1291. *Photo-oxygenation of 3-Acetoxyergosta-3,5,7,22-tetraene and Related Compounds.*[1]

By Peter Bladon and Thomas Sleigh

The photochemical reaction of 3-acetoxyergosta-3,5,7,22-tetraene with oxygen yields 3-acetoxyergosta-3,5,7,9(11),22-pentaene, 3-acetoxy-5α,8α-epidioxyergosta-6,22-diene, and 3-acetoxy-5α,14α-dihydroxyergosta 3,7,22 triene-6-one. The epidioxide on treatment with alkali undergoes rearrangement to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one.

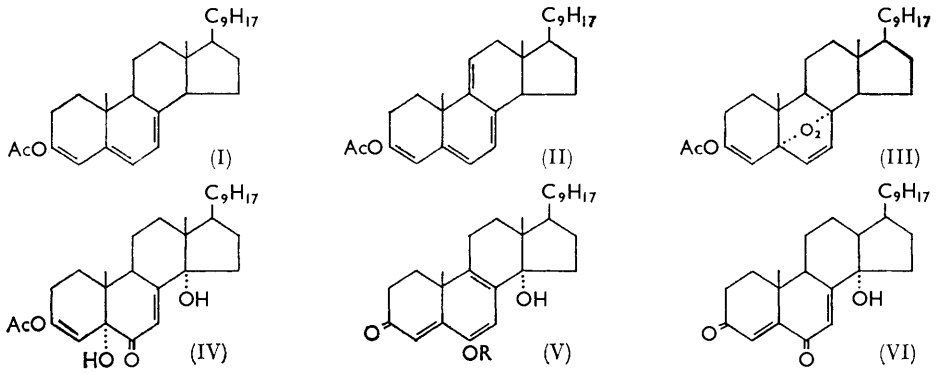
The eosin-sensitised, photochemical additions of oxygen to homoannular dienes to form epidioxides is well known,[2] and in the steroid series has been applied to ring-A,[3] ring-B,[4] and ring-C [5] diene systems. Transient formation of intermediate epidioxides probably occurs in the photo-oxygenation of the cisoid, heteroannular steroid 7,14-diene system also.[6] Ditertiary epidioxides, *e.g.*, ergosterol epidioxide, are in general stable to alkali.[7] In contrast, epidioxides in which one or both of the oxygen-bridge terminations is secondary are rearranged by alkali to form hydroxy-ketones.[3,5] Similar instability to alkali is also shown by ditertiary epidioxides in favourable stereochemical conditions, *e.g.*, in lumisterol epidioxide.[4c]

The work now reported was undertaken in order to study the reactions and properties of epidioxides in different environments, and the hitherto unknown 3-oxo-5,8-epidioxy-Δ^6-steroids and their derivatives are described for the first time.

3-Acetoxyergosta-3,5,7,22-tetraene (I) [8] was subjected to photo-oxygenation in the presence of eosin in ethanol–benzene solution, until the characteristic light absorption had disappeared. The mixture of products was chromatographed on silica gel, and three pure compounds were isolated. The first was 3-acetoxyergosta-3,5,7,9(11),22-pentaene (II), identified by its ultraviolet spectrum and by comparison with an authentic sample.[8] The second product (*ca.* 20% yield) was the expected epidioxide (III). The constitution of this compound follows from its analysis, its ultraviolet spectrum ($\lambda_{max.}$ 212 mμ), and the

[1] Preliminary communication, *Proc. Chem. Soc.*, 1962, 183.
[2] See A. Schönberg, "Präparative Organische Photochemie," Springer-Verlag, Vienna, 1958.
[3] E. L. Skau and W. Bergmann, *J. Org. Chem.*, 1938, **3**, 166.
[4] (*a*) A. Windaus and J. Brunken, *Annalen*, 1928, **460**, 225; (*b*) A. Windaus and O. Linsert, *ibid.*, 1928, **465**, 148; (*c*) P. Bladon, *J.*, 1955, 2176; (*d*) W. J. Dauben and G. J. Fonken, *J. Amer. Chem. Soc.*, 1959, **81**, 4060.
[5] G. D. Laubach, E. C. Schreiber, E. J. Agnello, and K. J. Brunings, *J. Amer. Chem. Soc.*, 1956, **78**, 4746.
[6] D. H. R. Barton and G. F. Laws, *J.*, 1954, 52.
[7] L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, 1959, p. 97.
[8] I. M. Heilbron, T. Kennedy, F. S. Spring, and G. Swain, *J.*, 1938, 869.

characteristic enol acetate peak at 1764 cm.$^{-1}$ in the infrared spectrum. The third compound is assigned the structure (IV) on the following evidence.



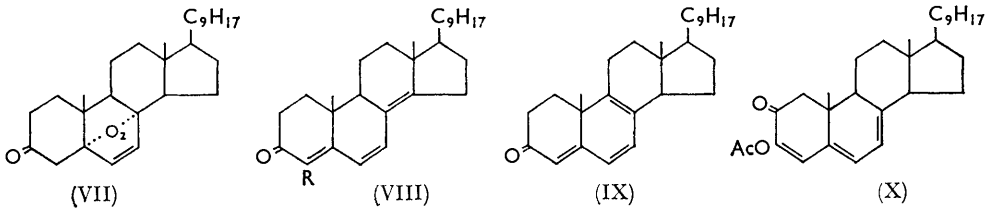
The ultraviolet absorption suggested an αβ-unsaturated ketone ($\lambda_{max.}$ 240 mμ), and this was confirmed by the infrared absorption ($\nu_{max.}$ 1692 cm.$^{-1}$), which also showed the presence of both hydrogen-bonded and free hydroxyl groups ($\nu_{max.}$ 3610, 3509, and 3360 cm.$^{-1}$) and showed that the enol acetate grouping ($\nu_{max.}$ 1767 cm.$^{-1}$) was still present. Analyses of the compound favour a molecular formula of $C_{30}H_{44}O_5$, showing that, in addition to the enol acetate group and the ketone, there are two hydroxyl groups. Neither of these could be acetylated with acetic anhydride and pyridine at room temperature, so they must both be tertiary. The αβ-unsaturated ketone system cannot be a Δ^5-7-ketone, as this would be in conjugation with the double bond of the enol acetate group; therefore it must be a Δ^8-7-ketone or a Δ^7-6-ketone.

