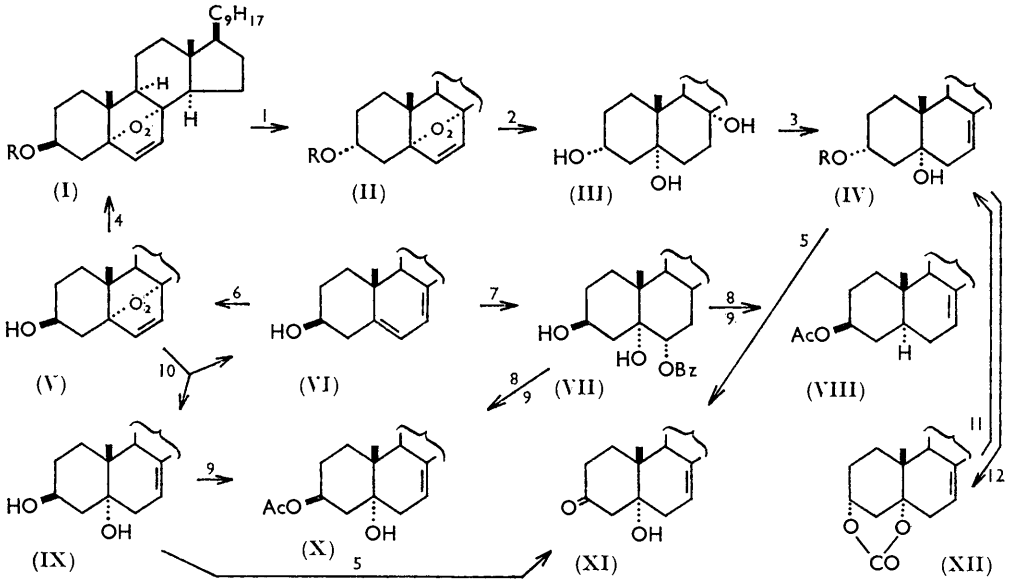
364. *Proof of Rear Approach in the Oxidation of Ergosterol.*

By F. Dalton and G. D. Meakins.

Many products obtained by oxidation of the ring B diene system in ergosterol have been formulated as arising from rear approach to the less hindered (α) side of the molecule. The present work provides chemical proof of the α-configuration of the 5-hydroxyl group in one of these products (the triol monobenzoate formed by oxidising ergosterol with perbenzoic acid).

In deducing the stereochemistry of products formed from steroids frequent use has been made of the "rule of rear attack," that certain reagents usually approach the steroid molecule from the less hindered (α) side.[1] Many exceptions to the rule are met even with natural steroids, especially when addition to ring A or B is involved,[2] and the rule is not applicable when the basic steroid stereochemistry is modified.[3] Results expected from the rule have been confirmed in some cases, such as the α-epoxidation of cholesterol,[4] by direct proof of the structures of the products. Elsewhere the stereochemical details are inferred by analogy, and the main support for the structures so deduced is that they lead to satisfactory explanations of the relations between the products. The series of compounds [1] formed by oxidising the 5,6-double bond of ergosterol and ergosteryl acetate fall into the latter category. While the accepted structures of the products are in accord with



Reagents: 1, Al_2O_3–KOH. 2, H_2–Pt. 3, HCl–MeOH. 4, p-$C_6H_4Me{\cdot}SO_2Cl$–C_5H_5N. 5, CrO_3–$COMe_2$. 6, O_2, irradiation. 7, BzO_2H–$CHCl_3$. 8, Li–NH_2Et. 9, Ac_2O–C_5H_5N. 10, $LiAlH_4$. 11, KOH–MeOH. 12, $COCl_2$–C_5H_5N.

their reactions and are supported by molecular-rotation data [5] there is no chemical proof that they arise by oxidation at the α-face of ergosterol and ergosteryl acetate.

