996. *Adducts of Maleic Anhydride with Ergosteryl Acetate.*

By D. Neville Jones and I. Thomas.

Evidence is presented which indicates that maleic anhydride reacts with ergosteryl acetate by addition to different faces of the latter. The anhydride portion is *endo* to the nuclear double bond in each adduct, these assignments being supported by nuclear magnetic resonance spectra and by reactions of derivatives of the adducts.

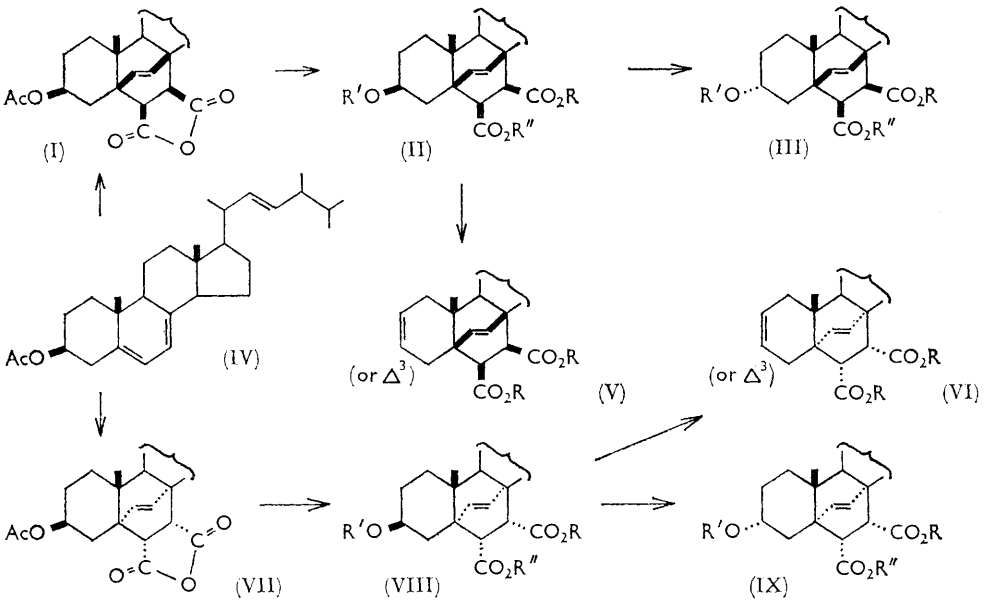
Three adducts of ergosteryl acetate and maleic anhydride have been described, the first by Windaus and Lütringhaus [1] (m. p. 200°) and the others by Inhoffen [2] (m. p. 216°) and by Hicks, Berg, and Wallis [3] (m. p. 176°). The first two were important for determination of the structure of ergosterol,[4] and have also been used to protect the diene system in reactions involving the side chain.[5] Distinction between the Windaus and the Inhoffen adduct is frequently lacking in the literature,[4] and it has been suggested that the adduct of m. p. 200° is a mixture of the other two.[6] The structure of the adducts was unknown, but it had been assumed that the Windaus and the Inhoffen adduct result from attack of maleic anhydride on the less hindered, rear face of ergosteryl acetate. This was a reasonable assumption since only the 5α,8α-peroxide has been isolated after reaction of oxygen with ergosterol,[7] and maleic anhydride presumably has greater steric requirements than oxygen.

We found that ergosteryl acetate and maleic anhydride in xylene at 135° give both the Windaus and the Inhoffen adduct, but we could not isolate the Wallis adduct, m. p. 176°. To clarify subsequent discussion the structures that we propose for the Windaus adduct, m. p. 200° (I), and the Inhoffen adduct, m. p. 216° (VII), are introduced at this point.* The nuclear magnetic resonance (n.m.r.) spectra of both the adducts had a signal at τ 4·65 which was assigned to the side-chain (Δ^{22}) vinyl protons since it was absent from the spectra of the 22,23-dihydro-hydroxy-acids (II and VIII; R = R′ = R″ = H; 22,23-dihydro) derived, respectively, from (I) and (VII) by hydrolysis and partial hydrogenation. The vinyl protons at positions 1′ and 2′ in the Inhoffen adduct (VII) gave rise to a pair of doublets at τ 3·67 (*J* 9 c./sec.) and 4·12 (*J* 9 c./sec.) whilst the corresponding protons in the Windaus adduct produced a complex signal at τ 4·24. In the corresponding

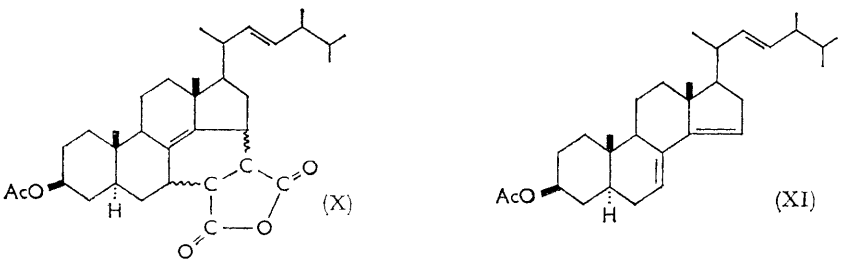
* The adducts are named as 5,8-etheno-derivatives of ergostane, the carbon atoms of the etheno-bridge being numbered 1′ and 2′.

[1] Windaus and Lüttringhaus, *Ber.*, 1931, **64**, 850.
[2] Inhoffen, *Annalen*, 1934, **508**, 81.
[3] Hicks, Berg, and Wallis, *J. Biol. Chem.*, 1946, **162**, 654.
[4] Fieser and Fieser, "Steroids," Reinhold Publ. Corp, New York, 1959, p. 109.
[5] (*a*) Bergmann and Stevens, *J. Org. Chem.*, 1948, **13**, 10; (*b*) deVries and Backer, *Rec. Trav. chim.*, 1952, **71**, 719.
[6] Elsevier's "Encyclopedia of Organic Chemistry," Series III, Vol. 14, Supplement, Elsevier Publ. Co., Amsterdam, 1954, p. 1743S.
[7] Dalton and Meakins, *J.*, 1961, 1880.

