# *The Action of Perphthalic Acid on 5-Dihydroergosteryl and Ergosteryl Acetates.*

By G. H. Alt and D. H. R. Barton.

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Treatment of 5-dihydroergosteryl acetate with perphthalic acid gives the corresponding 7 : 8-epoxide. Under controlled acid conditions the latter affords a mixture of ergosta-7 : 9 (11) : 22- and -7 : 14 : 22-trien-3β-yl acetates as well as a minor proportion of 7-oxoergost-22-en-3β-yl acetate.

Similar treatment of ergosteryl acetate affords a complex mixture, in which 3β-acetoxyergosta-7 : 22-diene-5α : 6β-diol has been identified. Evidence for the presence of the 6-(hydrogen phthalate) of this compound and of its 6-epimer has been adduced. Two other compounds of unknown structure have also been isolated.

The constitution of the minor yeast sterol, cerevisterol, has been elucidated as ergosta-7 : 22-diene-3β : 5α : 6β-triol.

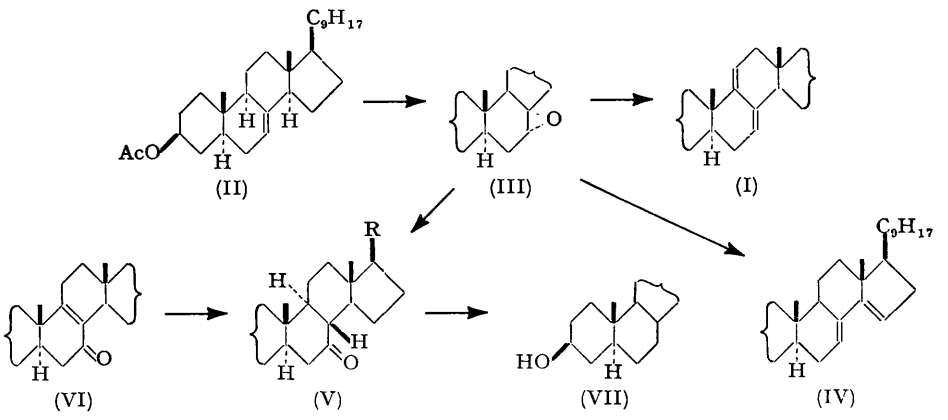
The conjugated diene 3β-acetoxyergosta-7 : 9(11) : 22-triene (ergosterol D acetate; Barton, *J.*, 1946, 512) (I) has become an important intermediate in potential cortisone syntheses (see Birch, *Ann. Reports*, 1951, 204; Cornforth, *ibid.*, 1952, 190). This compound is most efficiently prepared by dehydrogenation of 5-dihydroergosteryl acetate (II) with bromine or chlorine (Anderson, Budziarek, Newbold, Stevenson, and Spring, *Chem. and Ind.*, 1951, 1035 and subsequent papers) or with mercuric acetate (Windaus and Auhagen, *Annalen*, 1929, **472**, 185; Heilbron, Johnstone, and Spring, *J.*, 1929, 2248; see also Anderson *et al.*, *loc. cit.*). Dehydrogenation with selenium dioxide has also been reported (Callow and Rosenheim, *J.*, 1933, 387). We now describe an alternative route for the conversion of (II) into (I) (cf. Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119).

Treatment of (II) with one molecular proportion of perphthalic acid in ether gave the monoepoxide (III).* The α-configuration of the epoxide ring is based on the concept of preferred attack from the α-side of the molecule (Fieser, *Experientia*, 1950, **6**, 312; Gallagher and Kritchevsky, *J. Amer. Chem. Soc.*, 1950, **72**, 882). An alternative allylic

* The compound to which this structure was previously assigned (see Callow and Rosenheim, *loc. cit.*) has subsequently been shown by Fieser and Ourisson (*J. Amer. Chem. Soc.*, 1953, **75**, 4404) to be otherwise constituted.

alcohol formulation for (III) is excluded by its resistance to acetylation and by the absence of a hydroxyl band in the infra-red spectrum. Controlled acid-catalysed dehydration of (III) in aqueous dioxan afforded, as main product, a mixture of dienes. Treatment of this mixture with maleic anhydride in benzene solution gave, as the more insoluble product, the maleic anhydride adduct of ergosta-7 : 14 : 22-trien-3β-yl acetate (IV), whilst the more soluble product was identified as ergosta-7 : 9(11) : 22-trien-3β-yl acetate (I). The rotation of the diene mixture indicated that it contained 60% of the desired (I) and 40% of (IV). This was confirmed (*a*) by quantitative experiments with maleic anhydride (" dienometry ") as detailed in the Experimental section and (*b*) by the preparation of an artificial mixture of the dienes which had the same m. p., mixed m. p., and (by design) rotation.