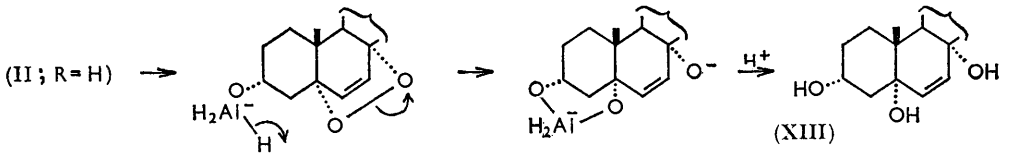
In the present work the α-orientation of the 5-hydroxyl group in the triol monobenzoate (VII) (obtained by oxidising ergosterol with perbenzoic acid [6]) was established by the

[1] For references see Fieser and Fieser, "Steroids," Reinhold Publ. Co., New York, 1959, pp. 14, 98.
[2] See, *inter al.*, Petrow, Rosenheim, and Starling, *J.*, 1943, 135; Henbest and Wilson, *J.*, 1956, 3289; Corey and Sneen, *J. Amer. Chem. Soc.*, 1956, **78**, 6289.
[3] Castells, Fletcher, Jones, Meakins, and Swindells, *J.*, 1960, 2627, and subsequent papers.
[4] Plattner and Lang, *Helv. Chim. Acta*, 1944, **27**, 1872.
[5] Zürcher, Heusser, Jeger, and Geistlich, *Helv. Chim. Acta*, 1954, **37**, 1562; Mills, *J.*, 1952, 4976.
[6] Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119.

following method, the 3β-hydroxyl group of ergosterol being used as a reference point. After inversion of the 3β-hydroxyl group in a suitable derivative of ergosterol, *i.e.*, the 5,8-epidioxide (V), the product was converted into the 3,5-diol (IV; R = H). In this diol the *cis*-orientation of the hydroxyl groups (and therefore the α-configuration of the 5-hydroxyl group) was demonstrated by the formation of a cyclic carbonate (XII). Oxidation of the diol gave the 5-hydroxy-3-ketone (XI). This hydroxy-ketone was also obtained by a route which related it to the triol monobenzoate, thus establishing the presence of a 5α-hydroxyl group in the latter compound. A somewhat similar sequence was used to establish the preference for attack at the β-face in the oxidation of lumisterol.[7]

In view of the failure of attempts [8] to epimerise the hydroxyl group of ergosterol itself the hydroxy-epidioxide [9] (V) in which the ring B diene system is masked was chosen as starting material: the epidioxide bridge is stable to the alkaline conditions used in the epimerisation of the 3-hydroxyl group, and is suitable for the production of a 5-hydroxyl group at a later stage. On treatment with alumina impregnated with potassium hydroxide [10] the corresponding toluene-*p*-sulphonate (I; R = *p*-$C_6H_4Me{\cdot}SO_2$) gave a new hydroxy-epidioxide (yield 43% after allowance for recovered toluene-*p*-sulphonate) which gave an acetoxy-epidioxide under mild conditions. The method of preparation and properties of these compounds show them to be the 3α-hydroxy- and 3α-acetoxy-epidioxides (II; R = H and Ac, respectively).

Reduction of the 3β-hydroxy-epidioxide (V) with lithium aluminium hydride followed by acetylation of the product yields the 3β-acetoxy-5α-hydroxy-compound (X) and ergosteryl acetate.[11] It was hoped that reduction of the 3α-hydroxy-epidioxide would similarly give the required 3α,5α-diol (IV; R = H), but the major product was a triol formulated as the 3α,5α,8α-trihydroxy-compound (XIII). [Only one of the triol's hydroxyl groups was acetylated on treatment with acetic anhydride in pyridine at 20°. The triol was also obtained from the 3α-hydroxy-epidioxide by reduction with zinc and methanolic sodium hydroxide, conditions which are known to convert the 3β-hydroxy-epidioxide (V) into the epimeric 3β,5α,8α-triol.[1] These facts, together with the α-orientation of the epidioxide bridge (see below), establish the structure of triol (XIII).] The preferential formation of the $\Delta^{6,22}$-3,5,8-triol (rather than the $\Delta^{7,22}$-3,5-diol) in the reduction of the 3α-hydroxy-epidioxide (II; R = H) with lithium aluminium hydride may be attributed to the proximity of the 3-hydroxyl and epidioxide groups, fission of the O–O bond occurring as in the annexed scheme.



