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REVISED STRUCTURES FOR THE ADDUCTS OF ERGOSTERYL ACETATE WITH MALEIC ANHYDRIDE

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Abstract—Revised structures are proposed for four adducts of ergosteryl acetate and maleic anhydride. One is a normal Diels–Alder adduct of *endo* configuration, in which the dienophilic addition has occurred from the α side. The other three adducts are produced by an addition-abstraction process, with addition at C-7 and concomitant abstraction of either the 9α or the 14α hydrogen to give 7α succinic anhydride derivatives of 3β-acetoxy-ergosta-5,8,22-triene and 3β-acetoxyergosta-5,8(14),22-triene.

FIVE adducts of ergosteryl acetate and maleic anhydride have been described since the first was isolated by Windaus and Lutringhaus in 1931,[1] but their structures have not been completely elucidated. Windaus[1] and Inhoffen[2] reported different adducts of m.p. 200° and 216° respectively, both of which were given the structure I, without complete stereochemical description. Another adduct of m.p. 176°, $[\alpha]_D$ −207° was later isolated by Wallis *et al.*,[3] but no structure was proposed. Schubert and Bohme also obtained these three adducts,[4] and in addition prepared two new adducts of m.p. 198°, $[\alpha]_D$ −134°, and m.p. 182°, $[\alpha]_D$ −51°. They proposed that the Inhoffen adduct, and the adduct m.p. 182°, $[\alpha]_D$ −51°, were Diels–Alder adducts typified by structure I, and suggested that the other three adducts were produced by an 'ene' type reaction[5] between the dienophile and the ring B diene, and put forward the partial structure II for these compounds. Lehmann later assigned the structure I to the Inhoffen adduct,[6] but obtained no evidence concerning the stereochemistry of addition. He allocated structures III and IV to the hydroxy acids derived from the Windaus and Wallis adducts respectively on the basis of their mode of pyrolysis together with a mechanistic interpretation of Schubert and Bohme's results. Recently two of us advanced different structures for the Windaus and the Inhoffen adducts,[7] concluding that they were formed by dienophilic attack of maleic anhydride from the α and β side of ergosteryl acetate respectively (V and VI).

Subsequently we obtained improved NMR spectra which were not consistent with these structures,* and further spectroscopic and chemical evidence showed them to be incorrect.† Briefly, some of this new evidence was as follows.

Integration of the NMR spectra of the Windaus and the Inhoffen acetoxy anhydrides revealed the presence of three and four vinyl protons in these compounds respectively,

* The NMR spectra recorded by Jones and Thomas were determined on a A.E.I. spectrometer, model R.S.2, without integrator. The spectra recorded in this paper were obtained on a Varian A60 spectrometer with integrator.

† When the re-investigation was almost complete, and we had arrived at the new structures presented in this paper, Professor Huisman and Dr. van der Gen wrote to us pointing out that the original structures were incorrect, and suggested the same new structures. We are grateful to them for agreeing to our suggestion that we publish now an account of our new findings.

which is in agreement with structure VI for the Inhoffen adduct, but not in agreement with structure V for the Windaus adduct. A diene, obtained in four steps from the Windaus adduct, and previously formulated as VII,[7] was subsequently shown to have the spectral characteristics of a conjugated heteroannular diene, λ_{max} 238 mμ (ε 19,100), so that structure VII is incorrect. Further investigations indicated that the structure of the diene is VIII. Since the diene VIII was obtained from the Windaus adduct by methods whereby skeletal rearrangements were very improbable, this evidence also rendered structure V for the Windaus adduct untenable. This in turn cast doubt on the stereochemistry indicated in VI for the Inhoffen adduct, since it was deduced partly by comparison of its properties with those of the Windaus adduct.

In a re-investigation of the reaction between ergosteryl acetate and maleic anhydride we separated the products chromatographically, since the fractional crystallization techniques adopted previously were tedious and not readily reproducible. The mixture of acetoxy anhydrides obtained by reaction in xylene at 135° was treated in sequence with aqueous methanolic potassium bicarbonate solution, ethereal diazomethane, and acetic anhydride in pyridine to give a mixture from which four acetoxy dimethyl esters were separated by TLC. The structures we propose for these acetoxy dimethyl esters are IX, X, XI and XII. Evidence for these structures will be discussed subsequently in the text. We obtained no evidence for the presence of a fifth adduct of Diels–Alder type structure (acetoxy anhydride m.p. 182°, $[\alpha]_D$ −51°) described by Schubert and Bohme.[4]

The acetoxy dimethyl ester IX had m.p. 164–166°, $[\alpha]_D$ −38°, and was identical in all respects with the acetoxy dimethyl ester prepared from the Inhoffen acetoxy anhydride.[2,7] Its NMR spectrum showed signals due to four vinyl protons, two occurring in an AB system (τ_A 3·86; τ_B 4·12; J_{AB} = 8·5 c/s; vinyl protons of the Δ^6 double bond) and two in an unresolved multiplet at τ 4·83 (side chain vinyl protons). A one-proton doublet at τ 7·29 (J = 10 c/s) was attributed to the high field part of an AB system due to the C-1′ and C-2′ protons, the associated doublet being overlapped by two 3-proton singlets at τ 6·49 and τ 6·58 (methoxycarbonyl Me groups). The UV spectrum, with λ_{max} 207 mμ (ε 2700) was consonant with the presence of two disubstituted unconjugated double bonds in the molecule.[8] This spectral evidence is consistent with the normal Diels–Alder structure IX for the acetoxy dimethyl ester, and hence points to the structure XIII for the Inhoffen acetoxy anhydride. In agreement with this, this adduct has been shown readily to undergo the retro-Diels–Alder reaction on heating to give ergosteryl acetate and maleic anhydride.[2] The NMR spectrum of the Inhoffen acetoxy anhydride XIII displayed an AB pattern (τ_A 3·77; τ_B 4·2; J_{AB} = 8·5 c/s) due to the vinyl protons of the double bond, and a 2-proton multiplet at τ 4·81 was attributed to the side chain vinyl protons. The 3α proton resonated at τ 4·9 (broad multiplet), and the C-1′ and C-2′ protons gave rise to another AB pattern at higher field (τ_A 6·43; τ_B 7·15; J_{AB} = 9 c/s). The acetate Me group gave a 3-proton singlet at τ 8·00, whilst the C-19 Me group resonated at τ 9·04, and the C-18 Me group at τ 9·26.