22,23-dihydrohydroxy-acids (II and VIII; R = R′ = R″ = H; 22,23-dihydro) these vinyl protons gave complex signals at τ 3·73 and 4·19, respectively.



This evidence for two disubstituted olefinic bonds in each of the adducts confirms the view that each is an adduct of maleic anhydride and ergosteryl acetate, and not an adduct of structure (X) from 3β-acetoxyergosta-7,14,22-triene (XI), which was considered a



possibility since it is known that maleic anhydride containing a trace of maleic acid isomerises ergosterol to ergosta-7,14,22-trien-3β-ol.[5b] The adduct (X) is reported to have m. p. 207°,[8] but neither a rotation nor a mixed melting point determination with either of the ergosteryl acetate–maleic anhydride adducts is recorded.

Since the Inhoffen and the Windaus adduct both furnished ergosteryl acetate on pyrolysis,[1,2] they had been formulated as adducts of ergosteryl acetate (and not of 3β-acetoxyergosta-7,14,22-triene). Whereas ergosteryl acetate was recovered nearly quantitatively after pyrolysis of the Inhoffen adduct,[2] the recovery from the Windaus adduct was very poor.[1] We had therefore been unable to exclude structure (X) for the Windaus adduct since the possibility of thermal isomerisation of 3β-acetoxyergost-7,14,22-triene to ergosteryl acetate during the pyrolysis had not been eliminated. Pyrolysis of the 3β-acetoxyergosta-7,14,22-triene–maleic anhydride adduct (X) has not been investigated.

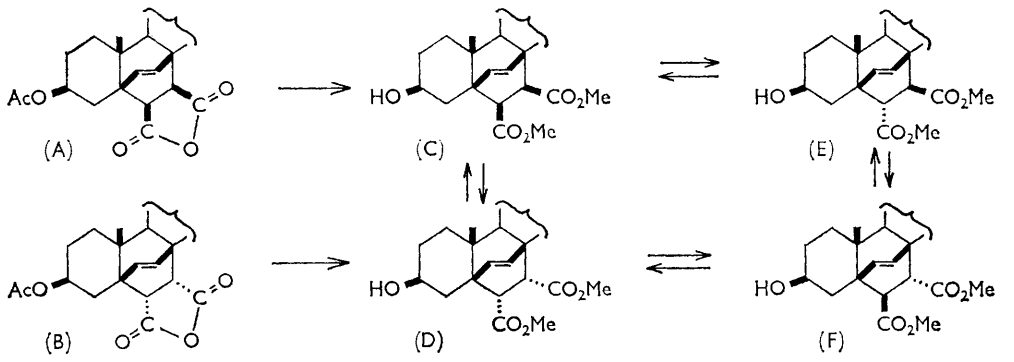
Mild hydrolysis of the Windaus adduct (I) with aqueous-methanolic potassium

[8] Windaus, Dithmar, Murke, and Suckfull, *Annalen*, 1931, **488**, 91.

hydrogen carbonate, followed by esterification of the resulting hydroxy-acid (II; R = R′ = R″ = H) with diazomethane, gave the dimethyl hydroxy-ester (II; R′ = H, R = R″ = Me). Similar reactions starting from the Inhoffen adduct (VII) gave the dimethyl hydroxy-ester (VIII; R′ = H, R = R″ = Me). Equilibration of the dimethyl hydroxy-ester (II; R′ = H, R = R″ = Me) with sodium ethoxide in boiling ethanol,[9] followed by hydrolysis and re-esterification, gave a new dimethyl hydroxy-ester with some recovered starting material. Similar equilibration of the dimethyl hydroxy-ester (VIII; R′ = H, R = R″ = Me) gave another two new dimethyl hydroxy-esters, with some recovered starting material. Thin-layer chromatography and comparison of infrared spectra confirmed that these were in fact five different dimethyl hydroxy-esters.

We consider that all these isomers are obtained by equilibration of the methoxycarbonyl groups, and not of the 3-hydroxyl group, since they were all obtained in yields greater than 20%. For other 3-hydroxy-steroids equilibration provides less than 10% of the axial isomer.[10] Examination of Dreiding models confirms that the 3-hydroxyl group of the dimethyl 3β-hydroxy-esters (II and VIII; R′ = H, R = R″ = Me) is equatorial and subject to the same non-bonded interactions as the 3β-hydroxyl group in 5α-cholestan-3β-ol. However, the non-bonded interactions of the hydroxyl group in the dimethyl 3α-hydroxy-esters (III and IX; R′ = H, R = R″ = Me) [which are epimeric at C-3 with (II and VIII; R′ = H, R = R″ = Me), respectively] are more severe than any of those of the 3α-hydroxyl group in 5α-cholestan-3α-ol. Thus equilibration of both the compounds (II and VIII; R′ = H, R = R″ = Me) should provide even less of the axial (3α-)isomer than is the case with the 3-hydroxy-derivatives of 5α-cholestane, in which equilibration gives ~10% of the axial (3α-)isomer.[10] In addition the dimethyl hydroxy-esters obtained by equilibration of the dimethyl 3β-hydroxy-ester (VIII; R′ = H, R = R″ = Me) were shown to differ from the authentic dimethyl 3α-hydroxy-ester (IX; R′ = H, R = R″ = Me) prepared by an unambiguous route (see below).

If the Windaus and the Inhoffen adduct were produced by addition of maleic anhydride at the less hindered α-side of ergosteryl acetate, then the anhydride group in one (A) would have an *endo*-relation to the nuclear double bond, and in the other (B) would have an *exo*-relation. Equilibration of the dimethyl hydroxy-esters (C) and (D) derived from two such adducts can provide only a total of four dimethyl hydroxy-esters, two *cis*- (C) and (D)



and two *trans*-diesters (E) and (F). That five different dimethyl hydroxy-esters were obtained suggests that these two adducts result from attack of maleic anhydride at different faces of ergosteryl acetate.