The required $\Delta^{7,22}$-3,5-diol (IV; R = H) was obtained from the 3α-hydroxy-epidioxide (II; R = H) by an alternative route which involved hydrogenation of the epidioxide in ethyl acetate to the Δ^{22}-3,5,8-triol (III) and dehydration of this compound with methanolic hydrogen chloride. (Similar treatment of the 3β-hydroxy-epidioxide gives the $\Delta^{7,22}$-3β,5α-diol.[12]) Proof that the diol (IV; R = H) is a 3,5- rather than a 3,8-dihydroxy-compound follows from results described below: if the positions of the hydroxyl groups are assumed their *cis*-relation is shown by the formation of a cyclic carbonate (XII) from the diol (IV; R = H). [The possibility that carbonyl chloride had coupled two molecules of

[7] Mayor and Meakins, *J.*, 1960, 2792.
[8] Barnett, Heilbron, and Jones, *J.*, 1940, 1390.
[9] Windaus and Brunken, *Annalen*, 1928, **460**, 225.
[10] Douglas, Ellington, Meakins, and Swindells, *J.*, 1959, 1720.
[11] Laws, *J.*, 1953, 4185.
[12] Clayton, Henbest, and Jones, *J.*, 1953, 2015.

the diol (IV; R = H) to give a " dimeric " carbonate was excluded by a molecular-weight determination.]

Mild oxidation of the diol (IV; R = H), in which the hydroxyl groups are now established to have the α-configuration, afforded the 5α-hydroxy-3-ketone (XI). To complete the work it was necessary to link the hydroxy-ketone (XI) to the triol monobenzoate (VII). Reduction of the latter compound with lithium in ethylamine and acetylation of the products gave mainly 5α-ergosta-7,22-dien-3β-yl acetate (VIII), the required 3β-acetoxy-5α-hydroxy-compound [12] (X) being formed in only low yield. However, the related 3β,5α-diol (IX) was readily obtained [by reduction of the 3β-hydroxy-epidioxide (V) with lithium aluminium hydride], and the acetylation [12] of this diol to the 3β-acetoxy-5α-hydroxy-compound (X) was confirmed. Oxidation of the 3β,5α-diol afforded the 5α-hydroxy-3-ketone (XI) already obtained from the 3α,5α-diol (IV; R = H).

Since the tertiary hydroxyl groups in the triol monobenzoate (VII) and the diol (IX) are known to be at position 5 the corresponding groups in the hydroxy-ketone (XI) and the diol (IV) must occupy the same position. The present work establishes the α-configuration of these groups, and thus confirms the α-orientation of the epidioxide bridge in ergosterol epidioxide (V) originally suggested from the rule of rear attack [1] and later supported by more direct evidence.[12]

Experimental

For general directions see *J.*, 1958, 2156.

5α,8α-Epidioxyergosta-6,22-dien-3α-ol (II; R = H).—Toluene-*p*-sulphonyl chloride (6 g.) was added to a solution of 5α,8α-epidioxyergosta-6,22-dien-3β-ol (V) (8 g.; m. p. 183—186°, $[\alpha]_D$ −35°, prepared directly from ergosterol by the method used in converting ergosteryl acetate into the acetate of the epidioxide [12]) in pyridine (40 ml.) at 0° and the mixture was kept at 0° for 2 days. After addition of water the insoluble material was collected and crystallised from ethanol, to give the *toluene*-p-*sulphonate* (I; R = *p*-$C_6H_4Me{\cdot}SO_2$) (10 g.) as plates, m. p. with decomp. dependent on rate of heating, $[\alpha]_D$ 0° (*c* 1·3) (Found: C, 71·7; H, 8·4. $C_{35}H_{50}O_5S$ requires C, 72·1; H, 8·65%), $\nu_{max.}$ (in Nujol) 1198, 1180, 971, and 693 cm.$^{-1}$.