A decision in favour of the second of these alternatives is possible on the basis of the nuclear magnetic resonance (n.m.r.) spectrum of the ketone which shows resonances due to six protons in the region τ 3·8—6·0: a sharp olefinic peak at τ 4·02 due to the C-7 proton; a second double olefinic peak centred at τ 4·27 due to the proton on C-4 [the coupling (J ~3 c./sec.) is to one of the protons at C-2]; a broad peak centred at τ 4·75 of area equivalent to two protons and characteristic of the side-chain unsaturated hydrogens; two further sharp peaks at τ 5·25 and 5·90, which disappeared when the sample was treated with deuterium oxide and are therefore due to the two hydroxyl protons. The absence of resonance in the region τ 6—7 confirms that the hydroxyl groups are tertiary, and these are assigned to positions 5 and 14 with probable α-configurations in both cases. The alternative structure, 3-acetoxy-5α,14α-dihydroxyergosta-3,8(9),22-trien-7-one would require only three olefinic proton peaks in the n.m.r. spectrum. Literature examples of steroid 5α-hydroxy-Δ^7-6-ketones [9] show principal absorption in the ultraviolet spectrum at 252 mμ. We presume that the hypsochromic shift in the present case is due to the presence of the extra hydroxyl group at C-14.

Brief alkaline hydrolysis of 3-acetoxy-5α,14α-dihydroxyergosta-3,7,22-trien-6-one (IV) gave an unsaturated ketone, which has been assigned the structure (V; R = H) for the following reasons. The ultraviolet and infrared spectra indicate a conjugated trienone and possibly two hydroxyl groups. The ultraviolet spectrum in alkaline solution shows two very broad peaks at 263 and 444 mμ. This is reminiscent of the behaviour of cholest-4-ene-3,6-dione [10] and, indeed, the ketone (V) can be considered to be an enol form of 14α-hydroxyergosta-4,7-diene-3,6-dione. Microanalysis suggests a molecular formula of $C_{28}H_{40}O_3$. Acetylation with acetic anhydride and pyridine at room temperature gave a monoacetate, which is assigned structure (V; R = Ac).

[9] L. Dorfman, *Chem. Rev.*, 1953, **53**, 47; A. I. Scott, " Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon, Oxford, 1964, p. 400.
[10] A. S. Meyer, *J. Org. Chem.*, 1955, **20**, 1240.

The epidioxide bridge in (III) probably has the α-configuration by analogy with ergosterol peroxide. The configuration of the epidioxide bridge in the latter compound was originally deduced by consideration of the "rule of rear attack." [11] Dalton and



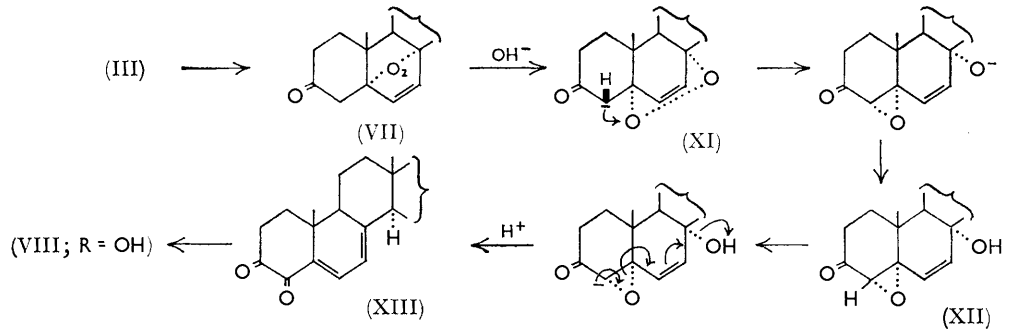
Meakins pointed out [12] that there are many exceptions to this rule, especially when ring A or B is involved,[13] but they provide chemical proof of rear approach in the oxidation of ergosterol.

It was expected that alkaline hydrolysis of the epidioxide (III) would give the 3-ketone (VII), although there is no record in the literature of the preparation of this ketone from ergosterol peroxide. On treatment with alkali, however, the epidioxide, in addition to being saponified, underwent rearrangement to yield 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH). This compound is identified mainly by its ultraviolet and infrared spectra, which taken together, indicate a conjugated trienone, with a hydroxyl group adjacent to the carbonyl group ($\lambda_{max.}$ 370 mμ; $\nu_{max.}$ 3400 and 1656 cm.$^{-1}$).

The ultraviolet absorption properties and the specific rotation (+733°) of the hydroxy-ketone (VIII; R = OH) are similar to those of ergosta-4,6,8(14),22-tetraen-3-one (VIII; R = H) rather than those of ergosta-4,6,8(9),22-tetraen-3-one (IX).[14] The hydroxy-ketone gave a 4-acetate (VIII; R = OAc) and a 4-benzoate (VIII; R = OBz).

The n.m.r. spectrum of the 4-acetate (VIII; R = OAc) (in CCl_4) contained two doublets due to the AB system of the C-6 (τ 3·82) and C-7 (τ 3·39) protons (J = 11 c./sec.), together with a broad peak due to the side-chain olefinic protons (τ 4·72). The absence of a peak in the olefinic region attributable to the C-4 proton, together with the high positive rotation and the ultraviolet spectrum, excluded the alternative structure (X) for the acetate.

The rearrangement probably proceeds by the following mechanism.



[11] L. F. Fieser, *Experientia*, 1950, **6**, 312; T. F. Gallagher and T. J. Kritchevsky, *J. Amer. Chem. Soc.*, 1950, **72**, 882.

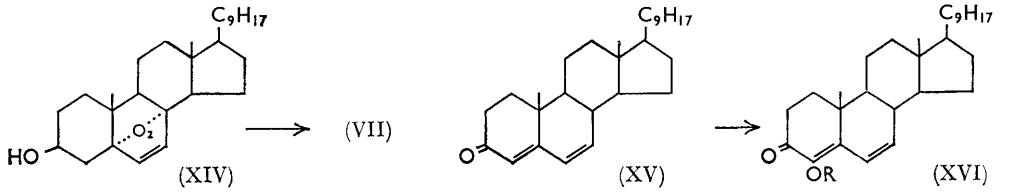
[12] F. Dalton and G. D. Meakins, *J.*, 1961, 1880.

[13] See, *inter al.*, V. Petrow, O. Rosenheim, and W. W. Starling, *J.*, 1943, 135; H. B. Henbest and R. A. L. Wilson, *ibid.*, 1956, 3289; W. S. Johnson, E. R. Rogier, J. Smuszkowicz, H. J. Hadler, J. Ackermann, B. K. Bhattacharyya, B. M. Bloom, L. Stalmann, R. A. Clement, B. Bannister, and H. Wynberg, *J. Amer. Chem. Soc.*, 1956, **78**, 6289.