We have depicted the Inhoffen acetoxy anhydride (XIII) as the product of dienophilic attack of maleic anhydride from the α side of the ergosteryl acetate molecule, with the anhydride moiety *endo* to the Δ^6 double bond. This would be the expected major product according to the widely accepted "rule of α attack" upon steroids, and

the well-known preference of maleic anhydride to add dienophilically in an *endo* manner.[9] We have obtained NMR spectroscopic evidence that this is the case. Partial hydrogenation of the Inhoffen acetoxy anhydride in ethyl acetate afforded the known dihydro acetoxy anhydride (XIV).[2,7] The NMR spectrum showed an AB pattern (τ_A 3·75; τ_B 4·20; J_{AB} = 8·5 c/s) due to the vinyl protons of the Δ^6 double bond. Further catalytic reduction of the acetoxy anhydride (XIV) with platinum in ethyl acetate containing a trace of perchloric acid gave the tetrahydro acetoxy anhydride (XV),[2] in which complete reduction was confirmed by the absence of the above AB pattern in the NMR spectrum. In the tetrahydro compound XV the C-19 Me group resonated at τ 8·87, whereas it resonated at τ 9·06 in the dihydro compound XIV. A pair of doublets attributed to the C-1′ and C-2′ protons was centred at τ 6·87 in the tetrahydro compound XV, but was centred at τ 6·68 in the dihydro compound XIV. Therefore saturation of the nuclear double bond produces a downfield shift of 19 c/s in the C-19 Me signal, and an upfield shift of 9 c/s in the C-1′ and C-2′ proton signals, indicating that the double bond in XIV exerts a shielding effect upon the C-19 Me group and a deshielding effect upon the C-1′ and C-2′ protons. Of the four possible Diels–Alder adducts (α-*endo*, α-*exo*, β-*endo*, β-*exo*) only the α-*endo* isomer has the required relationship between double bond, Me group, and anhydride moiety for this particular combination of effects to occur. In this case the C-19 Me group lies in the shielding cone of the Δ^6 double bond,[10a] and the C-1′ and C-2′ protons lie in a deshielding zone (Fig. 1). Analogous arguments have previously

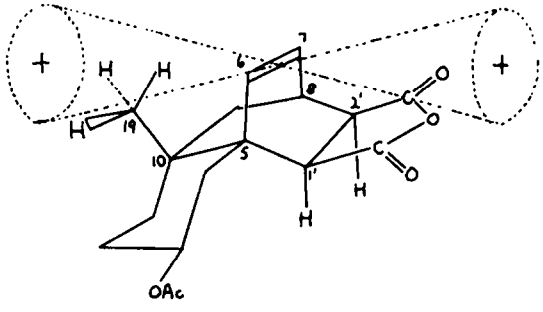


FIG. 1.

been used by Huisman *et al.* to allocate the α-*endo* structure to Diels–Alder adducts of 3β-acetoxycholesta-5,7,9(11)-triene with maleic anhydride.[11]

Hydrolysis of the acetoxy anhydride XIV with methanolic potassium hydroxide gave the hydroxy dicarboxylic acid (XVI),[2,7] which on treatment with bromine in chloroform afforded the hydroxy anhydride (XVII). We expected a bromolactone such as XVIII to be formed, by analogy with the behaviour of other unsaturated acids derived from *endo* adducts of maleic anhydride with dienes.[12] It appears that the steric compression on the β side of the Δ^6 double bond prevents formation of the intermediate bromonium ion, and therefore precludes bromolactonization. Partial hydrolysis of the acetoxy dimethyl ester (IX) gave the hydroxy dimethyl ester (XIX), which was oxidized by the Jones reagent[13] to the keto dimethyl ester (XX). In the NMR spectrum of this keto dimethyl ester the C-6 and C-7 vinyl protons manifest themselves as a two-proton singlet at τ 3·92, revealing that they were chemically

equivalent. The transition from non-equivalence in XIX to equivalence in XX may be due to transmission to ring B of the conformational distortion introduced by the 3 keto group.[14] The C-1′ and C-2′ protons gave rise to an AB pattern (τ_A 6·89; τ_B 7·24; J_{AB} = 11 c/s) whilst a two proton singlet at τ 7·20 overlapping this pattern was attributed to the C-4 methylene group. The methoxycarbonyl Me groups resonated at τ 6·52 and τ 6·55 (both 3-proton singlets) whilst the vinyl protons of the Δ^{22} double bond gave a two proton multiplet at τ 4·82. The NMR spectrum was therefore entirely consistent with structure XX for the keto dimethyl ester.

Hydrolysis of the acetoxy dimethyl ester (X), m.p. 78–81°, $[\alpha]_D$ −178°, gave the hydroxy dicarboxylic acid XXI, identical in all respects with the hydroxy dicarboxylic acid obtained by hydrolysis of the Windaus acetoxy anhydride.[1,7] The identity of these hydroxy acids was verified by partial hydrogenation of the two specimens to the same dihydro hydroxy dicarboxylic acid (XXII). Evidence for three double bonds in the Windaus adduct, one of which is Δ^5 has been obtained previously,[4] and arguments (based on mechanistic considerations) for the presence of a succinic anhydride residue at C-7 have been presented by Lehmann,[6] and in analogous cases by Huisman *et al.*[11,15] We have obtained chemical and spectroscopic evidence which established the positions of the three double bonds in the adduct, and illustrates that the succinic anhydride residue is at C-7 and is α orientated.