Supporting evidence was the similar difference in rotation of the two adducts and the rotations of the two series of derived dimethyl hydroxy-esters. The Windaus adduct, $[\alpha]_D$ −147°, gave two dimethyl hydroxy-esters of $[\alpha]_D$ −179 and −130°, whilst the

[9] Meinwald and Gassman, *J. Amer. Chem. Soc.*, 1960, **82**, 5445.
[10] Windaus and Uibrig, *Ber.*, 1914, **47**, 2384; 1915, **48**, 857; Windaus, *ibid.*, 1916, **49**, 1724.

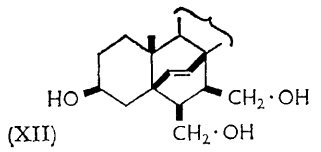
Inhoffen adduct, $[\alpha]_D$ −19°, gave three dimethyl hydroxy-esters of $[\alpha]_D$ −48, −41, and −53°. It seemed likely that this large difference between the two series was due to a significant difference in the carbon skeleton and not merely in the configuration of the functional groups, a conclusion substantiated by n.m.r. spectra.

The n.m.r. spectrum of the Windaus adduct (I) displayed a signal at τ 9·06 having about twice the intensity of that of the acetate-methyl protons at τ 7·94, while the Inhoffen adduct (VII) showed signals in this region having about the same intensity as that of the acetate-methyl signal at τ 7·93. Derivatives of the Windaus adduct (I) show this characteristic stronger signal between τ 9·01 and 9·13, whereas those of the Inhoffen adduct (VII) show a "plateau" with peaks between τ 8·85 and 9·28 having intensities similar to that of the acetate-methyl protons. In most 3-oxygenated steroids, the 19-methyl protons give rise to a signal at a lower field (τ 8·6—9·1) than those at C-18 (τ 9·2—9·4).[11]

The intense signal at τ 9·06 given by the Windaus adduct and its derivatives may be explained by an upfield shift of the 19-methyl signal so that it coincides with that of the 18-methyl group; other examples of this coalescence are known,[11,12] and it may be brought about by diamagnetic shielding by a suitably placed double bond, *e.g.*, 7,8-unsaturation in steroids,[11,13] and an analogous effect has recently been postulated in the maleic anhydride adduct of lævopimaric acid.[14]

Examination of Dreiding models shows that in the α-adduct of ergosteryl acetate (*i.e.*, that formed by attack from the α-side) the 19-methyl-hydrogen atoms fall within a cone of 57° apex angle drawn from the middle of the 1′,2′-double bond with its axis at right angles to the bond. Hydrogen atoms lying within such a cone are shielded and resonate at higher field strengths than in the absence of the olefinic bond;[15a] in the β-adduct these hydrogen atoms lie well outside the cone. Therefore, the Windaus (m. p. 200°) and the Inhoffen adduct (m. p. 216°) is the result of attack by maleic anhydride on the α- and β-face, respectively, of ergosteryl acetate.

Carbonyl groups can also shield, diamagnetically, suitably placed protons.[15b] However, the carbonyl groups of compounds (I) and (VII) have little effect upon the signals of the 19- and the 18-methyl group, since the triols (XII), (XII; 22,23-dihydro), and (XIII; 22,23-dihydro) displayed signals in the *C*-methyl region very similar to those of their parent adducts. The triols were obtained by reduction of the corresponding dimethyl esters (II; R′ = H, R = R″ = Me), (II; R′ = H, R = R″ = Me; 22,23-dihydro), and (VIII; R′ = Ac, R = R″ = Me; 22,23-dihydro) with lithium aluminium hydride.



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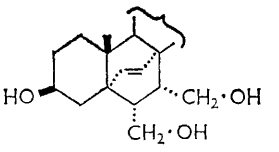


(XIII)

Saturation of the 1′,2′-double bond in the Windaus adduct should markedly weaken the signal at τ 9·06 by removing any diamagnetic shielding effect of the 19-methyl protons, which would resonate at lower fields and no longer coincide with the 18-methyl signal. We were unable to hydrogenate the nuclear double bond, although Inhoffen [2] had reported the preparation of a tetrahydro-derivative of the adduct (VII); so we were unable to confirm the shielding effect directly.

[11] Shoolery and Rogers, *J. Amer. Chem. Soc.*, 1958, **80**, 5121.
[12] Heller, McEvoy, and Bernstein, *J. Org. Chem.*, 1963, **28**, 1523; Knox, Velarde, Berger, Cuadriello, Landis, and Cross, *J. Amer. Chem. Soc.*, 1963, **85**, 1851.
[13] Cox, Bishop, and Richards, *J.*, 1960, 5118.
[14] Meyer and Huffman, *Tetrahedron Letters*, 1962, 691.
[15] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, 1959, (*a*) p. 129, (*b*) p. 124.

The above structural assignments were substantiated by the way in which the methanesulphonates (II and VIII; R′ = Me·SO_2, R = R″ = Me) reacted with acetate ions. Treatment of the former with tetrapropylammonium acetate in boiling ethyl methyl ketone [16] gave a 1 : 2 mixture of the 3α-acetoxy-compound (III; R′ = Me·CO, R = R″ = Me) and the unsaturated dimethyl ester (V; R = Me). The position of the newly introduced double bond in the latter product was not investigated. The dimethyl 3β-methanesulphonyloxy-ester (VIII; R′ = Me·SO_2, R = R″ = Me) and tetrapropylammonium acetate under identical conditions gave the dimethyl 3α-acetoxy-ester (IX; R′ = Ac, R = R″ = Me) and the unsaturated dimethyl ester (VI; R = Me) in the ratio 7 : 1. We consider that this large difference in ratios is due to greater steric hindrance to the development of a linear transition state during S_N2 attack of acetate ion at position 3 in compound (II; R′ = Me·SO_2, R = R″ = Me) than in (VIII; R′ = Me·SO_2, R = R″ = Me). Examination of Dreiding models show that the 6α-hydrogen atom in the former compound affords some protection of C-3 from attack from the rear by acetate ion; similar protection is absent from the compound (VIII; R = Me·SO_2, R = R″ = Me) because C-1′ (which takes the place of C-6) is now trigonal. An analogous explanation has been proposed to explain the higher substitution : elimination ratio (3 : 1) obtained on methanolysis of 3β-toluene-*p*-sulphonyloxy-5α-cholestane than that (7 : 12) for 3β-toluene-*p*-sulphonyloxycholestane-5α-ol.[17]