[14] J. Elks, *J.*, 1954, 468.

The initial step is, in fact, hydrolysis to the ketone (VII), which in alkaline solution forms the carbanion (XI). The negatively charged 4-position attacks the 5α,8α-epidioxide bridge, to form the intermediate epoxide (XII), which rearranges to form the diketone (XIII) the keto-form of the hydroxy-ketone (VIII; R = OH).

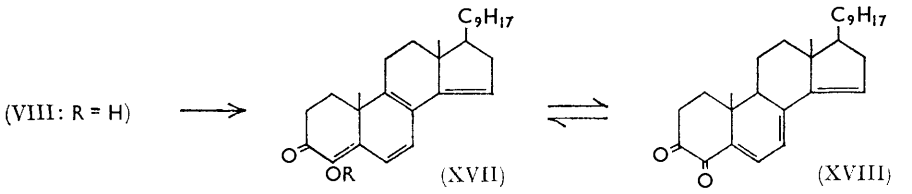
To establish a relationship between the epidioxide (III) and ergosterol peroxide (XIV) an attempt was made to oxidise the latter compound to 5α,8α-epidioxyergosta-6,22-dien-3-one (VII) in the hope that alkali treatment of this compound would give 4-hydroxy-ergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH). Oppenauer oxidation of ergosterol



peroxide did not give the expected 3-ketone, but there was isolated a trace of the hydroxy-ketone (VIII; R = OH), together with starting material. It was found, however, that chromium trioxide–pyridine oxidation gave the desired compound, if the mixture was kept approximately neutral during the working-up procedure. Alkaline hydrolysis of 5α,8α-epidioxyergosta-6,22-dien-3-one (VII) then gave the 4-hydroxy-3-ketone (VIII; R = OH).

An attempt was then made to obtain 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH) by aerial oxidation of ergosta-4,6,22-trien-3-one (XV) (cf. ref. 15). The trienone was dissolved in t-butyl alcohol containing potassium t-butoxide, and the solution stirred at room temperature for 24 hr. The product obtained, however, was 4-hydroxy-ergosta-4,6,22-trien-3-one (XVI; R = H), together with a little recovered starting material. The product was identified by its infrared absorption properties. The ultraviolet absorption ($\lambda_{max.}$ 288 mμ) is considerably lower than that predicted for this type of compound, and acetylation gave the 4-acetate (XVI; R = Ac), the ultraviolet absorption of which (307 mμ) is higher than expected. The anomalous behaviour of the hydroxy-compound may be due to its partial existence in a diketonic form.

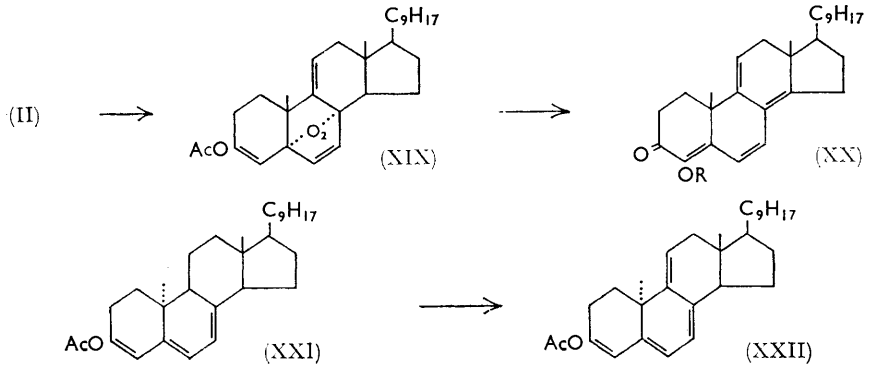
Aerial oxidation of ergosta-4,5,8(14),22-tetraen-3-one (VIII; R = H) under the same conditions gave a green, crystalline compound, which appears to contain one hydroxyl group and which has been assigned the structure (XVII; R = H). The deviation of the ultraviolet absorption of this compound from the calculated value, the presence of twin carbonyl peaks in its infrared spectrum, and its green colour are probably due to the



presence of the diketonic form (XVIII). This is supported by the visible absorption, which reveals a peak at 623 mμ (ε 5·1), the value of the extinction coefficient suggesting that the compound exists as the diketone to the extent of approximately 25%. [Simple 1,2-diketones, such as biacetyl show a series of absorption bands in the region between 420 and 450 mμ (ε *ca.* 20), and this absorption confers a yellow colour on these compounds.[16]] Acetylation of the compound (XVII; R = H) with acetic anhydride and pyridine at room temperature gave the 4-acetate (XVII; R = Ac).

[15] Cf. B. Camerino, B. Patelli, and R. Sciaky, *Tetrahedron Letters*, 1961, 554.
[16] A. E. Gillam and E. S. Stern, " Electronic Absorption Spectra," Arnold, London, 1957, p. 122.

The photo-oxygenation of 3-acetoxyergosta-3,5,7,9(11),22-pentaene in the manner described previously proceeded more quickly than in the case of its 9(11)-dihydro-analogue, and the sole product isolated after chromatography was 3-acetoxy-6α,7α-epidioxyergosta-



3,6,9(11),22-tetraene(XIX). The product shows no selective ultraviolet absorption, and the infrared spectrum shows the presence of the enol acetate group. On hydrolysis with

Compound	$\Delta[M]_D$ *	Ref.
Ergosterol	+379	*a*
Ergosteryl acetate	+300	4*c*
22,23-Dihydroergosterol	+458	*b*
22,23-Dihydroergosteryl acetate	+377	*c*
3,7-Diacetoxyandrosta-5,7-diene	+488	*c*
Lumisteryl acetate	−230	4*c*
3-Acetoxyergosta-3,5,7,22-tetraene	+668	*d*
3-Acetoxyergosta-3,5,7,9(11)22-pentaene	+1579	*d*

* For formation of epidioxide. (*a*) R. B. Clayton, H. B. Henbest, and E. R. H. Jones, *J.*, 1953, 2015. (*b*) A. Windaus and R. Langer, *Annalen*, 1934, **508**, 105. (*c*) Value from J. R. Mathieu and G. Petit, " Constants Selectionées, Pouvoir Rotatoire Naturel, vol. 1, Steroids," Masson, Paris, 1956. (*d*) Present work.

dilute alkali, the epidioxide underwent rearrangement to give 4-hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one (XX; R = H) which has similar light-absorption characteristics to those of 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH). Acetylation with acetic anhydride–pyridine at room temperature gave the 4-acetate (XX; R = Ac), and treatment with benzoyl chloride–pyridine gave the 4-benzoate (XX; R = Bz).