The minor product of the acid-catalysed dehydration of (III) was identified as 7-oxoergost-22-en-3β-yl acetate (V; R = $C_9H_{17}$). An authentic specimen of this compound was prepared from 7-oxoergosta-8 : 22-dien-3β-yl acetate (VI) (Heusser, Eichenberger, Kurath, Dällenbach and Jeger, *Helv. Chim. Acta*, 1951, **34**, 2106) by lithium and liquid ammonia reduction followed by reacetylation. The stability of this compound to attempted base-catalysed inversion of configuration at $C_{(8)}$ was demonstrated. Palladium-catalysed hydrogenation afforded 7-oxoergostan-3β-yl acetate (V; R = $C_9H_{19}$) which on Wolff–Kishner reduction gave ergostanol (VII; R = $C_9H_{19}$). The correctness of the α-configuration at $C_{(9)}$ in (V; R = $C_9H_{17}$ and $C_9H_{19}$) is thus confirmed. The molecular rotations observed for (V; R = $C_9H_{17}$) and (V; R = $C_9H_{19}$) were −301° and −211° respectively. These are in good agreement with those calculated (−308° and −206° respectively) from standard data (Barton and Cox, *J.*, 1948, 1354; Barton, Cox, and Holness, *J.*, 1949, 1771; Barton and Klyne, *Chem. and Ind.*, 1948, 755).



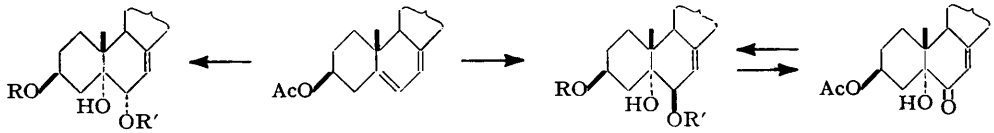

A complementary study of the action of perphthalic acid in ethereal solution on ergosteryl acetate (VIII) has also been undertaken. After separation of acid reaction products (hydrogen phthalates : see below) the neutral reaction products were chromatographed to give three compounds : $C_{30}H_{48}O_4$, $C_{30}H_{46}O_4$, and a conjugated diene $C_{30}H_{46}O_3$. The constitutions of the last-mentioned substances have not been elucidated with certainty, but the first has been identified as 3β-acetoxyergosta-7 : 22-diene-5α : 6β-diol (IX; R = Ac, R′ = H) on the basis of the following evidence. Mild acetylation furnished a diacetate (IX; R = R′ = Ac); similar benzoylation conditions gave an acetate benzoate (IX; R = Ac, R′ = Bz); alkaline hydrolysis afforded a triol (IX; R = R′ = H), converted into the diacetate on acetylation. Chromic acid oxidation gave 3β-acetoxy-5α-hydroxyergosta-7 : 22-dien-6-one (X) (Burawoy, *J.*, 1937, 409) from which (IX; R = R′ = H) was re-formed on lithium aluminium hydride reduction.* The assignment of

* Professors L. F. Fieser (Harvard) and C. W. Shoppee (Swansea) have very kindly informed us that they have also effected this reduction to (IX : R = R′ = H) : see Mary Fieser, Quilico, Nickon, Rosen, Tarlton, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4066; Blears and Shoppee, *Chem. and Ind.*, 1953, 947.

the 6β-configuration in (IX) was based, in our preliminary communication (Alt and Barton, *Chem. and Ind.*, 1952, 1103), on molecular-rotation arguments. These need not be repeated here, since compelling, and superior, chemical evidence for the correctness of this conclusion has recently been adduced by Fieser *et al.* (*loc. cit.*).

From the acid fraction (see above), after hydrolysis and reacetylation, a mixture of (IX; R = R′ = Ac) (30%) and of its 6-epimer (XI; R = R′ = Ac) (70%) was isolated. Since derivatives of (IX) do not isomerise to derivatives of (XI) under conditions at least as drastic as those involved in the perphthalic acid oxidation and subsequent processing, we conclude that (IX) and (XI) are initially present as the 6-(hydrogen phthalates) of (IX; R = Ac, R′ = H) and of (XI; R = Ac, R′ = H).