The toluene-*p*-sulphonate (10 g.) was adsorbed from benzene on a column of alumina (750 g., impregnated with potassium hydroxide) in the usual way.[10] The solution obtained by elution with ether–methanol (8 : 1; 700 ml.) was washed with water, dried, and evaporated, and the mixture (8·9 g.) so obtained was chromatographed on deactivated alumina (800 g.). Elution with light petroleum–benzene (3 : 2) afforded 5α,8α-*epidioxyergosta*-2(?),6,22-*triene* (210 mg.; m. p. 116—118° after crystallisation from methanol), $[\alpha]_D$ +7° (*c* 1·4) (Found: C, 81·8; H, 10·3. $C_{28}H_{42}O_2$ requires C, 81·9; H, 10·3%), $\nu_{max.}$ (in Nujol) 1031, 1015, and 976 cm.$^{-1}$. Elution with benzene gave unchanged toluene-*p*-sulphonate (I; R = *p*-$C_6H_4Me{\cdot}SO_2$) (5·18 g.), $[\alpha]_D$ 0° (*c* 1·2), identified by its infrared spectrum. Benzene–ether (9 : 1) eluted material (2·07 g.) which crystallised from ethanol to give 5α,8α-*epidioxyergosta*-6,22-*dien*-3α-*ol* (II; R = H) (1·52 g.), m. p. 192—195° depressed on admixture with the 3β-alcohol (V), $[\alpha]_D$ −30° (*c* 1·6) (Found: C, 78·3; H, 10·3. $C_{28}H_{44}O_3$ requires C, 78·45; H, 10·35%), $\nu_{max.}$ (in Nujol) 3478, 3425, 1042 sh, 1036, 1013, and 966 cm.$^{-1}$.

Acetylation of the 3α-hydroxy-epidioxide with acetic anhydride–pyridine at 20° afforded the 3α-*acetoxy-epidioxide* (II; R = Ac), m. p. 210—213° (from ethanol), $[\alpha]_D$ 0° (*c* 1·6) (Found: C, 76·6; H, 10·0. $C_{30}H_{46}O_4$ requires C, 76·6; H, 9·85%), $\nu_{max.}$ (in Nujol) 1730, 1266, 1241, and 965 cm.$^{-1}$.

Reduction of 5α,8α-*Epidioxyergosta*-6,22-*dien*-3α-*ol* (II; R = H).—(a) *With lithium aluminium hydride.* A solution of the hydroxy-epidioxide (800 mg.) in ether (100 ml.) was refluxed with lithium aluminium hydride (650 mg.) for 2 days. After addition of ethyl acetate and then 2N-sulphuric acid the products were isolated with ethyl acetate and chromatographed on deactivated alumina (100 g.). Benzene eluted material (260 mg.) which was repeatedly crystallised from acetone and then sublimed, to give an unidentified substance (40 mg.) with m. p. 168—175°, $[\alpha]_D$ +14° (*c* 1·5), $\nu_{max.}$ (in Nujol) 3257, 1044, 1020, and 966 cm.$^{-1}$, $\lambda_{max.}$ 288 mμ (ε 13,400). Benzene–ether (9 : 1) eluted *ergosta*-6,22-*diene*-3α,5α,8α-*triol* (XIII) (210 mg.; m. p.

178—181° after crystallisation from ethyl acetate), $[\alpha]_D$ −23° (*c* 1·7) (Found: C, 78·3; H, 10·9. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·8%), $\nu_{max.}$ (in Nujol) 3125, 1028, 1012, and 973 cm.$^{-1}$.