The NMR spectrum of the Windaus acetoxy anhydride (XXIII) showed a broad one proton multiplet at τ 4·55, attributed to the C-6 vinyl proton (which is coupled vicinally to the 7β proton and allylically to the two C-4 protons) and a two proton multiplet at τ 4·78 due to the side chain vinyl protons. The acetate Me group resonated at τ 8·04. The C-18 and C-19 Me group signals coincided in one intense singlet at τ 9·10. This is unusual since in the vast majority of steroids the C-19 Me group resonates at lower field than the 18 Me group.[16] However, it has been shown that the presence of a $\Delta^{8(14)}$ double bond causes an upfield shift in the C-19 Me resonance, and a downfield shift in the 18 Me resonance, relative to their position in 5α-androstane.[16] The coincidence of the two signals in this case may be understood if the compound contains a $\Delta^{8(14)}$ double bond, and if the magnitude of the resulting diamagnetic and paramagnetic shifts in the positions of the C-19 and C-18 Me signals respectively are such that they resonate at the same position. The presence of a $\Delta^{8(14)}$ double bond was substantiated by the UV spectrum of the acetoxy dimethyl ester (X), which had λ_{max} 212 mμ (ε 11,200). The contribution of the Δ^5 and Δ^{22} double bonds to this extinction coefficient may be estimated at 1500 and 500 respectively,[8] so the remaining value of 9200 is in excellent agreement with the extinction coefficient of about 10,000 observed at 210 mμ in $\Delta^{8(14)}$ steroids, but not with that of around 4500 exhibited by $\Delta^{8(9)}$ steroids at the same wavelength.[8] In the NMR spectrum of the acetoxy dimethyl ester (X) the vinyl protons of the Δ^6 and Δ^{22} double bonds overlapped to give a three proton multiplet at τ 4·75, while the 3α proton geminal to the acetate function gave rise to a broad signal at τ 5·48. The methoxycarbonyl methyl groups and the acetate Me group gave rise to a 6-proton and a 3-proton singlet at τ 6·42 and τ 7·98 respectively, whilst the C-19 and C-18 Me group signals coincided at τ 9·1 as before. The C-1′ and C-2′ hydrogens gave a 3-proton multiplet centred at τ 7·06.

Treatment of the hydroxy dicarboxylic acid (XXII) first with bromine in glacial acetic acid, and then with ethereal diazomethane, gave a product formulated as the bromolactone (XXIV) on the basis of analytical and spectroscopic evidence. The

IR spectrum had carbonyl bands at 1738 cm^{-1} (ester group) and 1730 cm^{-1} (δ-lactone), and the NMR spectrum contained a 3-proton singlet at τ 6·28 (Me of methoxycarbonyl). The IR assignments are substantiated by comparison with the IR spectra of the hydroxy dimethyl ester (XXV),[7] and the hydroxy lactone (XXVI),[17] which contain bands at 1740 cm^{-1} and 1728 cm^{-1} respectively. The data are not inconsistent with the presence of a 7-membered ring lactone, which could be formed if the C-2′ carboxyl group were involved in lactonization. However in view of the greater ease of formation of 6-membered lactone rings, and the close similarity between the spectral data of the two bromolactones (XXIV and XXVI), we consider it very unlikely that a 7-membered ring lactone has been formed. The NMR spectrum contained no vinyl proton signal, indicating that bromolactonization had occurred at the Δ^5 double bond. The UV spectrum of the bromolactone, with λ_{max} 210 mμ (ε 9100) confirmed the presence of the $\Delta^{8(14)}$ double bond, and contraindicated the presence of a $\Delta^{8(9)}$ double bond.[8] Examination of Dreiding models shows that lactone formation between a C-7 succinic acid residue and a Δ^5 double bond is possible only if the C-7 residue is α-orientated. A mechanistic consequence of ring closure at the 5α position is that the bromine atom is located at C-6 and has the β configuration, and this was verified by the occurrence of a one proton doublet (J = 1·5 c/s) at τ 5·75 in the NMR spectrum, as expected for the 6α proton adjacent to the bromine atom[10c] and vicinally coupled (dihedral angle 60°) with the 7β proton.[18] A three proton unresolved multiplet centred at τ 7·11 was attributed to the C-1′ and C-2′ protons adjacent to the carbonyl groups. A notable feature of the NMR spectrum was that the C-19 and C-18 Me groups resonated at τ 8·71 and τ 9·07 respectively. The coincidence of the two signals at τ 9·1, characterictic of all the compounds X, XXI, XXII, XXIII and XXV was destroyed in forming the bromolactone, the C-19 Me group signal undergoing a paramagnetic shift of 23 c/s. This shift is possibly due to the presence in the bromolactone of the 5α oxygen function and 6β bromine atom, features which are known to deshield the C-19 Me group;[16] this must be offset to some extent by the loss of the Δ^5 double bond, which itself deshields the C-19 Me group.[16] An additional factor is the presence under ring B of the lactonic carbonyl group, which is so orientated (Dreiding models) that the C-19 Me group lies in its deshielding zone.[10b] An assessment of the relative importance of these factors would be speculative, but at least the large paramagnetic shift observed can be rationalized in terms of the structural changes associated with the formation of the bromolactone (XXIV). The evidence so far presented established all the structural and stereochemical features of the Windaus adduct except the stereochemistry at C-1′. This will be discussed after considering the structure of the acetoxy ester (XI).