One of the minor yeast sterols, cerevisterol, $C_{28}H_{46}O_3$, was isolated and characterised as the diacetate by Honeywell and Bills (*J. Biol. Chem.*, 1932, **99**, 71; 1933, **103**, 515). It has also been obtained from *Amanita phalloides* and from ergot (Wieland and Coutelle, *Annalen*, 1941, **548**, 270). We noted a correspondence in properties between cerevisterol and its diacetate and (IX; R = R′ = H) and its diacetate. Through the courtesy of Dr. C. E. Bills, to whom we express our best thanks, it was possible to make a direct comparison and thus to confirm these identities. The constitution of cerevisterol as (IX; R = R′ = H) may therefore be taken as established (Alt and Barton, *loc. cit.*).




(XI) (VIII) (IX) (X)

## Experimental

For general experimental details see *J.*, 1952, 2339. $[\alpha]_D$ are in chloroform unless stated otherwise; ultra-violet absorption spectra are in ethanol. Infra-red spectra were kindly determined in carbon disulphide solution, unless specified to the contrary, by Messrs. Glaxo Laboratories Ltd. Light petroleum refers to that fraction of b. p. 40—60°.

*7-Oxoergost-22-en-3β-yl Acetate.*—7-Oxoergosta-8 : 22-dien-3β-yl acetate (400 mg.) (Heusser *et al.*, *loc. cit.*) in dry ether (40 ml.) was added to a solution of metallic lithium (200 mg.) in liquid ammonia (40 ml.) with vigorous stirring. The mixture was stirred for 30 min. and the excess of lithium destroyed by the addition of *n*-propanol. The product, after reacetylation (400 mg.), was chromatographed over alumina (12 g.; washed with ethyl acetate). Elution with 9 : 1 light petroleum–benzene gave *7-oxoergost-22-en-3β-yl acetate* (300 mg.). Recrystallised from methanol this had m. p. 185—187°, $[\alpha]_D$ −66° (*c*, 1·02) (Found: C, 78·95; H, 10·4. $C_{30}H_{48}O_3$ required C, 78·9; H, 10·6%). The acetate was recovered unchanged (m. p., mixed m. p., and rotation) after hydrolysis with 3% methanolic potassium hydroxide under reflux for 1 hr. followed by re-acetylation (pyridine–acetic anhydride overnight at room temperature).

*7-Oxoergostan-3β-yl Acetate.*—The unsaturated ketone (100 mg.) in ethyl acetate (20 ml.) was hydrogenated with a palladised calcium carbonate catalyst (5%; 100 mg.) for 4 hr., to give *7-oxoergostan-3β-yl acetate*, m. p. 178—180° (from methanol), $[\alpha]_D$ −46° (*c*, 1·10) (Found: C, 78·95; H, 10·9. $C_{30}H_{50}O_3$ requires C, 78·55; H, 11·0%). The ketone (65 mg.) in ethanol (2·5 ml.) was heated with a solution of sodium (200 mg.) in ethanol (3 ml.) and hydrazine hydrate (1 ml.) at 200° for 14 hr. Crystallisation from methanol afforded ergostan-3β-ol, identified by m. p., mixed m. p., and rotation {$[\alpha]_D$ + 15° (*c*, 1·24)}.

*7α : 8α-Epoxyergost-22-en-3β-yl Acetate.*—5-Dihydroergosteryl acetate (880 mg.) was treated with perphthalic acid (9 mg./ml.: 2 mols.) in ethereal solution at room temperature for 5 days (consumption of 1 mol. of per-acid). The product {800 mg.; m. p. 140—146°, $[\alpha]_D$ −14° (*c*, 1·32)} in light petroleum solution was filtered through alumina (ethyl acetate-washed). Crystallisation from light petroleum gave *7α : 8α-epoxyergost-22-en-3β-yl acetate* (200 mg.). This had m. p. 161—163°, $[\alpha]_D$ −19° (*c*, 1·32), and no ketonic absorption in the ultra-violet (Found: C, 78·7; H, 10·6. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%). It was recovered unchanged on attempted acetylation (pyridine–acetic anhydride at room temperature). Further elution of the alumina column with light petroleum afforded a small amount of a *compound*,

m. p. (from light petroleum) 190—192°, $[\alpha]_D$ −30° (*c*, 1·07) (Found: C, 76·0; H, 10·2. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·25%), which is probably the 7 : 8-22 : 23-diepoxide.