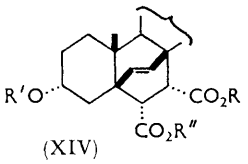
Treatment of the dimethyl 3α-acetoxy-ester (III; R′ = Ac, R = R″ = Me) with boiling methanolic potassium hydrogen carbonate for two hours gave a quantitative yield of the 3α-hydroxy-dicarboxylic acid monomethyl ester (III; R′ = R″ = H, R = Me), but identical treatment of the dimethyl 3β-acetoxy-ester (II; R′ = Ac, R = R″ = Me), which differs only in the configuration at C-3, gave only 40% of the hydrolysis product (II; R′ = R″ = H, R = Me), the remainder being dimethyl 3β-hydroxy-ester (II; R′ = H, R = R″ = Me). The dimethyl 3α- (IX; R′ = Ac, R = R″ = Me) and 3β-acetoxy-ester (VIII; R′ = Ac, R = R″ = Me), in the same hydrolytic conditions underwent only hydrolysis of the 3-acetoxy-groups to give, respectively, the 3α-hydroxy- (IX; R′ = H, R = R″ = Me) and 3β-hydroxy-ester (VIII; R′ = H, R = R″ = Me).

The reason for the differing behaviour of the above acetoxy-esters may be ascribed to the extents to which the 3-hydroxyl group (formed by initial hydrolysis of the acetate group) participates in the hydrolysis of the 6-methoxycarbonyl group, for hydrolysis of esters is facilitated by intramolecular hydrogen bonding with a hydroxyl group.[18] Dreiding models show that the smallest separation between the 3α-hydroxyl-hydrogen and the carbonyl-oxygen of the 6β-methoxycarbonyl groups in compound (III; R′ = H, R = R″ = Me) is 1·6 Å, a separation typical for compounds forming strong internal hydrogen bonds between hydroxyl and carbonyl groups.[19] In the same compound the distance of closest approach of the 3α-hydroxyl-hydrogen and the methoxyl-oxygen of the 6β-methoxycarbonyl group is 1·5 Å, again indicating that a strong hydrogen bond could be formed. Hence facilitation of hydrolysis of the 6β-methoxycarbonyl group by the 3α-hydroxyl group is possible whichever mode of hydrogen bonding is responsible for it.[18] The smallest separation between the 3β-hydroxyl-hydrogen and either of the oxygen atoms of the 6β-methoxycarbonyl group in the dimethyl 3β-hydroxy-ester (II; R′ = H, R = R″ = Me) is 3·6 Å, hitherto regarded as too great to permit hydrogen bonding.[20] The structures proposed for the dimethyl acetoxy-esters (III and IX; R′ = Ac, R = R″ = Me) are, therefore, compatible with the results obtained on hydrolysis.

We had considered the alternative structure (XIV; R′ = Ac, R = R″ = Me) for the

[16] Henbest and Jackson, *J.*, 1962, 954.
[17] Clayton, Henbest, and Smith, *J.*, 1957, 1982.
[18] Bartlett and Greene, *J. Amer. Chem. Soc.*, 1954, **76**, 1088; Henbest and Lovell, *J.*, 1957, 1965; West, Korst, and Johnson, *J. Org. Chem.*, 1960, **25**, 1976; Bender, *Chem. Rev.*, 1960, **60**, 53; Bruice and Fife, *J. Amer. Chem. Soc.*, 1962, **84**, 1973; Dalton, McDougall, and Meakins, *J.*, 1963, 4068.
[19] Kuhn, *J. Amer. Chem. Soc.*, 1952, **74**, 2492; 1954, **76**, 4323; 1958, **80**, 5950.
[20] Fales and Wildman, *J. Amer. Chem. Soc.*, 1963, **85**, 784.

dimethyl acetoxy-ester now formulated as (III; R′ = Ac, R = R″ = Me) because models indicate that internal alcoholysis of the 6α-methoxycarbonyl group could occur in this case to give the lactone (XV), which could give the 6α-carboxy-3α-hydroxy-7α-methoxycarbonyl compound (XIV; R′ = R″ = H, R = Me) by hydrolysis. However, the structure (XIV; R′ = Ac, R = R″ = Me) is impossible because the hydroxy-acid obtained, which would have structure (XIV; R′ = R″ = H, R = Me), was not lactonised on treatment with hydrochloric acid, acetyl chloride, or trifluoroacetic acid.



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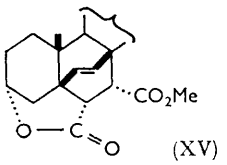

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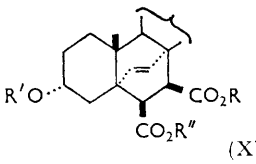

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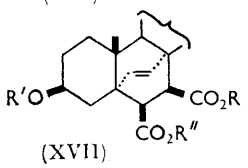

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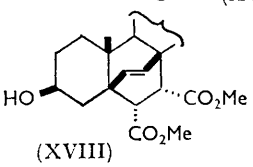

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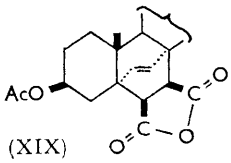

(XIX)

Dreiding models showed that hydrogen bonding between 3-hydroxyl-hydrogen and a 6-methoxycarbonyl group was sterically impossible in the hydroxy-esters (VIII, IX, XVI, and XVII; R′ = H, R = R″ = Me) which could in theory be derived from the β-adduct, and also in the hydroxy-ester (XVIII) derived from the α-adduct. We, therefore, understand why the methoxycarbonyl groups in the acetoxy-esters (VIII and IX; R′ = Ac, R = R″ = Me) are not hydrolysed under the conditions outlined above. Partial hydrolysis of the 3β-acetoxy-ester (II; R′ = Ac, R = R″ = Me) suggests some intramolecular facilitation of hydrolysis even at a separation of 3·6 Å between the hydroxyl and the carbonyl group.