It has been pointed out that the molecular-rotation increments for the addition of oxygen to steroidal ring-B dienes are of the same order in all cases.[17] It is interesting, therefore, to compare the molecular-rotation increments for the addition of oxygen to the two enol acetates with those of other ring-B dienes (Table).

Photo-oxygenation of 3-acetoxylumista-3,5,7,22-tetraene (XXI) gave, after chromatography on silica gel, 3-acetoxylumista-3,5,7,9(11),22-pentaene (XXII) as the only pure, crystalline product, identified by its ultraviolet and infrared spectra.

Experimental

Melting points were determined on a Kofler hot stage, and also, in certain specified cases, in a sealed evacuated tube. Optical rotations, unless otherwise stated were determined for chloroform solutions at room temperature. Ultraviolet spectra, unless otherwise stated refer to ethanol solutions. Alterations in spectra in alkaline solution were observed by diluting ethanol solutions of the compounds with 0·1N-sodium hydroxide. Blank solutions were prepared by

[17] B. R. Davis and T. G. Halsall, *J.*, 1962, 1833.

a similar dilution of pure ethanol. Infrared spectra unless otherwise stated are for potassium chloride discs. In certain specified cases, spectra were obtained in carbon tetrachloride solution at two concentrations; approx. 0·03M for the region 2—15 μ, and approx. 0·0025M for the region 2—4 μ, using 1-cm. infrared grade Vitreosil cells in the latter case.

Alumina (Spence Grade H) was deactivated.[18] The silica gel used for chromatography was Hopkin and Williams, M.F.C. The eosin used was found to vary in purity, some samples containing impurities which contaminated the photo-oxygenation products. Light petroleum had boiling point 60—80°.

Oxygenation and Irradiation of 3-*Acetoxyergosta*-3,5,7,22-*tetraene*.—3-Acetoxyergosta-3,5,7,22-tetraene [8] (10 g.) was dissolved in benzene (325 ml.) and ethanol (275 ml.). Eosin (a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (5 ml.) were added, and the solution was placed in a vertical Pyrex glass tube sealed at the lower end. Oxygen was passed through, while the tube was illuminated by a 22 in. 20 W " warm white " fluorescent tube placed vertically and parallel to the reaction tube. The reaction was allowed to proceed for 16 hr., although spectroscopic analysis showed that it was complete after 12 hr. Two further 10-g portions of enol acetate were treated in the same way, and the solutions from the three runs were combined and taken to dryness, to give a deep red gum which was dissolved as far as possible in 1 : 4 light petroleum–benzene, filtered, and chromatographed on silica gel (800 g.). Elution with 1 : 4 light petroleum–benzene gave a yellow crystalline solid which, after two recrystallisations from ethyl acetate–methanol, gave 3-acetoxyergosta-3,5,7,9(11),22-pentaene (II) (55 mg.) as yellow plates, m. p. 143—160° (158—161° *in vacuo*), $[\alpha]_D$ −234° (*c* 0·52) (Found: C, 82·9; H, 9·9. Calc. for $C_{30}H_{42}O_2$: C, 82·9; H, 9·7%), $\lambda_{max.}$ 206, 236, 338, 354, and 373 mμ (ε 6300, 12,300, 10,300, 13,700, and 10,500), $\nu_{max.}$ 1764 (enol acetate), 1656, 1458, 1368, 1211, and 1130 cm.$^{-1}$. Heilbron *et al.*[8] give m. p. 161°, $[\alpha]_D$ −232°, $\lambda_{max.}$ 356 mμ (ε 17,400). Elution with 9 : 1 benzene–ether gave yellowish crystalline material, which, after two recrystallisations from methanol, gave 3-*acetoxy*-5α,8α-*epidioxyergosta*-3,6,22-*triene* (III) as almost colourless needles (5·6 g.), m. p. 144—152° (148—149° *in vacuo*), $[\alpha]_D$ +0·4° (*c* 0·94), $\lambda_{max.}$ 212 mμ (ε 7700), $\nu_{max.}$ 1764 (enol acetate), 1681, 1458, 1374, and 1227 cm.$^{-1}$. A sample (500 mg.) was given three further recrystallisations from ethanol, to give colourless needles (243 mg.), m. p. 144—152·3 (148—149° *in vacuo*), $[\alpha]_D$ +1·1 (*c* 0·95) (Found: C, 77·3; H, 9·3. $C_{30}H_{44}O_4$ requires C, 76·9; H, 9·6%).

Elution with 7 : 3 benzene–ether gave orange crystalline material which, recrystallised twice from methylene chloride–isopropyl ether, gave 3-*acetoxy*-5α,14α-*dihydroxyergosta*-3,7,22-*trien*-6-*one* (IV) as almost colourless needles (1·01 g.), m. p. 182—200° (190—192° *in vacuo*), $[\alpha]_D$ −111° (*c* 0·55) (Found: C, 74·3; H, 9·3. $C_{30}H_{44}O_5$ requires C, 74·3; H, 9·15%), $\lambda_{max.}$ 240 mμ (ε 10,500), $\nu_{max.}$ 3250 (hydrogen-bonded OH), 1767 (enol acetate), 1692 (αβ-unsaturated ketone), 1634, and 1212 cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 3610 (free OH), 3509 (intermolecular hydrogen-bonded OH), and 3360 cm.$^{-1}$ (intramolecular hydrogen-bonded OH).

This photo-oxygenation reaction, and the separation of the products, is typical of several which were carried out.

Attempted Acetylation of 3-*Acetoxy*-5α,14α-*dihydroxyergosta*-3,7,22-*trien*-6-*one* (IV).—3-Acetoxy-5α,14α-dihydroxyergosta-3,7,22-trien-6-one (106 mg.) was dissolved in pyridine (1·5 ml.) and acetic anhydride (1·5 ml.) and allowed to stand overnight at room temperature. Water was added and the product isolated by ether extraction in the usual way, to give a slightly yellow crystalline solid (104 mg.), m. p. 179—199°. Recrystallisation from methylene chloride–isopropyl ether gave unchanged starting material as almost colourless needles (70 mg.).

