{35}H_{50}O_2$ requires C, 83·6; H, 10·0%). Alkaline hydrolysis furnished " β-dihydroergosterol," m. p. 117°, $[\alpha]_D$ −9° (*c*, 2·04), which on acetylation by the pyridine method gave " β-dihydroergosteryl acetate," m. p. 104·5°, $[\alpha]_D$ −18° (*c*, 1·38; micro-tube), both compounds being apparently identical with those described above.

" *β-Ergostadienone.*"—3 G. of " β-dihydroergosterol " were oxidised by the Oppenauer procedure, and the reaction product was chromatographed over alumina (12 fractions). Only one band was observed, although Δ^4-3-ones are normally separated very efficiently by this process (Barton and Jones, *loc. cit.*; cf. below) from the corresponding stanones. Except the first three (gummy) fractions, all were combined and recrystallised from ethanol to give " β-ergostadienone," m. p. 125—126°, $[\alpha]_D$ +4° (*c*, 1·19), $[M]_D$ +16°. The 2 : 4-*dinitrophenylhydrazone*, prepared in the usual way, gave only a broad orange band on chromatography over alumina and no trace of a red band such as would correspond to any Δ^4-3-one present as contaminant; after slow recrystallisation from chloroform–methanol it had m. p. 203° (Found: N, 9·5. $C_{34}H_{48}O_4N_4$ requires N, 9·7%).

Ozonolysis of " *β-Dihydroergosterol.*"—1·0 G. of " β-dihydroergosterol " was suspended in AnalaR acetic acid (30 ml.), and ozonised oxygen was bubbled through the liquid for 30 minutes (all steroid had

passed into solution after 15 minutes). The solution was poured into water (30 ml.), and the mixture was warmed for 10 minutes with occasional addition of zinc dust. Finally the liquid was distilled, and to the first 20 ml. of aqueous distillate was added 1 g. of semicarbazide hydrochloride, followed by sufficient sodium hydrogen carbonate to render the solution only slightly acid. The mixture was set aside at 0° for 18 hours. The precipitate was filtered off and recrystallised from benzene–light petroleum. The product had m. p. 129·5°, $[\alpha]_D$ −52° (*c*, 1·30; micro-tube; in ethanol). Guiteras, Nakamiya, and Inhoffen (*Annalen*, 1932, **494**, 116) give for (−)-methyl*iso*propylacetaldehyde semicarbazone, m. p. 129—130°, $[\alpha]_D$ −52° (in ethanol).

Hydrogenation of "β-Dihydroergosteryl Acetate" in Acid Solution.—(i) 130 Mg. of "β-dihydroergosteryl acetate" were dissolved in 1 : 1 ether–acetic acid (60 ml.) and hydrogenated during 7 hours in presence of 100 mg. of platinum oxide catalyst. The product was recrystallised from ethanol to give ergostanyl acetate, m. p. 143°, $[\alpha]_D$ +6° (*c*, 1·30; micro-tube), in 25% yield.

(ii) 150 Mg. of "β-dihydroergosteryl acetate" were hydrogenated as described above. The crude product, m. p. 110—120°, was crystallised once to give (*a*) 60 mg., m. p. 130—133°, $[\alpha]_D$ +5° (*c*, 2·49; micro-tube), and complete evaporation of the mother-liquors of this crystallisation afforded (*b*) 90 mg., m. p. *ca.* 100°, $[\alpha]_D$ +3° (*c*, 1·83; micro-tube). These m. p.s and rotations accord best with those for mixtures of ergostanyl acetate, m. p. 144°, $[\alpha]_D$ +6° (see below), and "α"-ergostenyl acetate, m. p. 109°, $[\alpha]_D$ +1° (*J.*, 1948, 783). The ratio of the two, calculated from the observed rotations, is about 1 : 1.

(iii) 160 Mg. of "β-dihydroergosteryl acetate" were hydrogenated as described above and the crude product was treated by the Anderson–Nabenhauer procedure (*J. Amer. Chem. Soc.*, 1924, **46**, 1957) to remove unsaturated material. In this way 80 mg. (50%) of ergostanyl acetate, m. p. 144°, $[\alpha]_D$ +6° (*c*, 1·80; micro-tube), were recovered. Again this corresponds to a 1 : 1-mixture of ergostanyl and "α"-ergostenyl acetates.