Hydrolysis of the acetoxy dimethyl ester (XI), m.p. 116–118°, $[\alpha]_D$ −175° gave the dicarboxylic acid (XXVII), which on treatment with acetic anhydride gave the acetoxy anhydride (XXVIII). The physical constants of the anhydride are in fair agreement with those obtained for the ergosteryl acetate/maleic anhydride adduct prepared independently by Wallis *et al.*,[3] and by Schubert and Bohme.[4] The acetoxy esters (X and XI) displayed very similar NMR, IR and UV spectra, indicating that their structures differed only in minor detail. They were interconverted by treatment with methanolic potassium hydroxide followed by reacetylation and re-esterification, indicating that they probably differed only in the configuration about C-1′. This was confirmed in the following manner. Partial hydrogenation of the acetoxy ester (XI)

gave the acetoxy dimethyl ester (XXIX), which was hydrolysed to the hydroxy dicarboxylic acid (XXX). Treatment of this compound with bromine in chloroform gave the bromolactone (XXXI), confirming that the succinic acid residue at C-7 was α orientated. The structure of the bromolactone (XXXI) was established spectroscopically. The NMR spectrum contained no signal due to vinyl protons, and contained a 3-proton singlet at τ 6·32 attributed to the methoxycarbonyl Me group. The 6α proton resonated at τ 5·67, as expected for a proton geminal to a halogen atom,[10c] but it occurred as a broad singlet (width at half intensity 4 c/s). Coupling of the 6α proton with the vicinal 7β proton might be expected to give rise to a doublet with coupling constant between 1·8 and 2·5 c/s,[18] but there is analogy for the reduction in the magnitude of coupling constants in the presence of electronegative substituents.[19] The NMR spectrum of the bromolactone (XXXI) was almost identical with that of the bromolactone (XXIV) in the τ 8·5 to 10 region, indicating a close similarity in their skeletal structure. In particular the C-19 and C-18 Me groups again resonated at τ 8·72 and τ 9·07 respectively. However in the bromolactone (XXXI) the C-1′ proton was associated with an incompletely resolved doublet at τ 7·15, whereas the two C-2′ protons gave rise to a multiplet at τ 6·69. This is in marked contrast to the spectrum of the bromolactone (XXIV), where the C-1′ and C-2′ protons give rise to a multiplet centred at τ 7·11. This difference in NMR spectral characteristics is associated with the difference in stereochemistry at C-1′ in the bromolactones, and furnishes evidence for a tentative assignment of configuration at this chiral centre. The greater deshielding of the C-2′ protons in the bromolactone (XXXI) than in the bromolactone (XXIV) may be associated with the R configuration at C-1′ in XXXI,

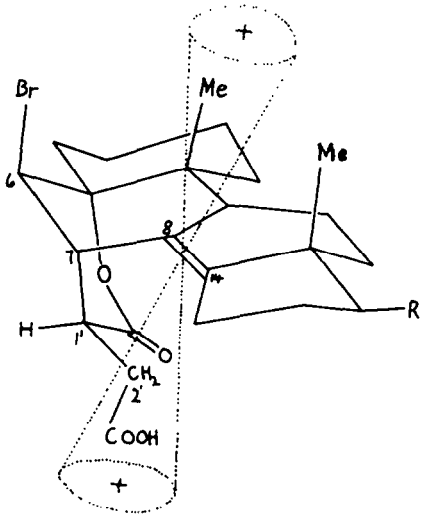


FIG. 2.

and the S configuration at C-1′ in XXIV. In XXIV the C-2′ methylene protons are held in the shielding cone of the $\Delta^{8(14)}$ double bond (Fig. 2), whereas in the compound XXXI it is remote from the double bond (Fig. 3) and is possibly slightly deshielded by it. The greater deshielding of the C-2′ methylene group in the bromolactone

(XXXI) is also consistent with its axial configuration, since it appears that an axial Me group adjacent to a carbonyl group in a 6-membered ring is subject to greater deshielding than an equatorial Me group.[20] In both cases we have assumed that the lactone ring has the chair conformation. Conformational analysis indicates that this is certainly true for the bromolactone (XXIV) (severe non-bonded interaction

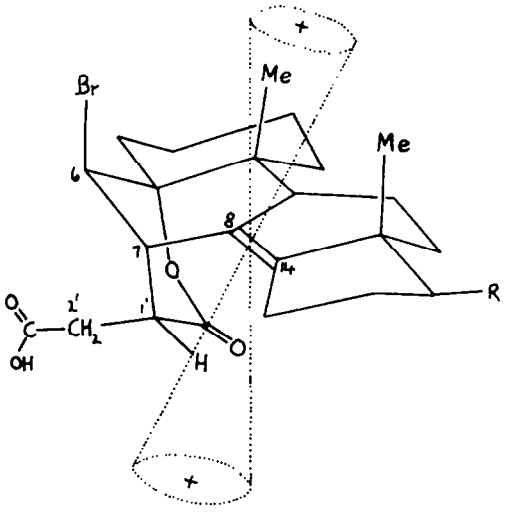


FIG. 3.

between the C-2′ methylene group and the 9α hydrogen destabilizes the boat conformation) but the more favoured conformation of the lactone ring in the bromolactone (XXXI) is not as readily apparent. However models reveal that if the lactone ring adopts the boat conformation in XXXI the C-2′ methylene group is still slightly deshielded by the $\Delta^{8(14)}$ double bond. In this boat conformation the spatial relationship between the C-2′ methylene group and the lactone carbonyl group is identical to that in the bromolactone (XXIV), so that adoption of this boat conformation in XXXI, or a conformation intermediate between boat and chair, would diminish, but not reverse the differential shielding effect of the lactone carbonyl group upon the C-2′ methylene group in the two compounds. Hence the arguments leading to the assignment of configuration at C-1′ are not invalidated by the conformational uncertainty of the lactone ring.