6,14α-*Dihydroxyergosta*-4,6,8(9),22-*tetraen*-3-*one* (VI; R = H).—3-Acetoxy-5α,14α-dihydroxyergosta-3,7,22-trien-6-one (IV) (300 mg.) was suspended in ethanol (12·5 ml.), and potassium hydroxide (1 g.) in water (5 ml.) was added with stirring. The resulting red solution was stirred at room temperature for 1 hr., then acidified with concentrated hydrochloric acid, to give a yellow solid which was filtered off, washed well with water, and dried *in vacuo*. This was pure 6,14α-*dihydroxyergosta*-4,6,8(9),22-*tetraen*-3-*one* (V; R = H) (222 mg.), m. p. 182—203° (decomp.) (202—206° with decomp. *in vacuo*), $[\alpha]_D$ +172° (*c* 0·53 in pyridine) (Found: C, 78·8; H, 9·3. $C_{28}H_{40}O_3$ requires C, 79·2; H, 9·5%), $\lambda_{max.}$ 206, 240, 266, and 375 mμ (ε 7800, 8200, 8900, and 15,500), $\lambda_{max.}$ (in NaOH) 263 and 444 mμ (ε 2900 and 11,300), $\nu_{max.}$ 3410 (OH), 1642 (unsaturated ketone), 1597, 1582, 1456, and 1229 cm.$^{-1}$.

[18] K. R. Farrer, J. C. Hamlet, H. B. Henbest, and E. R. H. Jones, *J.*, 1952, 2657.

Acetylation with acetic anhydride and pyridine at room temperature gave 6-*acetoxy*-14α-*hydroxyergosta*-4,6,8(9),22-*tetraen*-3-*one* (V; R = Ac) as tiny colourless needles, m. p. 157—159° (from n-hexane), $[\alpha]_D$ +263° (*c* 0·52) (Found: C, 77·45; H, 9·1. $C_{30}H_{42}O_4$ requires C, 77·2; H, 9·1%), $\lambda_{max.}$ 345 mμ (ε 26,200), $\lambda_{max.}$ (in NaOH) 265 and 440 mμ (ε 2900 and 3200), $\nu_{max.}$ 3500 (OH), 1770 (enol acetate), 1658 (unsaturated ketone), 1603, and 1585 cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 3597 cm.$^{-1}$.

4-*Hydroxyergosta*-4,6,8(14)22-*tetraen*-3-*one* (VIII; R = OH).—3-Acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (1 g.) was dissolved in ethanol (40 ml.), and a solution of potassium hydroxide (2 g.) in water (5 ml.) added. The resulting orange-red solution was refluxed for 10 min. during which time it became deep red-brown. The solution was cooled, acidified with concentrated hydrochloric acid, and extracted twice with ether. The ether extracts were washed with water, dried over sodium sulphate, and evaporated to dryness, to give an orange crystalline solid (875 mg.) which was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave a yellow crystalline solid which was recrystallised from methylene chloride–isopropyl ether, to give 4-*hydroxyergosta*-4,6,8(14),22-*tetraen*-3-*one* (VIII; R = OH) as fine bright yellow needles (211 mg.), m. p. 187—198° (197—204° *in vacuo*), $[\alpha]_D$ +733° (*c* 0·84) (Found: C, 82·6; H, 9·9. $C_{28}H_{40}O_2$ requires C, 82·3; H, 9·9%), $\lambda_{max.}$ 205, 262, and 370 mμ (ε 9200, 6600, and 21,000), $\lambda_{max.}$ (in NaOH) 225, 277, and 390 mμ (ε 36,100, 6600, and 14,500), $\nu_{max.}$ 3400 (hydrogen-bonded OH), 1656 (hydrogen-bonded unsaturated ketone), 1600, and 1370 cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 3448 cm.$^{-1}$ (hydrogen-bonded OH).

Acetylation with acetic anhydride and pyridine at room temperature gave 4-*acetoxyergosta*-4,6,8(14),22-*tetraen*-3-*one* (VIII; R = OAc) as yellow needles, m. p. 130—133° (from methylene chloride–methanol), $[\alpha]_D$ +624° (*c* 0·60) (Found: C, 80·1; H, 9·5. $C_{30}H_{42}O_3$ requires C, 79·95; H, 9·3%), $\lambda_{max.}$ 250 and 362 mμ (ε 4700 and 17,900), $\lambda_{max.}$ (in NaOH) 218 and 398 mμ (ε 13,200 and 11,200), $\nu_{max.}$ 1773 (enol), 1681 (unsaturated ketone), 1597, 1319, and 1206 cm.$^{-1}$.

The 4-*benzoate* (VIII; R = OBz), prepared with benzoyl chloride and pyridine, formed pale yellow crystals, m. p. 147—154°, $[\alpha]_D$ +520° (*c* 0·59), from methylene chloride–methanol (Found: C, 82·35; H, 8·7. $C_{35}H_{44}O_3$ requires C, 82·0; H, 8·65%), $\lambda_{max.}$ 205, 232, 278, and 355 mμ (ε 15,500, 18,000, 6800, and 25,200), $\lambda_{max.}$ (in NaOH) 218 and 355 mμ (ε 42,500 and 22,600), $\nu_{max.}$ 1751 (enol benzoate), 1581 (3-ketone), 1642, 1600, and 701 cm.$^{-1}$.

Oppenauer Oxidation of Ergosterol Peroxide (XIV).—Ergosterol peroxide (750 mg.) in acetone (20 ml.) was refluxed for 4 hr. with a 25% solution of aluminium t-butoxide in toluene (7·5 ml.). The solution was cooled and diluted with ether, the ether extract washed with water, dried over sodium sulphate, and evaporated to dryness several times with xylene to remove mesityl oxide. The product was obtained as a yellow gum (634 mg.), which was dissolved in 1 : 1 light petroleum–benzene and chromatographed on silica gel (30 g.).

Elution with 1 : 9 light petroleum–benzene gave yellow crystalline material (64 mg.) which was recrystallised twice from isopropyl ether, to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH) as fine pale yellow needles (9 mg.), m. p. 193—198° *in vacuo*, $\lambda_{max.}$ 204, 214, 256, and 371 mμ (ε 5800, 4400, 1900, and 22,000), $\lambda_{max.}$ (in NaOH) 218, 225, 272, and 400 mμ (ε 36,100, 31,000, 5100, and 15,000), $\nu_{max.}$ 3378 (OH), 1645 (3-ketone), and 1590 cm.$^{-1}$. The infrared spectrum was identical with that of the pure hydroxy-ketone. The combined mother-liquors gave a second crop of yellow needles (5 mg.), m. p. 146—197°, $[\alpha]_D$ +539° (*c* 0·45).