Hydrogenation of "β-Dihydroergosteryl Acetate" in Neutral Solution.—220 Mg. of "β-dihydroergosteryl acetate" in ethyl acetate (60 ml.) were shaken with 200 mg. of used platinum catalyst in an atmosphere of hydrogen for 27·5 hours. The product, recrystallised from methanol, melted constantly at 93·5—94·5° and had $[\alpha]_D$ +6° (*c*, 0·68). A mixed m. p. with the "αβ-ergostenyl acetate" complex, m. p. 93·5—94·5° (see below), also melted sharply at 93·5—94·5°. The hydrogenation product was hydrolysed and the resulting alcohol mixture benzoylated. The mixed benzoates were crystallised from chloroform–methanol till the m. p. reached 150°. A mixed m. p. with almost pure "β"-ergostenyl benzoate, m. p. 154°, prepared as below, gave m. p. 151°. The combined mother-liquors from the crystallisation of the mixed benzoates were concentrated, and successive crops of crystals removed. The last two crops, m. p. 98—100°, were recrystallised several times from chloroform–methanol to give "α"-ergostenyl benzoate, m. p. 110—111·5°; the mixed m. p. with an authentic specimen, m. p. 112—112·5° (*J.*, 1948, 783), was 110—111·5°.

Rearrangement of "α"-Ergostenyl Acetate.—2 G. of "α"-ergostenyl acetate, m. p. 109°, $[\alpha]_D$ −1° (*c*, 1·96), were isomerised as described above for the preparation of "β-dihydroergosteryl acetate." The "αβ-ergostenyl acetate," recrystallised from methanol, had m. p. 93·5—94·5°, $[\alpha]_D$ +8° (*c*, 5·5), +7° (*c*, 2·9), +6° (*c*, 1·16), unchanged on further recrystallisation. Alkaline hydrolysis and benzoylation of the product furnished, after repeated crystallisation from chloroform–methanol, almost pure "β"-ergostenyl benzoate, m. p. 153—154°.

"αβ-*Ergostenyl Acetate.*"—27·7 Mg. of "α"-ergostenol and 28·0 mg. of "β"-ergostenol (for prep. see *J.*, 1948, 783) were dissolved in pyridine (3 ml.), and acetylated and worked up in the usual way. After one recrystallisation from methanol, "αβ-ergostenyl acetate" was obtained in almost quantitative yield. It had m. p. 93·5—94·5°, undepressed on admixture with the neutral hydrogenation product, m. p. 93·5—94·5°, from "β-dihydroergosteryl acetate."

Preparation of iso*Ergosterone.*—Carefully purified ergosterone (10 g.) was dissolved in chloroform (100 ml.) and treated with dry hydrogen chloride at 0° for 1 hour. The reaction mixture was poured into cold sodium hydrogen carbonate solution, the chloroform solution washed with water, and the chloroform removed *in vacuo*. Crystallisation from ethyl acetate–methanol afforded *iso*ergosterone, m. p. 105°, in 60% yield. The yield was much reduced if ergosterone contaminated with mesityl oxide (from the Oppenauer oxidation) was employed. In our hands the procedure of Wetter and Dimroth (*Ber.*, 1937, **70**, 1665; cf. Heilbron, Kennedy, Spring, and Swain, *J.*, 1938, 869) gave *iso*ergosterone from ergosterone in only 20% yield.

Hydrogenation of iso*Ergosterone.*—5·8 G. of *iso*ergosterone and 150 mg. of platinum oxide in ethyl acetate (120 ml.) were shaken under hydrogen until 500 ml. had been absorbed (10 minutes). After removal of the catalyst by filtration and of the solvent by evaporation *in vacuo*, the crystalline product was dissolved in light petroleum (b. p. 40—60°) and chromatographed over alumina (Birlec grade H). A typical chromatogram is illustrated in the figure.

Coproergost-22(23)-en-3-one.—*Ketone* (B) (see text), recrystallised from acetone–methanol, had m. p. 110·5°, $[\alpha]_D$ −2° (*c*, 2·16), −1° (*c*, 1·75), $[M]_D$ −4° (Found: C, 84·2; H, 11·3. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6%). The ketone showed no absorption over the range 220—260 mμ. The 2 : 4-dinitrophenylhydrazone, prepared in the usual way, was yellow and, after chromatography and recrystallisation from chloroform–methanol, had m. p. 199°.