The stereospecific α substitution at C-7 during the formation of the Windaus and Wallis adducts is fully consistent with the concerted, four centre, cyclic mechanism previously advanced for "indirect substitutive addition" reactions,[21] the optical asymmetry at C-14 (14α hydrogen abstracted) in the reactant being transferred to C-7 (7α substitution) in the adduct, as required by the mechanism.[21c] The adducts differ in configuration at C-1′ because in the concerted addition the maleic anhydride can orientate itself in two ways with respect to the steroid molecule (Figs. 4 and 5) in a manner analogous to that leading to *endo* and *exo* adducts in Diels–Alder reactions of maleic anhydride with dienes.

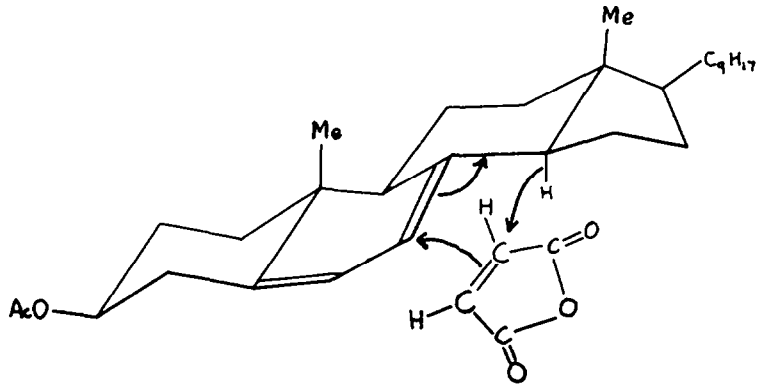


FIG. 4.

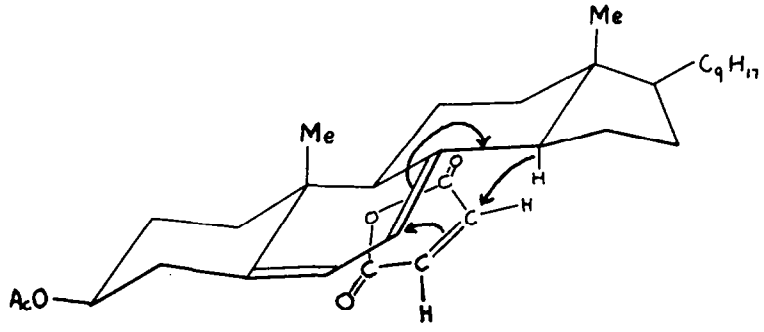


FIG. 5.

Hydrolysis of the acetoxy dimethyl ester (XII), m.p. 141°, $[\alpha]_D$ −78°, gave a hydroxy dicarboxylic acid (XXXII), which after treatment with boiling acetic anhydride gave and acetoxy anhydride (XXXIII). The physical constants of this anhydride, and of the acid XXXII correspond with those of an acetoxy anhydride and its derived dicarboxylic acid which were prepared by Schubert and Bohme,[4] who showed that these compounds contained three double bonds, one of which was Δ^5. The NMR spectrum of the acetoxy dimethyl ester (XII) differed markedly from those of the acetoxy dimethyl esters (X and XI). It displayed a 3-proton multiplet at τ 4·87 due to overlapping of the 6 vinyl proton signal and the signals of the vinyl protons of the Δ^{22} double bond. The two methoxycarbonyl Me groups resonated at τ 6·32 and τ 6·38, and the acetate Me group at τ 8·05. The most significant piece of information was that the C-19 and C-18 Me group resonances were separated by 32 c/s, being at τ 8·78 and τ 9·32 respectively. This is consistent with the presence of a $\Delta^{8(9)}$ double bond, which is known to exert a deshielding effect on the 19 Me group, and a shielding effect on the 18 Me group,[16] so that the signals due to these groups, which are separated by 6 c/s. in 5α-androstane, occur further apart. This was supported by the UV spectrum of the acetoxy ester (XII), which had λ_{max} 208 mμ (ε, 5400), the extinction coefficient of which, after subtraction of 2000 due to the contributions of the Δ^5 and Δ^{22} double bonds,[8] is consistent with the presence of a $\Delta^{8(9)}$ double bond, but not with that of a $\Delta^{8(14)}$ double bond.[8] A mechanistic interpretation of the formation of

the acetoxy anhydride (XXXIII) involves the concurrent abstraction of the 9α allylic hydrogen, and formation of the single bond to C-7, with migration of the Δ^7 double bond. (Fig. 6). This leads to the assignment of the α configuration for the C-7 succinic anhydride residue, as in the case of the acetoxy anhydrides (XXII and XXVIII).

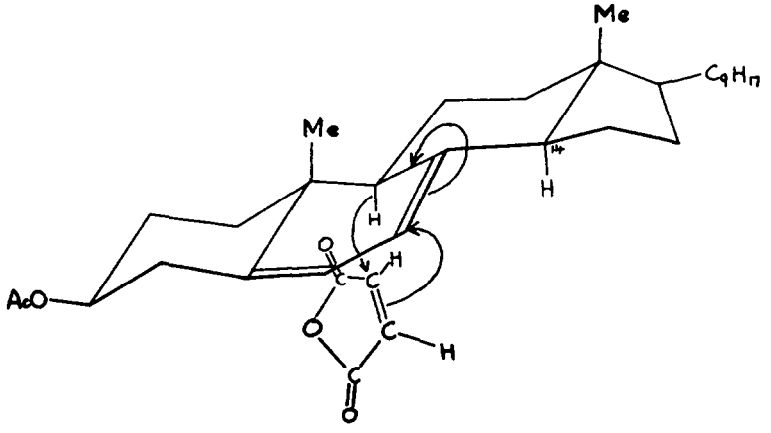
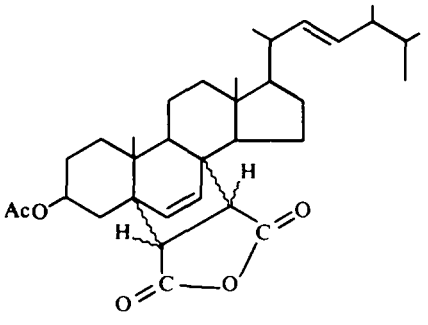


FIG. 6.