Treatment of this triol with acetic anhydride–pyridine at 20° afforded a compound with m. p. 159—161°, $\nu_{max.}$ (in CS_2) 3571, 1751, 1244, 1212, 1014, and 974 cm.$^{-1}$. The molecular extinction coefficient (ε 550) of the acetate peak at 1751 cm.$^{-1}$ showed that only one acetoxyl group was present.

(b) *With zinc and alkali.* A solution of the hydroxy-epidioxide (200 mg.) in 5% methanolic sodium hydroxide (60 ml.) was refluxed with zinc dust (500 mg.) for 1 hr. The mixture was filtered and the filtrate extracted with ether, to give the triol (XIII) (110 mg.), m. p. 175—178°, identified by mixed m. p. and comparison of infrared spectra with an authentic specimen.

(c) *Catalytic reduction.* A solution of the hydroxy-epidioxide (800 mg.) in the minimum volume of ethyl acetate was shaken in hydrogen with prereduced Adams catalyst (70 mg.). *Ergost-22-ene-3α,5α,8α-triol* (III) was precipitated during the hydrogenation, which was stopped after 70 ml. of hydrogen had been adsorbed. After the addition of more ethyl acetate the mixture was warmed to dissolve the triol, and filtered. The filtrate was concentrated and cooled to give the triol (620 mg.) as needles, m. p. 180—203°. Two crystallisations from ethyl acetate afforded material (450 mg.) with m. p. 195—208°, $[\alpha]_D$ −75° (*c* 2·0) (Found: C, 77·8; H, 11·4. $C_{28}H_{48}O_3$ requires C, 77·7; H, 11·2%), $\nu_{max.}$ (in Nujol) 3110, 1030, 1013, and 967 cm.$^{-1}$.

Ergosta-7,22-diene-3α,5α-diol (IV; R = H).—A trace of methanolic hydrochloric acid was added to a solution of the preceding triol (III) (500 mg.) in the minimum volume of methanol. The mixture was shaken at 20° until a precipitate appeared. Water was added and the mixture extracted with ether to give the *diol* (IV; R = H) (250 mg.), m. p. 196—198° (after two crystallisations from ethyl acetate), $[\alpha]_D$ −1° (*c* 1·7) (Found: C, 80·8; H, 11·2. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%), $\nu_{max.}$ (in Nujol) 3115, 1033, 1021, 968, and 864 cm.$^{-1}$. Acetylation at 20° afforded the *acetate* (IV; R = Ac), m. p. 153—155° (plates from methanol), $[\alpha]_D$ −22° (*c* 1·3) (Found: C, 78·5; H, 10·6. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%), $\nu_{max.}$ (in Nujol) 3478, 3430, 1732, 1703, 1258, 1233, 1022, and 967 cm.$^{-1}$.

Ergosta-7,22-dien-3α,5α-ylene Carbonate (XII).—Toluene (75 ml.), saturated with carbonyl chloride at 5°, was added to a solution of the diol (IV; R = H) (150 mg.) in a mixture of pyridine (3 ml.) and chloroform (50 ml.). After being kept at 20° for 2 days the mixture was washed successively with saturated aqueous potassium hydrogen carbonate (to remove excess of reagent), 2N-hydrochloric acid (2 × 50 ml.), aqueous potassium hydrogen carbonate, and water. After evaporation of the dried solution the residue crystallised from acetone, to give the *carbonate* as needles (120 mg.), m. p. 235—239°. Recrystallisation from chloroform–methanol afforded material (80 mg.), m. p. 245—248°, $[\alpha]_D$ 0° (*c* 1·3) [Found: C, 78·7; H, 10·0%; *M* (Rast), 421. $C_{29}H_{44}O_3$ requires C, 79·0; H, 10·1%; *M*, 441], $\nu_{max.}$ (in Nujol) 1718 (C=O; value in CCl_4, 1764), 1252, 1229, 1185, 1078, 1064, and 967 cm.$^{-1}$.

Hydrolysis of the carbonate (35 mg.) with boiling 5% methanolic potassium hydroxide (5 ml.) for 1 hr. gave the 3α,5α-diol (IV; R = H) (20 mg.), m. p. and mixed m. p. 194—196°, $[\alpha]_D$ −1°.