The hydrolyses described above provide no evidence about the configuration at positions 6 and 7 in the dimethyl acetoxy-esters derived from the Inhoffen adduct, *i.e.*, they do not distinguish between structures (VIII) and (XVII), or (IX) and (XVI) (R′ = Ac, R = R″ = Me in each case). However, we assign structure (VII) to the Inhoffen adduct because a Dreiding model of this *endo*-anhydride showed no strong destabilising non-bonded interactions, whereas a model of the alternative *exo*-anhydride (XIX) revealed powerful steric interaction between the *exo*-anhydride group and the 18- and the 19-methyl groups, the anhydride–methyl separations being 0·4 and 1·0 Å, respectively. We have made the reasonable assumption that the adducts are formed under thermodynamic control. This assignment of configuration is also in agreement with the observation that the *endo*-isomer predominates in every addition of maleic anhydride to a cyclic diene.[21] The structures of the dimethyl acetoxy-esters (VIII and IX; R′ = Ac, R = R″ = Me) then follow from that of the Inhoffen adduct.

Experimental

Rotations cited are for $CHCl_3$ solutions. M. p.s were determined on a Kofler hot-stage apparatus. Preparative thin-layer chromatography was performed on glass plates 25 cm. square, with a layer of adsorbent (Merck silica gel G) ~1 mm. thick. Infrared spectra were measured for KBr discs, unless stated otherwise, on a Unicam S.P. 100 spectrophotometer. N.m.r. spectra were determined on an A.E.I. spectrometer, model R.S.2, for CCl_4 solutions unless stated otherwise, and are recorded on the τ scale.

3β-Acetoxy-5α,8α-ethenoergost-22-ene-6α,7α-dicarboxylic Anhydride (VII).—Ergosteryl acetate (66 g.) and maleic anhydride (22 g.) in xylene (528 ml.) were heated at 135° under nitrogen for

[21] Martin and Hill, *Chem. Rev.*, 1961, **61**, 537.

18 hr. The solvent was evaporated under reduced pressure at 100°, and the bulk of the maleic anhydride removed at 100°/0·1 mm. Careful fractional crystallisation from acetone gave the acetoxy-anhydride (VII) (12·7 g.) as cubes, m. p. 216—218° (lit.,[2] 216°), $[\alpha]_D$ −19° (*c* 1·3) (lit.,[2] −19°), $\nu_{max.}$ 1856, 1843, 1784, 1735, 1270, and 975 cm.$^{-1}$, n.m.r. (in pyridine) τ 9·28, 9·09, 9·04, 8·95, 8·86, and 8·36 (all overlapping and of similar intensity) 3·67 (doublet *J* 9 c./sec.) and 4·12 (doublet, *J*/9 c./sec.) (vinyl protons of the 1′,2′-double bond) 4·65 (vinyl protons of the Δ^{22}-double bond) 7·93 (CH_3·CO) (Found: C, 76·1; H, 9·1. Calc. for $C_{34}H_{48}O_5$: C, 76·1; H, 9·0%).

3β-*Hydroxy*-5β,8β-*ethenoergost*-22-*ene*-6β,7β-*dicarboxylic Acid* (II; R = R′ = R″ = H) *and its Dimethyl Ester*.—Evaporation of the mother-liquors from the above experiment gave a syrup which was boiled with 5% methanolic potassium hydroxide (1 l.) for 30 min. The mixture was poured into water, acidified with hydrochloric acid, and extracted twice with ether. The extracts were washed with water, dried (Na_2SO_4), and evaporated to yield a gum which on repeated crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) afforded the 3β-hydroxy-dicarboxylic acid (2·0 g.) as needles, m. p. 206—208° (lit.,[1] 202—206°), $[\alpha]_D$ −142° (*c* 0·5) (lit.,[1] −178°), $\nu_{max.}$ 1731, 1714, and 975 cm.$^{-1}$, n.m.r. 9·05 (18- and 19-Me), 6·58 (3β-OH), 4·73 (Δ^{22}-vinyl protons), and 4·18 ($\Delta^{1'}$-vinyl protons) (Found: C, 74·7; H, 9·1. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%).

This acid (597 mg.) with ethereal diazomethane (15 min.) gave the *dimethyl* 3β-*hydroxy-ester* (II; R′ = H, R = R″ = Me) as an oil $[\alpha]_D$ −179° (*c* 0·3), $\nu_{max.}$ (in CCl_4) 1740 and 973 cm.$^{-1}$, homogeneous on chromatoplate analysis (Found, after distillation at 150°/0·1 mm.: C, 75·1; H, 9·9. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%]. With acetic anhydride in pyridine it gave the oily 3β-*acetate*, $[\alpha]_D$ −175° (*c*, 0·5) (homogeneous on thin-layer chromatography) (Found, after distillation at 150°/0·1 mm.: C, 74·2; H, 9·3. $C_{36}H_{54}O_6$ requires C, 74·8; H, 9·2%].

3β-*Hydroxy*-5α,8α-*ethenoergost*-22-*ene*-6α,7α-*dicarboxylic Acid* (VIII; R = R′ = R″ = H), *and its Dimethyl Ester*.—3β-Acetoxy-5α,8α-ethenoergost-22-ene-6α,7α-dicarboxylic anhydride (VII) (4·04 g.) in aqueous-methanolic sodium hydrogen carbonate (400 ml.) was refluxed for 2 hr. Isolation in the usual way and two recrystallisations from acetone yielded the 3β-hydroxy-dicarboxylic acid (VIII; R = R′ = R″ = H) as an amorphous solid, m. p. 170—172° (crystals formed in the melt, m. p. 196—198°), $[\alpha]_D$ −48° (*c* 1·1), $\nu_{max.}$ 1719, 1700, and 980 cm.$^{-1}$, n.m.r. 9·20, 9·06, and 8·98 (overlapping and of comparable intensity), 6·43 (doublet, 3β-OH), 4·65 (Δ^{22}-vinyl protons), and 3·58 ($\Delta^{1'}$-vinyl protons) (Found: C, 75·10; H, 9·7. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%). Inhoffen [2] reports m. p. 198—199°, Wetter and Dimroth [22] report m. p. 120°, $[\alpha]_D$ −46°.