*Treatment of* 7α : 8α-*Epoxyergost-22-en-3β-yl Acetate with Acid.*—The oxide (4·0 g.) in dioxan (200 ml.; redistilled over sodium) and aqueous 2N-sulphuric acid (10 ml.) was left at room temperature for 48 hr. (no further increase in the intensity of the ultra-violet absorption spectrum). The product (4·0 g.), which had $\lambda_{max.}$ 236 and 243 mμ (ε 9200 and 10,200 respectively), in ethanol (150 ml.) was refluxed with semicarbazide hydrochloride (1·2 g.) and anhydrous sodium acetate (1·0 g.) for 20 min. The total product in benzene solution (50 ml.) was filtered through alumina (100 g.). Elution with benzene (100 ml.) and 19 : 1 benzene–ether gave a product (3·6 g.) which was crystallised from chloroform–methanol, to furnish a diene mixture (2·0 g.), m. p. 155—160°, $[\alpha]_D$ −70° (*c*, 1·80), $\lambda_{max.}$ 236 and 243 mμ (ε 11,800 and 13,000 respectively). Elution of the column with ether–methanol, hydrolysis with 5N-ethanolic sulphuric acid at room temperature for 24 hr., and re-acetylation (pyridine–acetic anhydride) gave a product which was chromatographed over alumina (34 g.). Elution with mixtures of light petroleum and benzene gave diene mixtures (200 mg.). Elution with 4 : 1 benzene–ether afforded fractions (200 mg.), which on crystallisation from methanol gave 7-oxoergost-22-en-3β-yl acetate, identified by m. p., mixed m. p., and rotation.

The diene mixture of $[\alpha]_D$ −70° (see above)(100 mg.) in dry benzene (5 ml.) was refluxed with maleic anhydride (15 mg.; redistilled) for 4 hr. Preliminary experiments showed that this time of reflux was adequate for the quantitive conversion of ergosta-7 : 14 : 22-trien-3β-yl acetate into its maleic anhydride adduct. The benzene was removed *in vacuo* and the residue extracted with light petroleum. Evaporation of the light petroleum and crystallisation from chloroform–methanol furnished ergosta-7 : 9(11) : 22-trien-3β-yl acetate (50 mg.), m. p. 172—174°, $[\alpha]_D$ +29° (*c*, 1·34), $\lambda_{max.}$ 236 and 242 mμ (ε 15,300 and 17,000 respectively), undepressed in m. p. on admixture with an authentic specimen of the same physical constants. The residue from the extraction with light petroleum was recrystallised from benzene, to give ergosta-7 : 14 : 22-trien-3β-yl acetate–maleic anhydride adduct, m. p. 197—203°, undepressed in m. p. on admixture with an authentic specimen of the same m. p. range.

To confirm that the diene mixture consisted solely of ergosta-7 : 9(11) : 22- and -7 : 14 : 22-trien-3β-yl acetates, the rotation of the diene mixture before and after refluxing with maleic anhydride was determined and the resultant rotational shift was compared with that which would be expected on the basis that the diene mixture was a two-component system of composition determined by its initial rotation. All rotatons were taken in benzene. The pure compounds had: ergosta-7 : 9(11) : 22-trien-3β-yl acetate, $[\alpha]_D$ +23° (*c*, 2·08); ergosta-7 : 14 : 22-trien-3β-yl acetate, $[\alpha]_D$ −212° (*c*, 1·12); derived maleic anhydride adduct, $[\alpha]_D$ −96° (*c*, 0·77). The results obtained, shown in the Table, indicate that the diene mixture

| | Control mixture of ergosta-7 : 9(11) : 22- (56·5%) and -7 : 14 : 22-(43·5%)-trien-3β-yl acetates | | Mixture of dienes. Calc. by assuming ergosta-7 : 9(11) : 22- (60%) and -7 : 14 : 22 (40%) -trien-3β-yl acetates | |
|---|---|---|---|---|
| | Obsd. | Calc. from known composition of mixture | Obsd. | Calc. on above assumption |
| $[\alpha]_D$ (in $C_6H_6$) of mixture | −78° (*c*, 3·20) | −79° | −68° (*c*, 3·26) | −71° |
| $[\alpha]_D$ (in $C_6H_6$) after refluxing with 2·5 mols. of maleic anhydride | −35° (*c*, 1·92) | −37° | −33° (*c*, 1·96) | −33° |