Elution with 4 : 1 benzene–ether gave almost colourless crystalline material (105 mg.), m. p. 174—180°, which, on recrystallisation from methanol, gave unchanged ergosterol peroxide as flat colourless needles (80 mg.), m. p. 172—181°, $[\alpha]_D$ −23·2° (*c* 0·52), $\nu_{max.}$ 3425 (OH), 1453, and 1370 cm.$^{-1}$. The infrared spectrum was identical with that of the starting material.

5α,8α-*Epidioxyergosta*-6,22-*dien*-3-*one* (VII).—Ergosterol peroxide (1 g.) in pyridine (15 ml.) was added to a slurry of chromium trioxide (1 g.) in pyridine (10 ml.), and the mixture allowed to stand overnight at room temperature. A large volume of water (approx. 600 ml.) was added, and the product extracted with ether. Before the ether and aqueous layers would separate, however, the solution had to be centrifuged for 10 min. The ether layer was pipetted off and the aqueous layer decanted into a separating funnel and re-extracted with ether. The combined ether layers were dried over sodium sulphate and evaporated at room temperature until only a small quantity of pyridine remained. Trituration with methanol gave almost colourless crystalline material (280 mg.), m. p. 166—179°. Recrystallisation from ether–methanol (1 drop of pyridine) gave 5α,8α-*epidioxyergosta*-6,22-*dien*-3-*one* (VII) as colourless plates (150 mg.)

m. p. 162—180° (with a change of form to needles and decomposition) (161—165° *in vacuo*), $[\alpha]_D$ +8·4° (*c* 0·55) (Found: C, 78·6; H, 10·0. $C_{28}H_{42}O_3$ requires C, 78·8; H, 9·9%), $\nu_{max.}$ 1709 (3-ketone), 1449, 1381, 1370, and 966 cm.$^{-1}$. The mother-liquor gave a second crop of plates (15 mg.), m. p. 165—180°.

Treatment of 5α,8α-*Epidioxyergosta*-6,22-*dien*-3-*one* (VII) *with Ethanolic Potassium Hydroxide Solution.*—5α,8α-Epidioxyergosta-6,22-dien-3-one (106 mg.) was dissolved in ethanol (2·5 ml.), and a solution of potassium hydroxide (0·2 g.) in distilled water (1 ml.) added. The reddish-brown solution was refluxed for 1 hr., cooled, acidified with concentrated hydrochloric acid, and extracted twice with ether. The combined extracts were washed with water, dried over sodium sulphate, and evaporated to dryness, to give a reddish crystalline solid (101 mg.) which was dissolved in benzene and chromatographed on silica gel (5 g.). Elution with benzene gave yellow crystalline material (55 mg.) which was recrystallised twice from methylene chloride–isopropyl ether, to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (VIII; R = OH) as fine bright yellow needles (16 mg.), m. p. 186—199° (decomp.), $[\alpha]_D$ +774° (*c* 0·43) (Found: C, 82·1; H, 9·9. Calc. for $C_{29}H_{40}O_2$: C, 82·3; H, 9·9%), $\lambda_{max.}$ 206, 264, and 368 mμ (ε 6600, 5400, and 10,900), $\lambda_{max.}$ (in NaOH) 218, 282, and 404 mμ (ε 28,000, 5400, and 9100), $\nu_{max.}$ 3344 (OH), 1642 (3-ketone), 1587, and 1368 cm.$^{-1}$. Combined mother-liquors gave a second crop of bright yellow needles (8 mg.), m. p. 144—187° (decomp.).

Oxidation of Ergosta-4,6,22-*trien*-3-*one* (XV) *in Alkaline Solution.*—Potassium (468 mg.) was dissolved in t-butyl alcohol (15 ml.), then ergosta-4,6,22-trien-3-one [19] (1 g.) was added and dissolved by warming the mixture slightly. The resulting solution quickly became yellow and after 1 hr. solid came down, but the solution was allowed to stand at room temperature for 24 hr. in an open flask. Water was added, the solution acidified, and the product extracted twice with ether. The ether extracts were washed well with water and evaporated to dryness, to give yellow crystalline material (1·03 g.) which melted up to 148°. This material was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave yellow crystalline material (541 mg.) which, after two recrystallisations from methylene chloride–methanol, gave 4-*hydroxyergosta*-4,6,22-*trien*-3-*one* (XVI; R = H) as pale yellow needles (420 mg.), m. p. 156—159°, $[\alpha]_D$ −151° (*c* 0·66) (Found: C, 81·4; H, 9·9. $C_{28}H_{42}O_2$ requires C, 81·9; H, 10·3%), $\lambda_{max.}$ 228 and 288 mμ (ε 16,900 and 14,300), $\lambda_{max.}$ (in NaOH) 220, 257, and 300 mμ (ε 7500, 17,500, and 10,000), $\nu_{max.}$ 3378 (hydrogen-bonded OH), 1661, 1639, 1616, 1577 cm.$^{-1}$ (hydrogen-bonded unsaturated ketone), $\nu_{max.}$ (in CCl_4) 3401 (hydrogen-bonded OH), 1672, 1656, 1618, and 1575 cm.$^{-1}$ (hydrogen-bonded unsaturated ketone). The mother-liquor gave a second crop of pale yellow needles (74 mg.), m. p. 148—156°.

Further elution with benzene gave less-pure material (174 mg.), which was recrystallised from methylene chloride–methanol, to give 4-hydroxyergosta-4,6,22-trien-3-one as yellow needles (153 mg.), m. p. 155—158°.

Acetylation with acetic anhydride and pyridine at room temperature gave 4-*acetoxyergosta*-4,6,22-*trien*-3-*one* (XVI; R = Ac) as almost colourless needles, m. p. 161—163° (from methylene chloride–methanol), $[\alpha]_D$ −72·0° (*c* 0·48) (Found: C, 79·4; H, 9·6. $C_{30}H_{44}O_3$ requires C, 79·6; H, 9·8%), $\lambda_{max.}$ 214, 267, and 307 mμ (ε 14,300, 9700, and 11,100), $\lambda_{max.}$ (in NaOH) 260 and 302 mμ (ε 15,200 and 10,300), $\nu_{max.}$ 1761 (enol acetate), 1658, 1645, 1610, and 1575 (unsaturated ketone) cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 1779 (enol acetate), 1678, 1623, and 1585 cm.$^{-1}$ (unsaturated ketone).