This acid (560 mg.) with ethereal diazomethane (15 min.) gave, after two crystallisations from acetone, the dimethyl ester as cubes, m. p. 164—166° (lit., 163°,[1] 72° [22]) $[\alpha]_D$ −48° (*c* 0·4,) $\nu_{max.}$ 1744 and 974 cm.$^{-1}$ (Found: C, 75·3; H, 9·9. Calc. for $C_{34}H_{52}O_2$: C, 75·5; H, 9·7%).

Treatment of the 3β-hydroxy-ester with methanesulphonyl chloride in pyridine, and crystallisation of the product from methanol, gave the 3β-*methanesulphonate* as cubes, m. p. 103—105°, $[\alpha]_D$ −45° (*c* 0·3), $\nu_{max.}$ 1757, 1180, and 972 cm.$^{-1}$ (Found: C, 67·7; H, 9·0. $C_{35}H_{54}O_7S$ requires C, 67·9; H, 8·8%).

3β-*Acetoxy*-5β,8β-*ethenoergost*-22-*ene*-6β,7β-*dicarboxylic Anhydride* (I).—The 3β-hydroxy-dicarboxylic acid (II; R = R′ = R″ = H) (352 mg.) was boiled with acetic anhydride (15 ml.) for 1 hr., then poured into water, and the precipitate was filtered off, dissolved in ether, washed with water, dried (Na_2SO_4), and recovered. Two crystallisations of the residue from acetone–di-isopropyl ether furnished the 3β-acetoxy-anhydride (I) as needles, m. p. 198—200° (lit.,[1] 200—201°), $[\alpha]_D$ −164° (*c* 0·4) (lit.,[1] −147°), $\nu_{max.}$ 1865, 1795, 1738, 1262, and 975 cm.$^{-1}$, n.m.r. 9·06 (18- and 19-Me), 7·94 (CH_3·CO), 4·65 (Δ^{22}-vinyl protons), and 4·34 ($\Delta^{1'}$-vinyl protons) (Found: C, 75·9; H, 9·3. Calc. for $C_{34}H_{48}O_5$: C, 76·1; H, 9·0%).

Equilibration of Dimethyl 3β-*Hydroxy*-5α,8α-*ethenoergost*-22-*ene*-6α,7α-*dicarboxylate* (VIII; R′ = H, R = R″ = Me).—Sodium (0·75 g.) was dissolved in dry ethanol (90 ml.); the ester (VIII; R′ = H, R = R″ = Me) (1·51 g.) was added, and the mixture refluxed for 40 hr. Water (5 ml.) was added and the whole was boiled for 24 hr. Isolation in the usual manner gave a foam (1·43 g.) which was treated with ethereal diazomethane. The product was chromatographed on a thin layer of silica. Elution with ether–benzene (1 : 19) gave three bands, which were severally extracted with refluxing ether. Band 1 gave an oily, new *dimethyl* 3β-*hydroxy-ester* (428 mg.), $[\alpha]_D$ −41° (*c* 0·3), $\nu_{max.}$ (in CCl_4) 1755 and 1744 cm.$^{-1}$, homogeneous on chromatoplate analysis (Found, after distillation at 150°/0·05 mm.: C, 75·5;

[22] Wetter and Dimroth, *Ber.*, 1937, **70**, 1665.

H, 9·8. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%]. Band 2 gave another new *dimethyl 3β-hydroxy-ester* (370 mg.) as needles, m. p. 104—107° (from acetone), $[\alpha]_D$ −53° (*c* 0·2), $\nu_{max.}$ 1757 cm.$^{-1}$ (Found: C, 75·6; H, 9·8). Band 3 gave the dimethyl 3β-hydroxy-ester (VIII; R′ = H, R = R″ = Me) (244 mg.), m. p. and mixed m. p. 164—166°, $[\alpha]_D$ −47° (*c* 0·4), also identified chromatographically (thin-layer technique) and by its infrared spectrum.

Equilibration of Dimethyl 3β-Hydroxy-5β,8β-ethenoergost-22-ene-6β,7β-dicarboxylate (II; R′ = H, R = R″ = Me).—The ester (II; R′ = H, R = R″ = Me) (632 mg.) was treated with sodium ethoxide (from 320 mg. of sodium in 40 ml. of ethanol) as above. The product, after hydrolysis and esterification as in the previous experiment, was chromatographed on silica-gel chromatoplates. Development with ether–benzene (1 : 4) and drying was repeated three times, to give two bands which were severally extracted with refluxing ether. Band 1 gave an oily, new *dimethyl 3β-hydroxy-ester* (103 mg.), $[\alpha]_D$ −130° (*c* 0·4), $\nu_{max.}$ (in CCl_4) 1750 cm.$^{-1}$. Band 2 gave the 3β-hydroxy-dimethyl ester (II; R′ = H, R = R″ = Me) (151 mg.), $[\alpha]_D$ −176° (*c* 0·1), characterised by chromatoplate analysis in a variety of solvents and by its infrared spectrum.

Dimethyl 3α-Acetoxy-5β,8β-ethenoergost-22-ene-6β,7β-dicarboxylate (III; R′ = Ac, R = R″ = Me) *and 5β,8β-Ethenoergost-2(or 3),22-diene-6β,7β-dicarboxylate* (V; R = Me).—The ester (II; R′ = H, R = R″ = Me) with methanesulphonyl chloride in pyridine gave the 3β-methanesulphonate as an oil, $[\alpha]_D$ −147° (*c* 0·3), $\nu_{max.}$ 1738, 1175, and 975 cm.$^{-1}$, homogeneous on chromatoplate analysis. This product (II; R′ = Me·SO_2, R = R″ = Me) (741 mg.) and tetrapropylammonium acetate (1·63 g.) in ethyl methyl ketone (20 ml.) were boiled for 20 hr. The mixture was diluted with ether and washed twice with water. Evaporation of the dried organic layer afforded an oil which on thin-layer chromatography (development with ether–benzene, 1 : 19) gave, as the less polar component, the unsaturated *ester* (V; R = Me) (275 mg.) as needles, m. p. 71—73° (from methanol), $[\alpha]_D$ −256° (*c* 0·4), $\nu_{max.}$ 1736 and 975 cm.$^{-1}$ (Found: C, 78·0; H, 10·0. $C_{34}H_{50}O_4$ requires C, 78·1; H, 9·6%). The more polar component, the *dimethyl 3α-acetoxy-ester* (III; R′ = Ac, R = R″ = Me) (140 mg.) was an oil, $[\alpha]_D$ −135° (*c* 0·2), $\nu_{max.}$ 1745 and 1255 cm.$^{-1}$ (Found, after distillation at 140°/0·02 mm.: C, 74·1; H, 9·5. $C_{36}H_{54}O_6$ requires C, 74·2; H, 9·3%).