consisted of 60% of ergosta-7 : 9(11) : 22-trien-3β-yl acetate and 40% of the 7 : 14 : 22-isomer (the validity of the method is shown by the results given in the Table for the artificial 56·5 : 43·5 mixture made up from pure components). This was confirmed by the preparation of an artificial mixture of the two acetates in these proportions which had m. p. 155—160°, $[\alpha]_D$ −69° (*c*, 1·47), and gave no depression in m. p. on admixture with the diene mixture of $[\alpha]_D$ −70°.

*Treatment of Ergosteryl Acetate with Perphthalic Acid.*—Ergosteryl acetate (8·8 g.) was treated with perphthalic acid (25 mg./ml.; 3 mols.) in ether at 0° for 18 hr. (uptake of 1·5 mols.). The solution was washed with 5% aqueous sodium hydroxide and water. Removal of the ether *in vacuo* and azeotropic drying with benzene furnished a solid (5·5 g.), m. p. 150—170°, $\lambda_{max.}$ 253 mμ (ε 4000). This was dissolved in benzene (150 ml.) and filtered through alumina (120 g.; ethyl acetate-washed). Elution with benzene gave material (2·4 g.) of m. p. 165—185°. Elution with 1 : 1 benzene–ether afforded material (2·5 g.), m. p. 253—257°, which, on

crystallisation from chloroform–methanol, furnished 3β-*acetoxyergosta*-7 : 22-*diene*-5α : 6β-*diol*, m. p. 255—258°, $[\alpha]_D$ −52° (*c*, 1·09) (Found : C, 75·25; H, 10·15. $C_{30}H_{48}O_4,\frac{1}{2}CH_3{\cdot}OH$ requires C, 74·95; H, 10·3%), giving a pale yellow colour with tetranitromethane. Acetylation with pyridine–acetic anhydride overnight at room temperature gave the *diacetate*, m. p. 168—170° (from ethanol), $[\alpha]_D$ −149° (*c*, 1·58) (Found : C, 74·25; H, 9·65. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%), undepressed in m. p. on admixture with authentic cerevisterol diacetate of m. p. 169—171°, $[\alpha]_D$ −143° (*c*, 1·07). Hydrolysis of the monoacetate (150 mg.) with methanolic potassium hydroxide (5%; 10 ml.) gave ergosta-7 : 22-diene-3β : 5α : 6β-triol. Recrystallised from ethyl acetate this had m. p. 253—256°, $[\alpha]_D$ −84° (*c*, 1·39 in pyridine), undepressed in m. p. on admixture with an authentic specimen of cerevisterol, m. p. 256—259°, $[\alpha]_D$ −83° (*c*, 0·89 in pyridine); reacetylation gave the diacetate mentioned above (m. p. and mixed m. p.).

Treatment of the monoacetate (100 mg.) with pyridine–benzoyl chloride overnight at room temperature gave 3β-*acetoxy*-6β-*benzoyloxyergosta*-7 : 22-*dien*-5α-*ol*, m. p. 179—182° (from ether–methanol), $[\alpha]_D$ −156° (*c*, 0·84) (Found : C, 77·4; H, 9·2. $C_{37}H_{52}O_5$ requires C, 77·05; H, 9·1%), depressed in m. p. on admixture with an authentic specimen of 3β-acetoxy-6α-benzoyloxyergosta-7 : 22-dien-5α-ol, m. p. 186—187°, $[\alpha]_D$ +44° (*c*, 1·14), prepared by the method of Windaus and Lüttringhaus (*loc. cit.*).