Since it appears that the stereochemistry of this addition-abstraction process is controlled by steric factors,[21c] we assign the S configuration to the C-1′ chiral centre. The non-bonded interactions between the constituent parts of the reactant molecules appear to be less in the transition state leading to the S adduct (Fig. 6) than in the transition state leading to the R adduct, which is relatively destabilized by interaction between the incoming maleic anhydride molecule and the 14α hydrogen.



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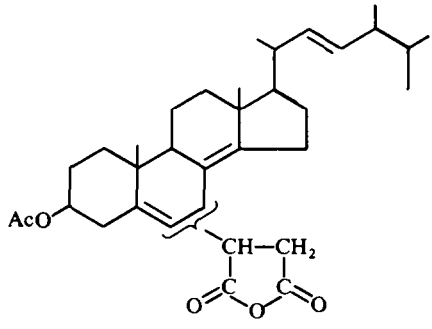

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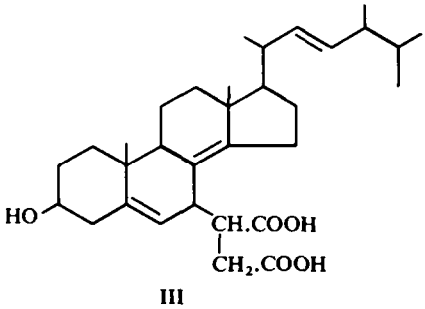
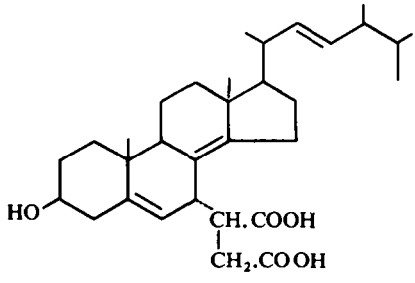


III



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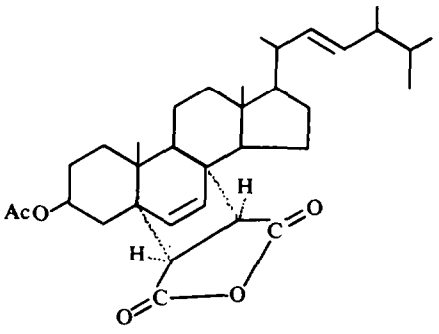

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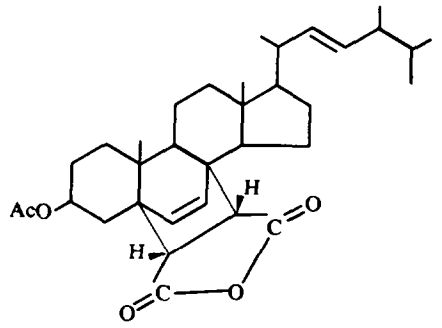
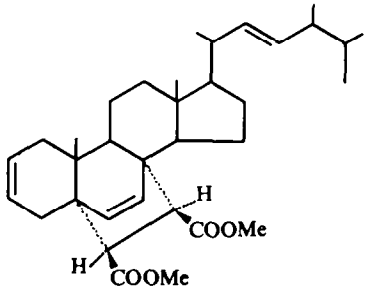
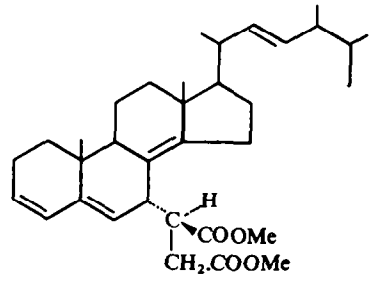