Coproergost-22(23)-en-3(α)-ol.—The above ketone (300 mg.) in *n*-propanol (30 ml.) was reduced, on the boiling water-bath, by the addition of sodium (3 g.). The steroidal product was taken up in 95% ethanol (50 ml.), treated with excess of digitonin in the same solvent, and worked up in the usual way (Schönheimer, *Z. physiol. Chem.*, 1933, **215**, 59). A small amount of insoluble digitonide was rejected and the coproergost-22(23)-en-3(α)-ol was recrystallised from ethyl acetate–methanol; m. p. 149—150°, $[\alpha]_D$ −4° (*c*, 2·13), −4° (*c*, 1·29; micro-tube), $[M]_D$ −16° (Found: C, 83·8; H, 11·9. $C_{28}H_{48}O$ requires C, 84·0; H, 12·0%).

Coproergost-22(23)-en-3(α)-yl acetate, recrystallised from ethyl acetate–methanol, had m. p. 114—115°, $[\alpha]_D$ +16° (*c*, 1·43), $[M]_D$ +71° (Found: C, 81·3; H, 11·1. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%).

Coproergost-22(23)-en-3(α)-yl benzoate, recrystallised from chloroform–methanol, had m. p. 146°, $[\alpha]_D$

+9° (*c*, 1·44; micro-tube), $[M]_D$ +45° (Found: C, 83·4; H, 10·1. $C_{35}H_{52}O_2$ requires C, 83·3; H, 10·3%).

Coproergost-22(23)-en-3(α)-yl Acetate 22 : 23-Dibromide.—Coproergost-22(23)-en-3(α)-yl acetate (40 mg.) was dissolved in 1 : 1 ether–acetic acid (3 ml.) and treated with a slight excess of bromine in the same solvents. The ether was removed by evaporation at room temperature *in vacuo*, and the dibromide isolated by the addition of methanol and recrystallised from chloroform–methanol; it had m. p. 190° without decomposition (Found: Br, 26·9. $C_{30}H_{50}O_2Br_2$ requires Br, 26·6%).

Hydrogenation of Coproergost-22(23)-en-3-one in Neutral Solution.—The ketone (40 mg.) in ethyl acetate (20 ml.) was hydrogenated for 3 hours in presence of 50 mg. of platinum oxide. After removal of the catalyst by filtration and of the solvent by evaporation *in vacuo*, the product was chromatographed over alumina to give traces of hydrocarbon (eluted first) and the bulk as a sterol fraction m. p. 125—135°, saturated to $C(NO_2)_4$. The sterol was treated with digitonin in the usual way (see above), a small quantity of insoluble digitonide removed, and the bulk worked up (Schönheimer, *loc. cit.*) to give, after crystallisation from methanol, coproergostan-3(α)-ol, m. p. 139°. This was converted into the acetate, recrystallised from methanol, m. p. 99°, $[\alpha]_D$ +39° (*c*, 1·02; micro-tube), undepressed in m. p. by admixture with authentic material, m. p. 99°, $[\alpha]_D$ +39° (Barton and Miller, this vol., p. 337).

Ergost-22(23)-en-3-one.—Ketone D (see text), recrystallised from acetone–methanol, had m. p. 165·5°, $[\alpha]_D$ +8° (*c*, 1·90; micro-tube), +6° (*c*, 1·55; micro-tube), $[M]_D$ +28° (Found: C, 84·5; H, 11·3. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6%). The ketone showed no absorption over the range 220—260 mμ. The yellow 2 : 4-dinitrophenylhydrazone, prepared in usual way, had m. p. 208—209°, after chromatography over alumina and crystallisation from chloroform–methanol.