The monoacetate (100 mg.) in " AnalaR " acetic acid (5 ml.) was treated with chromium trioxide (17 mg.; 1·2 mols.) in the minimum of water and left overnight at room temperature. Recrystallisation of the product from ethyl acetate furnished 3β-acetoxy-5α-hydroxyergosta-7 : 22-dien-6-one, m. p. 262—264°, $[\alpha]_D$ −4° (*c*, 1·46), $\lambda_{max.}$ 250 mμ (ε 13,500), undepressed in m. p. on admixture with an authentic specimen (Burawoy, *J.*, 1937, 409) of the same m. p. and rotation. This ketone (200 mg.) was extracted from the thimble of a Soxhlet extractor into a solution of lithium aluminium hydride (100 mg.) in dry ether (50 ml.). Working up in the usual way and crystallisation from ethyl acetate furnished ergosta-7 : 22-diene-3β : 5α : 6β-triol, m. p. and mixed m. p. 253—256°, $[\alpha]_D$ −83° (*c*, 1·19 in pyridine).

The material (2·4 g.), m. p. 165—185°, mentioned above was rechromatographed over alumina (60 g.; ethyl acetate-washed). Elution with light petroleum afforded a *compound* (1·5 g.) which, after recrystallisation from ethyl acetate–methanol, had m. p. 209—212°, $[\alpha]_D$ −103° (*c*, 1·94), no selective absorption in the range 220—320 mμ (Found : C, 76·85; H, 9·75. $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%).

Elution with 1 : 1 light petroleum–benzene furnished a further *compound* (250 mg.) which, recrystallised from methanol, had m. p. 126—128°, $[\alpha]_D$ +33° (*c*, 1·65 in $CCl_4$), $\lambda_{max.}$ 253 mμ (ε 25,500) (Found : C, 79·3; H, 10·3. $C_{30}H_{46}O_3$ requires C, 79·25; H, 10·2%). This substance deteriorates rapidly to a yellow gum and is also very sensitive to acid. To show that it was not an αβ-unsaturated ketone the compound (70 mg.) in dry ether (50 ml). was refluxed with lithium aluminium hydride (70 mg.) for 24 hr. Working up, reacetylation (pyridine–acetic anhydride at room temperature overnight), and crystallisation from ethyl acetate–methanol gave back starting material, identified by m. p., mixed m. p., rotation, and absorption spectrum.

*Examination of the Sodium Hydroxide Wash-liquors.*—The 5% sodium hydroxide wash-liquors (see above; from a preparation starting with 2·2 g. of ergosteryl acetate) were acidified with aqueous hydrochloric acid and extracted with ether. The product was refluxed with 5% methanolic potassium hydroxide (100 ml.) for 1 hr. Crystallisation of the hydrolysate from ethanol gave a product (800 mg.), m. p. 235—237°, $[\alpha]_D$ −6° (*c*, 1·10 in pyridine). This was acetylated (pyridine–acetic anhydride overnight at room temperature) and chromatographed over alumina (15 g.; ethyl acetate-washed). Elution with light petroleum and mixtures therewith containing up to 50% of benzene gave 3β : 6α-diacetoxyergosta-7 : 22-dien-5α-ol, m. p. (from ethanol) 178—180°, $[\alpha]_D$ +34° (*c*, 2·24), undepressed in m. p. on admixture with an authentic specimen of m. p. 179—181°, $[\alpha]_D$ +38° (*c*, 1·15), prepared according to the method of Windaus and Lüttringhaus (*loc. cit.*). Elution with benzene gave 3β : 6β-diacetoxyergosta-7 : 22-dien-5α-ol, m. p. (from ethanol) 168—170°, $[\alpha]_D$ −146° (*c*, 1·11), undepressed in m. p. on admixture with an authentic specimen (see above).

The assumption that the material of m. p. 235—237°, $[\alpha]_D$ −6° (see above), was a mixture of ergosta-7 : 22-diene 3β : 5α : 6β- and 3β : 5α : 6α-triol showed from the rotation that it must be of the composition 30 : 70. An authentic mixture of these proportions had, after crystallisation, m. p. and mixed m. p. 235—237°.

*Attempted Epimerisation of* 6β-*Compounds.*—3β-Acetoxyergosta-7 : 22-diene-5α : 6β-diol (53 mg.) in chloroform (5 ml.) underwent no change during 2 days at room temperature or 1 hr. under reflux. Similarly the corresponding 6β-benzoate was unchanged in refluxing chloroform solution (2 hr.) with or without the addition of benzoic acid (2 mols.). The 3-acetate 6β-benzoate

(10 mg.) was also unchanged when left in chloroform (3 ml.) solution containing toluene-*p*-sulphonic acid (0·6 mg.) at room temperature for 3 days.

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BIRKBECK COLLEGE, LONDON, W.C.1. [*Received, October 21st,* 1953.]