3α-Hydroxy-7β-methoxycarbonyl-5β,8β-ethenoergost-22-ene-6β-carboxylic Acid (III; R′ = R″ = H, R = Me).—The ester (III; R′ = Ac, R = R″ = Me) (75 mg.) in 4 : 1 aqueous methanol (15 ml.) saturated with potassium hydrogen carbonate was refluxed for 2 hr. The mixture was poured into water, acidified, and extracted with ether. The extract was washed with water, dried, and evaporated. Crystallisation of the oily residue twice from methanol afforded the *3α-hydroxy-7β-methoxycarbonyl-6β-carboxylic acid* (60 mg.) as rods, m. p. 164—166°, $[\alpha]_D$ −209° (*c* 0·1), $\nu_{max.}$ 1724, 1708sh, 1734sh, and 972 cm.$^{-1}$ (Found: C, 75·4; H, 9·7. $C_{33}H_{50}O_5$ requires C, 75·2; H, 9·6%). Thin-layer chromatography of the mother-liquors showed only one spot, which corresponded to that of the crystalline product.

Hydrolysis of Dimethyl 3β-Acetoxy-5β,8β-ethenoergost-22-ene-6β,7β-dicarboxylate (II; R′ = Ac, R = R″ = Me).—The ester (II; R′ = Ac, R = R″ = Me) (459 mg.) in 4 : 1 aqueous methanol (92 ml.) saturated with potassium hydrogen carbonate was refluxed for 2 hr. The mixture was worked up as above to give a gum (397 mg.) which was chromatographed on silica gel (thin-layer technique). Development with ether gave two bands which were severally extracted with ether. The band of highest R_F was an oil (143 mg.), $[\alpha]_D$ −177° (*c* 0·4), identical with dimethyl 3β-hydroxy-5β,8β-ethenoergost-22-ene-6β,7β-dicarboxylate ester (II; R′ = H, R = R″ = Me) according to thin-layer chromatography and infrared spectroscopy. The band of lower R_F gave 3β-hydroxy-7β-methoxycarbonyl-5β,8β-ethenoergosta-22-ene-6β-carboxylic acid (II; R′ = R″ = H, R = Me) (96 mg.) as an oil, $[\alpha]_D$ −175° (*c* 0·6). With diazomethane it gave the dimethyl ester, identified by thin-layer chromatography and infrared spectroscopy.

Attempted lactonisation of this monomethyl ester under the following conditions failed:

(i) Ester (12 mg.) in dioxan (10 ml.) was saturated with dry hydrogen chloride and then kept at 100° for 6 hr.

(ii) Ester (15 mg.) with boiling acetyl chloride (3 ml.) for 4 hr.

(iii) Ester (269 mg.) and trifluoroacetic anhydride (1·5 ml.) at room temperature for 25 min.

Dimethyl 3α-Acetoxy-5α,8α-ethenoergost-22-ene-6α,7α-dicarboxylate (IX; R′ = Ac, R = R″ = Me) *and 5α,8α-Ethenoergost-2(or 3),22-diene-6α,7α-dicarboxylate* (VI; R = Me).—The dimethyl 3β-methanesulphonyloxy-ester (VIII; R′ = Me·SO_2, R = R″ = Me) (743 mg.) and tetrapropylammonium acetate (1·63 g.) in ethyl methyl ketone (20 ml.) were refluxed for 20 hr.

The mixture, after dilution with ether, washing with water, drying, and evaporation, afforded an oil which was chromatographed on a thin layer of silica gel (development with ether–benzene, 1 : 19). Band 1 yielded the oily unsaturated *dimethyl ester* (VI; R = Me) (76 mg.), $[\alpha]_D$ −46° (*c* 0·2), $\nu_{max.}$ (in CCl_4) 1759 and 972 cm.$^{-1}$ (Found, after distillation at 170°/0·02 mm.: C, 78·0; H, 9·5. $C_{34}H_{50}O_4$ requires C, 78·1; H, 9·6%). Band 2 yielded the *dimethyl* 3α-*acetoxy-ester* (IX; R′ = Ac, R = R″ = Me) (491 mg.) as broad needles, m. p. 116—118° (after three crystallisations from methanol), $[\alpha]_D$ −71° (*c* 0·6), $\nu_{max.}$ 1758, 1754, 1242, and 973 cm.$^{-1}$ (Found: C, 73·8; H, 9·6. $C_{36}H_{54}O_6$ requires C, 74·2; H, 9·3%).

Dimethyl 3α-*Hydroxy*-5α,8α-*ethenoergost*-22-*ene*-6α,7α-*dicarboxylate* (IX; R′ = H, R = R″ = Me).—The dimethyl 3α-acetoxy-ester (IX; R′ = Ac, R = R″ = Me) (132 mg.) in 4 : 1 aqueous methanol (15 ml.) saturated with potassium hydrogen carbonate was refluxed for 2 hr. Isolation as above afforded a homogeneous (thin-layer chromatography) gum. Crystallisation from methanol gave the *dimethyl* 3α-*hydroxy-ester* (IX; R′ = H, R = R″ = Me) as cubes, m. p. 164—166°, $[\alpha]_D$ −52° (*c* 0·4), $\nu_{max.}$ 1758, 1741, 1714, and 972 cm.$^{-1}$ (Found: C, 75·4; H, 10·0. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%).