Ergost-22(23)-en-3(β)-ol.—200 Mg. of the above ketone in *n*-propanol (20 ml.) were reduced by the addition of sodium (2 g.) on the boiling water-bath. The steroidal product, in hot 95% ethanol, furnished an insoluble digitonide which was decomposed in the usual way (Schönheimer, *loc. cit.*) to give *ergost-22(23)-en-3(β)-ol*, recrystallised from chloroform–methanol, m. p. 152°, $[\alpha]_D$ −9° (*c*, 2·07; micro-tube), −10° (*c*, 1·97, micro-tube), $[M]_D$ −40° (Found: C, 82·3; H, 12·0. $C_{28}H_{48}O,\frac{1}{2}H_2O$ requires C, 82·2; H, 12·0%).

Ergost-22(23)-en-3(β)-yl acetate, recrystallised from ethyl acetate–methanol, had m. p. 155·5°, $[\alpha]_D$ −17° (*c*, 1·42; micro-tube), $[M]_D$ −75° (Found: C, 80·9; H, 11·0. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%).

Ergost-22(23)-en-3(β)-yl benzoate, recrystallised from chloroform–methanol, had m. p. 140°, $[\alpha]_D$ −8° (*c*, 2·27; micro-tube), $[M]_D$ −40° (Found: C, 82·7; H, 10·4. $C_{35}H_{52}O_2$ requires C, 83·3; H, 10·3%).

Ergost-22(23)-en-3(β)-yl Acetate 22 : 23-Dibromide.—Ergost-22(23)-en-3(β)-yl acetate (40 mg.) in 1 : 1 ether–acetic acid (3 ml.) was treated with a slight excess of bromine in the same solvents. The *dibromide* crystallised from the reaction mixture and was purified by recrystallisation from chloroform–methanol; decomp. *ca.* 226° (Found: Br, 26·1. $C_{30}H_{50}O_2Br_2$ requires Br, 26·6%).

Hydrogenation of Ergost-22(23)-en-3-one in Neutral Solution.—The ketone (*D*) (see above) (13 mg.) was hydrogenated in ethyl acetate (20 ml.) for 3 hours, 25 mg. of platinum oxide being used. After the mixture had been worked up in the usual way, acetylation and recrystallisation from chloroform–methanol gave ergostanyl acetate, m. p. 144°, undepressed in m. p. when admixed with authentic material, m. p. 144°.

Ergosta-4(5) : 22(23)-dien-3-one.—Ketone (F) (see text), recrystallised from acetone–methanol, had m. p. 127·5—128·5°, $[\alpha]_D$ +43° (*c*, 4·63), +44° (*c*, 3·10; micro-tube), $[M]_D$ +174° (Found: C, 84·6; H, 11·0. $C_{28}H_{44}O$ requires C, 84·8; H, 11·1%). The ketone showed a strong absorption band in the ultra-violet in ethanol solution, $\lambda_{max.}$ 242 mμ., ε 18,200, characteristic for an αβ-unsaturated ketone. The red 2 : 4-*dinitrophenylhydrazone*, prepared in the usual way and purified by chromatography and recrystallisation from chloroform–methanol, had m. p. 242—243° (Found: N, 9·7. $C_{34}H_{48}O_4N_4$ requires N, 9·7%). The 4-phenylsemicarbazone, prepared as described previously (*J.*, 1948, 783) and recrystallised from chloroform–methanol had m. p. 229° (decomp.).

Oxidation of Brassicasterol.—Brassicasterol (150 mg.), obtained by hydrolysis of the brassicasteryl acetate, m. p. 151—153°, kindly supplied by Dr. H. E. Stavely, was oxidised by the Oppenauer method. The product was chromatographed over alumina and gave the following fractions (each after one recrystallisation from methanol):

(i) 100 ml. of light petroleum (b. p. 40—60°)	Nothing.
(ii) 350 ml. of 4 : 1 light petroleum (b. p. 40—60°)–benzene...	Traces of gum (mesityl oxide).
(iii) 25 ml. of 1 : 1 light petroleum (b. p. 40—60°)–benzene...	Ketonic material, m. p. 129°.
(iv) 25 ml. of benzene	Gum.
(v)—(xiii) 200 ml. of benzene each	All ketonic fractions, m. p. 123°.
(xiv) 50 ml. of 1 : 1 ether–methanol	Little unchanged brassicasterol.

Fractions (v)—(xiii) were combined and recrystallised from acetone–methanol, to give brassicastadienone, m. p. 127°, $[\alpha]_D$ +49° (*c*, 1·88; micro-tube), undepressed in m. p. by admixture with ergosta-4(5) : 22(23)-dien-3-one prepared as above. Recrystallisation of fraction (iii) yield 2 mg. of a ketone, m. p. *ca.* 135°.