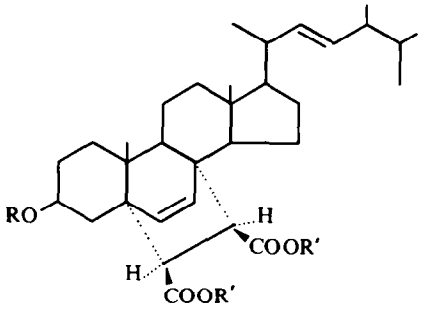
VI



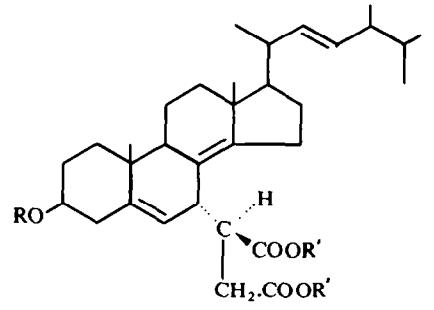
VII



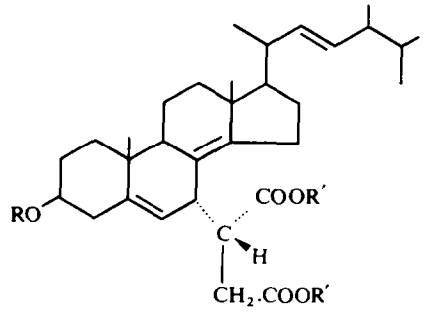
VIII



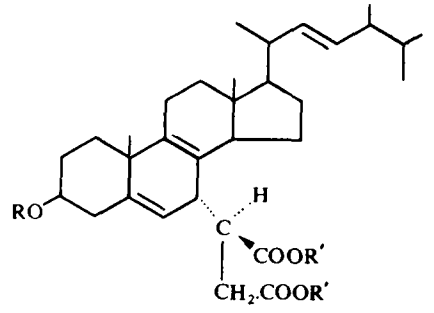
IX: R = Ac, R′ = Me
XIX: R = R′ = H



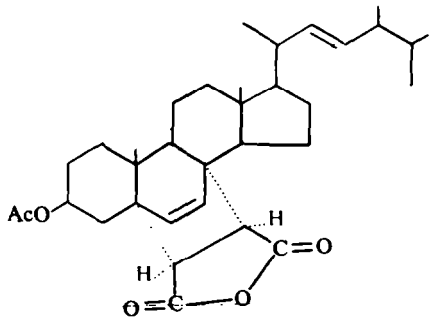
X: R = Ac, R′ = Me
XXI: R = R′ = H



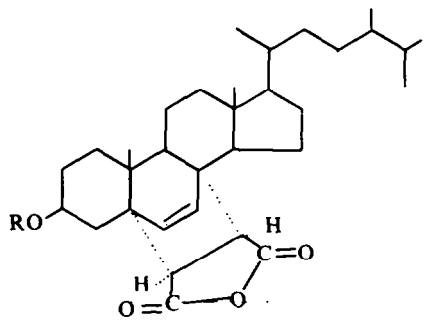
XI: R = Ac, R′ = Me
XXVII: R = R′ = H



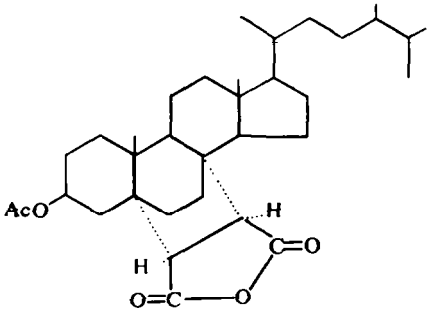
XII: R = Ac, R′ = Me
XXXII: R = R′ = H



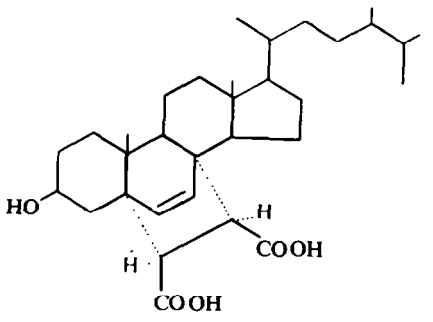
XIII



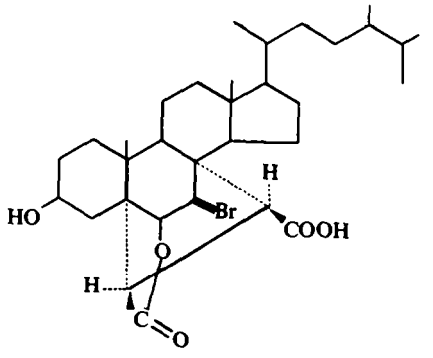
XIV: R = Ac
XVII: R = H



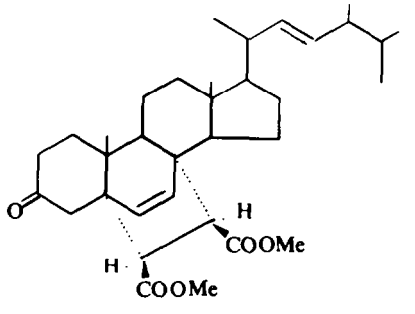
XV



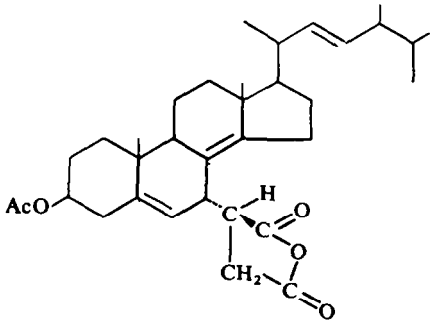
XVI



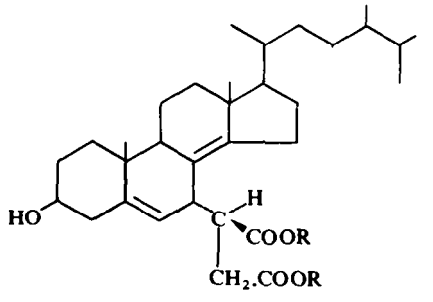
XVIII



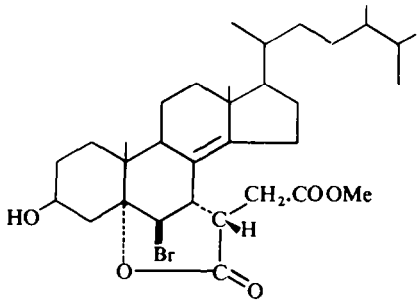
XX



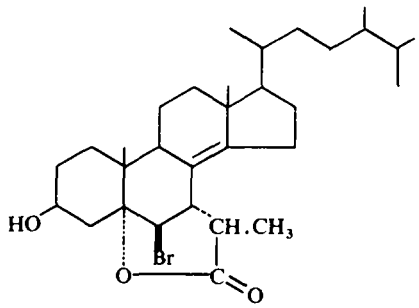
XXIII



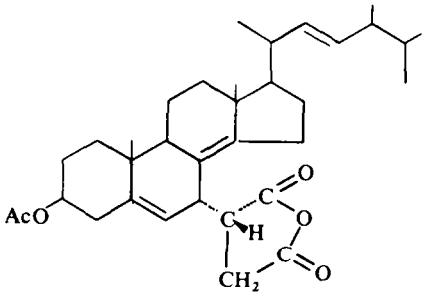
XXII: R = H
XXV: R = Me



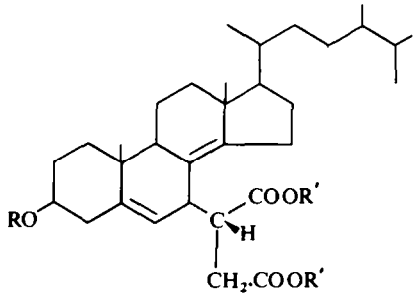
XXIV



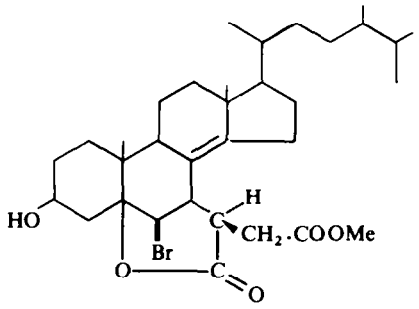
XXVI



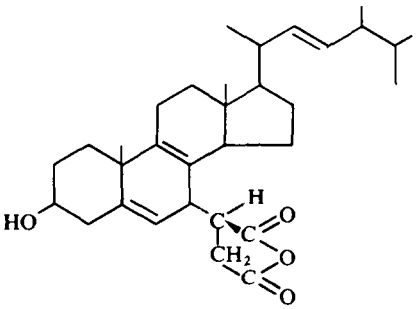
XXVIII



XXIX: R = Ac, R' = Me
XXX: R = R' = H



XXXI



XXXIII

EXPERIMENTAL

Rotations cited are for $CHCl_3$ sols unless stated otherwise. M.ps were determined on a Kofler hot-stage apparatus. Preparative TLC was performed on glass plates 25 cm square, with a layer of silica gel G (Merck) 1 mm thick. IR spectra were measured on a Unicam SP 100 spectrophotometer, and UV spectra on a Perkin–Elmer ultracord 137 spectrophotometer. NMR spectra were determined on a Varian A-60 spectrometer, and are recorded on the τ scale.

Acetoxy dimethyl esters IX, X, XI and XII. Ergosteryl acetate (23·9 g) and maleic anhydride (8 g) were heated together in boiling zylene for 19 hr. The solvent was evaporated under reduced press, and the maleic anhydride was removed at 100°/0·1 mm. The residual gum was dissolved in sat $KHCO_3$ aq in aqueous MeOH (1:4, 1 l.) and boiled for 2 hr. The mixture was poured into water, acidified with dil HCl and extracted with ether. The ethereal extract was washed with water, dried (anh. Na_2SO_4), concentrated to small bulk and treated with ethereal diazomethane. Evaporation of the ether gave a gum (27·7 g) which was