3β-*Hydroxy*-5α,8α-*ethenoergostane*-6α,7α-*dicarboxylic Acid* (VIII; R = R′ = R″ = H, side chain saturated).—The acetoxy-anhydride (VII) (1·53 g.) in ethyl acetate (150 ml.) was hydrogenated in the presence of platinum oxide (206 mg.). After 1 mol. of hydrogen had been absorbed, filtration through "Hyflosupercel," and evaporation of the filtrate gave the amorphous acetoxy-22,23-dihydroanhydride (VII; side chain saturated), m. p. 172—173° (crystals form in the melt with m. p. 183—185°) (from acetone) (lit.,[2] sinters 172—174° and melts 202—203°), $[\alpha]_D$ −9° (*c* 1·2), $\nu_{max.}$ 1850, 1782, 1737, and 1260 cm.$^{-1}$ (Found: C, 75·7; H, 9·7. Calc. for $C_{34}H_{50}O_5$: C, 75·8; H, 9·4%). The acetoxy-dihydro-anhydride (VII; side chain saturated) (931 mg.) in 10% methanolic potassium hydroxide (50 ml.) was refluxed for 0·5 hr. The solid product (888 mg.) obtained after the usual working up and crystallised from acetone furnished the 22,23-*dihydro-hydroxy-dicarboxylic acid* (VIII; R = R′ = R″ = H; side chain saturated) as cubes, m. p. 168—171°, $[\alpha]_D$ −31° (*c* 0·9), $\nu_{max.}$ 1735 and 1715sh cm.$^{-1}$, n.m.r. 9·01 to 9·09 (broad overlapping signals), 6·47 and 6·66 (doublet, J = 5 c./sec.; OH), and 3·7 ($\Delta^{1'}$-vinyl protons) (Found: C, 74·9; H, 10·1. Calc. for $C_{32}H_{50}O_5$: C, 74·7; H, 9·8%).

The Dimethyl 22,23-*Dihydro*-3β-*hydroxy-ester* (VIII; R′ = H, R = R″ = Me; side chain saturated), prepared with diazomethane and crystallised from acetone, had m. p. 114—116°, $[\alpha]_D$ −26° (*c* 0·2), $\nu_{max.}$ 1770sh, 1745sh, and 1760 cm.$^{-1}$ (Found: C, 74·8; H, 10·4. $C_{34}H_{54}O_5$ requires C, 75·2; H, 10·0%).

The dimethyl 22,23-dihydro-3β-acetoxy-ester (VIII; R′ = Ac, R = R″ = Me), prepared with acetic anhydride in pyridine, had m. p. 110—112° (lit.,[2] 116—117°), $[\alpha]_D$ −29° (*c*, 0·6), $\nu_{max.}$ 1760, 1716sh, 1753sh, 1730, and 1257 cm.$^{-1}$ (Found: C, 74·0; H, 9·7. Calc. for $C_{36}H_{56}O_6$: C, 74·0; H, 9·7%).

3β-*Hydroxy*-5β,8β-*ethenoergostane*-6β,7β-*dicarboxylic Acid* (II; R = R′ = R″ = H; side chain saturated).—The 3β-hydroxy-dicarboxylic acid (II; R = R′ = R″ = Me) was hydrogenated at room temperature in ethyl acetate with a platinum catalyst. After 1 mol. of hydrogen was taken up, the platinum was removed by filtration through "Hyflosupercel." Concentration of the liquor gave the 3β-*hydroxy-dicarboxylic acid* as needles (from ethyl acetate), m. p. 194—197°, $[\alpha]_D$ −137° (*c* 0·3) (from ethanol), $\nu_{max.}$ 1748 and 1700 cm.$^{-1}$, n.m.r. 9·02 (18- and 19-Me), and 4·19 ($\Delta^{1'}$-vinyl protons) (Found: C, 75·1; H, 9·9. $C_{32}H_{50}O_5$ requires C, 74·7; H, 9·8%).

6β,7β-*Di*(*hydroxymethyl*)-5β,8β-*ethenoergost*-22-*en*-3β-*ol* (XII).—Dimethyl 3β-hydroxy-5β,8β-ethenoergost-22-ene-6β,7β-dicarboxylate (472 mg.) in refluxing ether (50 ml.) was treated with an excess of lithium aluminium hydride for 30 min. The usual isolation procedure afforded the *triol* (XII), m. p. 160—163° (needles from acetone–di-isopropyl ether), $[\alpha]_D$ −183° (*c* 0·4), n.m.r. 9·01 (18- and 19-Me), 5·88 (3β-OH), 4·67 (Δ^{22}-vinyl protons), and 4·34 ($\Delta^{1'}$-vinyl protons) (Found: C, 79·0; H, 10·5. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%).

6β,7β-*Di*(*hydromethyl*)-5β,8β-*ethnoergostan*-3β-*ol* (XII; side chain saturated).—The 22,23-dihydro-3β-hydroxy-ester (II; R′ = H, R = R″ = Me; 22,23-dihydro) was reduced with lithium aluminium hydride as above to the *triol* (XII; 22,23-dihydro), m. p. 173—176° (rods, from acetone), $[\alpha]_D$ −152° (*c* 0·3), n.m.r. 9·03 (18- and 19-Me), 5·90 (3β-OH), 4·31 and 4·04 (doublet, $\Delta^{1'}$-vinyl protons) (Found: C, 78·8; H, 11·3 $C_{32}H_{54}O_3$ requires C, 79·0; H, 11·2%).

6β,7β-*Di*(*hydroxymethyl*)-5β,8β-*ethenoergostan*-3β-*ol*. (XIII; 22,23-dihydro).—Reduction of the ester (VIII; R′ = Ac, R = R″ = Me; 22,23-dihydro) with lithium aluminium hydride as

above gave the *triol* (XIII; 22,23-dihydro), m. p. 209—211° (needles, from acetone), $[\alpha]_D$ −29° (EtOH) (*c* 0·7), n.m.r. 9·06, 9·13, 9·24 (overlapping and of similar intensity), 5·88 (3β-OH), and 4·08 ($\Delta^{1'}$-vinyl protons) (Found: C, 79·1; H, 11·5. $C_{32}H_{54}O_3$ requires C, 79·0; H, 11·2%).

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