Reduction of 6α-Benzoyloxy-7,22-diene-3β,5α-diol (VII).—Freshly cut lithium (1·05 g.) was added in portions to a stirred solution of the triol monobenzoate [6] (1·5 g.) in ethylamine (125 ml.) containing a few drops of liquid ammonia, the reaction flask being fitted with a " cold finger " filled with acetone and solid carbon dioxide. Stirring was continued for 10 min. after the solution had developed a blue colour: water was then added and the mixture extracted with ether. The material so obtained was dissolved in pyridine (10 ml.) and acetic anhydride (10 ml.) and kept at 20° for 24 hr. The acetylated product was chromatographed on deactivated alumina (150 g.). Light petroleum–benzene (3 : 1) eluted 5α-ergosta-7,22-dien-3β-yl acetate (VIII) (382 mg. after crystallisation from methanol), m. p. and mixed m. p. 182—184°. Elution with benzene (30 ml. portions) gave materials which were collected into two main fractions of about equal weight. The later fraction crystallised from ethyl acetate to give 3β-acetoxy-ergosta-7,22-dien-5α-ol (X) as needles (200 mg.), m. p. and mixed m. p. with authentic material (see below) 227—231°, $[\alpha]_D$ +2° (*c* 1·1).

Ergosta-7,22-diene-3β,5α-diol (IX).[11,12]—A solution of ergosterol epidioxide (V) (1·5 g.) in ether was refluxed with lithium aluminium hydride (1·25 g.) for 2 days. After addition of ethyl acetate and then 2N-sulphuric acid the products were isolated with ethyl acetate and chromatographed on deactivated alumina (100 g.). Benzene–ether (3 : 1) eluted ergosterol (250 mg.), m. p. 156—160° (from methanol). The material eluted with ether crystallised from ethyl

acetate to give the 3β,5α-diol as plates (583 mg.), m. p. 229—233°, $[\alpha]_D$ +2° (*c* 1·3), $\nu_{max.}$ (in Nujol) 3400, 3284, 1055, 1026, and 975 cm.$^{-1}$. Acetylation with acetic anhydride–pyridine at 20° gave the 3β-acetate (X), m. p. 228—232° (from ethyl acetate), $[\alpha]_D$ +2° (*c* 1·2), $\nu_{max.}$ 3571, 3443, 1735, 1250, and 977 cm.$^{-1}$. Clayton *et al.*[12] found m. p. 227—234°, $[\alpha]_D$ +1° for the diol, and m. p. 228—233°, $[\alpha]_D$ +2° for the monoacetate: Laws's values [11] were m. p. 237—238°, $[\alpha]_D$ +0·5° and m. p. 230—232°, $[\alpha]_D$ 0°, respectively.

5α-*Hydroxyergosta*-7,22-*dien*-3-*one* (XI).—A solution of the 3α,5α-diol (IV; R = H) (95 mg.) in acetone (50 ml.) was cooled by addition of solid carbon dioxide. 8N-Chromic acid (0·2 ml.) was added, and the mixture swirled for 30 sec. and then poured into water. The material isolated with ether was chromatographed on deactivated alumina (10 g.). Benzene eluted the *hydroxy-ketone* (XI) (50 mg.), m. p. 242—244° (from ethanol), $[\alpha]_D$ +5° (*c* 1·6) (Found: C, 81·3; H, 10·65. $C_{28}H_{44}O_2$ requires C, 81·5; H, 10·75%), $\nu_{max.}$ 3400, 1718, 1020, and 965 cm.$^{-1}$.

Oxidation of the 3β,5α-diol (IX) (135 mg.) in acetone (50 ml.) with 8N-chromic acid (0·3 ml.) for 75 sec. at 20° gave the same hydroxy-ketone (75 mg.), m. p. 241—244°.

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THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, November 3rd*, 1960.]