Hydrogenation of Ergosta-4(5) : 22(23)-dien-3-one in Neutral Solution.—1·28 G. of pure ketone (*F*) (see above) were shaken in ethyl acetate (30 ml.) with platinum oxide (100 mg.) in an atmosphere of hydrogen until 85 ml. had been absorbed (1 minute). The product was chromatographed over alumina and gave coproergost-22(23)-en-3-one, m. p. 108—109°, $[\alpha]_D$ +1° (*c*, 1·48), undepressed in m. p. by admixture with ketone (*B*) above (m. p. 110°), and ergost-22(23)-en-3-one, m. p. 164—165°, $[\alpha]_D$ +8° (*c*, 0·50; micro-tube), undepressed in m. p. on admixture with ketone (*D*) above (m. p. 165°).

Ergost-22(23)-ene 22 : 23-Dibromide.—The hydrocarbon mixture (*A*) (see text) in 1 : 1 ether–acetic acid (5 ml.) was treated with a slight excess of bromine in the same solvents. The *dibromide* crystallised from the reaction mixture and after two recrystallisations from chloroform–acetone attained a reproducible decomposition point of 218° (Found: Br, 29·3. $C_{28}H_{48}Br_2$ requires Br, 29·4%).

Ergost-22(23)ene.—The above dibromide was treated in chloroform–acetic acid on the water-bath

for ½ hour with successive small portions of zinc dust. After filtration from excess zinc dust and working up of the product by recrystallisation from acetone, ergost-22(23)-ene, m. p. 107—108°, $[\alpha]_D$ −10° (*c*, 2·27; micro-tube), $[M]_D$ −38°, was obtained (Found: C, 87·1; H, 12·6. $C_{28}H_{48}$ requires C, 87·5; H, 12·5%).

Hydrogenation of Ergost-22(23)-ene in Neutral Solution.—25 Mg. of ergost-22(23)-ene were hydrogenated in ethyl acetate (30 ml.) for 3 hours, 100 mg. of platinum oxide being used. After removal of the catalyst by filtration and of the solvent by evaporation *in vacuo*, the product was recrystallised from chloroform–methanol to give ergostane, m. p. 82—83°, $[\alpha]_D$ +15° (*c*, 0·76; micro-tube), $[M]_D$ +58°.

Action of Hydrogen Chloride on Stigmasteryl Acetate.—After several hours' treatment of 300 mg. of stigmasteryl acetate, (m. p. 143°) in chloroform with excess of dry hydrogen chloride at 0° and working up of the product as described above for "β-dihydroergosteryl acetate," the starting material was (almost quantitatively) recovered, having m. p. 143°, not depressed by admixture with pure stigmasteryl acetate.

Hydrogenation of Ergost-14(15)-en-3(β)-ol.—Ergost-14(15)-en-3(β)-ol (0·3 g.) (*J.*, 1948, 783) was hydrogenated overnight in anhydrous ether (50 ml.) in presence of fresh platinum oxide. After being worked up in the usual way the product gave no colour in the Liebermann–Burchard reaction. One recrystallisation from methanol afforded ergostan-3(β)-ol, m. p. 141·5°, $[\alpha]_D$ +15° (*c*, 1·82), in almost quantitative yield. Use of 1 : 1 ether–acetic acid as solvent gave essentially the same result.

Ergostan-3-one.—This compound was prepared by chromic acid oxidation of ergostanol in the usual way and purified by chromatography over alumina. Recrystallised from ethyl acetate–methanol, it had m. p. 160°, $[\alpha]_D$ +33° (*c*, 1·45; micro-tube), $[M]_D$ +132°.

We thank the Chemical Society for a grant which defrayed the cost of the ergosterol used, and Dr. E. A. Braude and Mrs. I. Boston for determining the absorption spectra. The work was carried out during the tenure of an I.C.I. Fellowship by one of us (D. H. R. B.). We also acknowledge a maintenance grant from the D.S.I.R. (J. D. C.).

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
LONDON, S.W.7.

[*Received, November 24th*, 1948.]