Oxidation of Ergosta-4,6,8(14),22-*tetraen*-3-*one* (VIII; R = H) *in Alkaline Medium.*—Potassium (440 mg.) was dissolved in t-butyl alcohol (15 ml.), then ergosta-4,6,8(14),22-tetraen-3-one [14] (VIII; R = H) (1 g.) was added, with stirring, to give a deep red solution which gradually became darker on standing. The mixture was allowed to stand at room temperature for 24 hr. in an open flask, with occasional swirling. Water was added, the solution acidified, and the product extracted with ether. The ether extract was washed well with water, dried over sodium sulphate, and evaporated to dryness, to give a dark red froth (1·07 g.) which was dissolved in light petroleum and chromatographed on silica gel (40 g.).

Elution with benzene gave crystalline material (175 mg.) which, after three recrystallisations from methylene chloride–methanol, gave 4-*hydroxyergosta*-4,6,8(9),14,22-*pentaen*-3-*one* (XVII; R = H) as green needles (51 mg.), m. p. 165—168°, $[\alpha]_D$ +419° (*c* 0·27) (Found: C, 82·9; H, 9·55. $C_{28}H_{38}O_2$ requires C, 82·7; H, 9·4%), $\lambda_{max.}$ 208, 270, 371, and 623 mμ (ε 13,200, 19,900, 9300, and 5·1), $\lambda_{max.}$ (in NaOH) 229, 287, and 348 mμ (ε 12,300, 16,500, and 11,000),

[19] D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. R. Holysz, G. Slomp, J. E. Stafford, R. L. Pederson, and A. C. Ott, *J. Amer. Chem. Soc.*, 1955, **77**, 1212.

$\nu_{max.}$ 3378 (hydrogen-bonded OH), 1664, 1629, and 1590 cm.$^{-1}$ (hydrogen-bonded unsaturated ketone), $\nu_{max.}$ (in CCl_4) 3425 (hydrogen-bonded OH), 1678, 1642, 1637, and 1590 (hydrogen-bonded unsaturated ketone).

The combined mother-liquors gave a second crop of green needles (27 mg.), m. p. 164—167°, and a third crop (21 mg.), m. p. 163—167°.

A second experiment was carried out on the same scale as above, but the reaction was stopped after 3 hr. The crude product (1·08 g.) was dissolved in benzene and chromatographed on silica gel (40 g.). Elution with benzene gave crystalline material (132 mg.) which was recrystallised from methylene chloride–methanol, to give 4-hydroxyergosta-4,6,8(9),14,22-pentaen-3-one (XVII; R = H) as green needles (118 mg.), m. p. 163—169°. A second recrystallisation of a sample (45 mg.) again gave green needles (28 mg.), m. p. 164—168°, $[\alpha]_D$ +405° (*c* 0·43).

Elution with 19 : 1 benzene–ether gave a red gum (354 mg.) which crystallised from methylene chloride–methanol, to give starting material as brown crystals (200 mg.), m. p. 111—115°, $\nu_{max.}$ 1667, 1639, and 1587 cm.$^{-1}$. The infrared spectrum was identical with that of the starting material.

The 4-*acetate* (XVII; R = Ac), prepared with acetic anhydride–pyridine, was obtained as pale yellow needles (from methanol), m. p. 115—119°, $[\alpha]_D$ +470° (*c* 0·45) (Found: C, 80·1; H, 9·0. $C_{30}H_{40}O_3$ requires C, 80·3; H, 9·0%), $\lambda_{max.}$ 222, 261, and 374 mμ (ε 8600, 22,400, and 12,800), $\lambda_{max.}$ (in NaOH) 221, 269, and 356 mμ (ε 15,700, 20,800, and 14,000), $\nu_{max.}$ 1773 (enol acetate), 1669, 1647, and 1590 cm.$^{-1}$ (3-ketone), $\nu_{max.}$ (in CCl_4) 1773 (enol acetate), 1669, 1653, and 1590 cm.$^{-1}$ (3-ketone).

3-*Acetoxyergosta*-3,5,7,9(11),22-*pentaene* (II).—9(11)-Dehydroerogosterol [40] was converted into ergosta-4,7,9(11),22-tetraen-3-one.[8] The crude material was used directly to prepare 3-acetoxyergosta-3,5,7,9(11),22-pentaene.[8]

Oxygenation and Irradiation of 3-*Acetoxyergosta*-3,5,7,9(11),22-*pentaene* (II).—3-Acetoxyergosta-3,5,7,9(11),22-pentaene (II) (10·0 g.) was dissolved in benzene (645 ml.) and ethanol (650 ml.). Eosin (100 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (5 ml.) were added, and the solution was oxygenated and irradiated as described previously. Spectroscopic analysis showed that the reaction was complete in 5·5 hr. The solution was taken to dryness, to give a red gum (11·8 g.) which was dissolved in chloroform and passed quickly through a column of deactivated alumina (100 g.). This removed most of the eosin, and evaporation of the eluate gave a red gum which was recrystallised from ether–methanol, to give pink crystalline material (6·32 g.), m. p. 129—155°. The mother-liquor gave two further crops of yellow needles (575 mg., m. p. 150—154°; 326 mg., m. p. 120—147°).

Two recrystallisations of the first crop (6·32 g.) from ether–methanol gave 3-*acetoxy*-5α,8α-*epidioxyergosta*-3,6,9(11),22-*tetraene* (XIX) as pale orange needles (4·62 g.), m. p. 152—155°, $[\alpha]_D$ +113° (*c* 0·52) (Found: C, 77·3; H, 9·0. $C_{30}H_{42}O_4$ requires C, 77·2; H, 9·1%), $\nu_{max.}$ 1761 (enol acetate), 1681, and 1212 cm.$^{-1}$.

The recrystallisation mother-liquors gave pink crystalline material (1·35 g.), m. p. 110—150°.

The initial mother-liquors, on evaporation, gave a red gum (2·38 g.) which was dissolved in benzene and chromatographed on silica gel (100 g.). The only crystalline material which was obtained, however, was a further small quantity of epidioxide (XIX). The other fractions were red oils which gave no crystalline material.

4-*Hydroxyergosta*-4,6,8(14),9(11),22-*pentaen*-3-*one* (XX; R = H).—3-Acetoxy-5α,8α-epidioxyergosta-3,6,9(11),22-tetraene (1 g.) was dissolved in ethanol (28 ml.), and a solution of potassium hydroxide (1·4 g.) in water (7 ml.) added. The solution was refluxed for 5 min., during which time it quickly became deep red. The solution was cooled, acidified with concentrated hydrochloric acid, and extracted twice with ether. The extracts were washed three times with water, dried over sodium sulphate, and evaporated to dryness, to give a deep yellow crystalline solid (868 mg.) which was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave a yellow crystalline solid (552 mg.) which was recrystallised from methylene chloride–isopropyl ether, to give 4-*hydroxyergosta*-4,6,8(14),9(11),22-*pentaen*-3-*one* (XX; R = H) as bright yellow needles (391 mg.), m. p. 187—198° (decomp.). A sample (94 mg.) was given a second recrystallisation from methylene chloride–isopropyl ether, to give bright yellow needles (79 mg.), m. p. 189—201° (decomp.) (193—200° with decomp. *in vacuo*), $[\alpha]_D$ +230° (*c* 0·57) (Found: C, 82·0, 81·8; H, 9·5, 9·6. $C_{28}H_{38}O_2$,0·25H_2O requires C, 81·9;

H, 9·4), $\lambda_{max.}$ 202, 281, and 400 mμ (ε 9600, 8400, and 11,900), $\lambda_{max.}$ (in NaOH) 221 and 310 mμ (ε 17,600 and 11,700), $\nu_{max.}$ 3378 (hydrogen-bonded OH), 1656, 1642, 1610, and 1582 (hydrogen-bonded unsaturated ketone), and 1527 cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 3442 cm.$^{-1}$ (hydrogen-bonded OH).

The combined mother-liquors gave a second crop of yellow needles (70 mg.), m. p. 185—197° (decomp.), and a third crop (36 mg.), m. p. 173—196°.

Acetylation with acetic anhydride and pyridine at room temperature gave the 4-*acetate* (XX; R = Ac) as yellow prisms (from methylene chloride–methanol), m. p. 199—209° (205—209° with decomp. *in vacuo*), $[\alpha]_D$ +62° (*c* 0·57) (Found: C, 80·2; H, 9·1. $C_{30}H_{40}O_3$ requires C, 80·3; H, 9·0%), $\lambda_{max.}$ 207, 271, and 405 mμ (ε 11,200, 8600, and 12,100), $\lambda_{max.}$ (in NaOH) 221, 270, and 407 mμ (ε 16,100, 10,800, and 10,500), $\nu_{max.}$ 1764 (enol acetate), 1653, 1623, and 1585 (unsaturated ketone), and 1534 cm.$^{-1}$.

The 4-*benzoate* (XX; R = Bz), prepared with benzoyl chloride and pyridine, formed pale yellow feathery needles, m. p. 197—213° (212—215° *in vacuo*), $[\alpha]_D$ +123° (*c* 0·56) (from methylene chloride–acetone) (Found: C, 82·3; H, 8·2. $C_{33}H_{42}O_3$ requires C, 82·3; H, 8·3), $\lambda_{max.}$ 235, 271, and 404 mμ (ε 18,800, 7600, and 10,900), $\lambda_{max.}$ (in NaOH) 235, 280, and 400 mμ (ε 16,400, 12,000, and 7600), $\nu_{max.}$ 1732 (enol benzoate), 1667, and 1626 (unsaturated ketone), and 1543 cm.$^{-1}$.

3-*Acetoxylumista*-3,5,7,22-*tetraene* (XXI).—Lumisterol, m. p. 118°, $[\alpha]_D$ −182°, obtained by hydrolysis of the 3,4-dinitrobenzoate, was converted by Oppenauer oxidation into lumista-4,7,22-trien-3-one, m. p. 135—189°, $[\alpha]_D$ +18·6° (*c* 0·59), and thence, by treatment with pyridine and acetic anhydride, into 3-acetoxylumista-3,5,7,22-tetraene (XXI).[8]

Oxygenation and Irradiation of 3-*Acetoxylumista*-3,5,7,22-*tetraene* (XXI).—3-Acetoxylumista-3,5,7,22-tetraene (3 g.) was dissolved in benzene (333 ml.) and ethanol (335 ml.). Eosin (30 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (2 ml.) were added, and the solution was oxygenated and irradiated as described previously. Spectroscopic analysis showed that the reaction was 85% complete after 16 hr. The reaction was stopped and the solution taken to dryness, to give a red gum which could not be crystallised; it was dissolved in light petroleum and chromatographed on silica gel (100 g.).

Elution with 1 : 9 light petroleum–benzene gave yellow crystalline material (282 mg.) which was recrystallised twice from methylene chloride–methanol, to give 3-*acetoxylumista*-3,5,7,9(11),22-*pentaene* (XXII) as yellow needles (154 mg.), m. p. 119·5—129° (126—129° *in vacuo*), $[\alpha]_D$ +639° (*c* 0·55) (Found: C, 82·8; H, 9·5. $C_{30}H_{42}O_2$ requires C, 82·9; H, 9·7%), $\lambda_{max.}$ 234, 320, 334, and 353 mμ (ε 10,500, 12,200, 12,400, and 9800), ν_{max} 1757 (enol acetate), 1661, 1562, 1224, and 1124 cm.$^{-1}$.

Elution with 4 : 1 benzene–ether gave an orange semicrystalline solid (1·59 g.) which could not be recrystallised. Elution with 1 : 1 benzene-ether gave slightly pink crystalline material (445 mg.) which was recrystallised twice from methylene chloride–isopropyl ether, to give an αβ-unsaturated ketone as almost colourless needles (77 mg.), m. p. 165—177°, $[\alpha]_D$ +59° (*c* 0·58) (Found: C, 76·7; H, 9·25%), $\lambda_{max.}$ 240 mμ ($E^{1\%}_{1\,cm.}$ 382), $\nu_{max.}$ 3378 (hydrogen-bonded OH) and 1669 (αβ-unsaturated ketone) cm.$^{-1}$, $\nu_{max.}$ (in CCl_4) 3590 (free OH) and 3478 cm.$^{-1}$ (intermolecular hydrogen-bonded OH). This material was not examined further. Other fractions from the chromatography gave traces of poorly crystalline material which were not examined.

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