dissolved in pyridine and acetylated with Ac_2O at room temp overnight. The usual work-up gave a gum (27·6 g), a portion of which (8 g) was chromatographed on thin layer silica plates. Development with ether–benzene (1:12) gave three bands which were severally extracted with ether. Band 1 gave an oil (1·49 g) which on crystallization from MeOH gave IX as needles, m.p. 164–166° (lit.,[2] m.p. 164°), $[\alpha]_D$ −38° (*c*, 1·3), ν_{max} 1762, 1733 cm^{-1}, λ_{max} 207 mμ (ε, 2,700), NMR spectrum (in CCl_4) an AB system τ_A 3·77, τ_B 4·20, J_{AB} = 8·5 c/s (C-6 and C-7 vinyl protons), 4·81 (2H, multiplet, vinyl protons of Δ^{22} double bond), 4·90 (1H, broad multiplet, 3α proton), an AB system τ_A 6·43, τ_B 7·15, J_{AB} = 9 c/s (C-1′ and C-2′ protons), 8·00 (3H, singlet, acetate Me), 9·04 (C-19 Me), 9·26 (C-18 Me), (Found: C, 74·6; H, 9·2. Calc. for $C_{36}H_{54}O_6$: C, 74·2; H, 9·3%.) Band 2 gave a gum which was shown by TLC to consist of two very similar compounds. Careful rechromatography (thin layer) on a preparative scale gave XII as needles (from MeOH) m.p. 140–141°, $[\alpha]_D$ −78° (*c*, 1·3), ν_{max} 1738, 1244, 1038 cm^{-1}, λ_{max} 208 mμ (ε, 5400), NMR spectrum (in CCl_4) τ 4·87 (3H, multiplet, C-6, C-22, and C-23 vinyl protons), 5·55 (1H, multiplet, 3α proton), 6·32 and 6·38 (singlets, methoxycarbonyl Me groups), 8·05 (singlet, acetate Me), 8·78 (C-19 Me) and 9·32 (C-18 Me), (Found: C, 74·1; H, 9·5%), and X as needles (from MeOH) m.p. 78–81°, $[\alpha]_D$ −178° (*c*, 1·3), ν_{max} 1737, 1245, 1144 cm^{-1}, λ_{max} 212 mμ (ε, 11,200), NMR spectrum (in CCl_4) τ 4·75 (3H, multiplet, C-6, C-22, and and C-23 vinyl protons), 5·48 (1H, multiplet, 3α proton), 6·42 (6H, singlet, methoxycarbonyl Me groups), 7·06 (3H, multiplet, C-1′ and C-2′ protons), 7·98 (3H, singlet, acetate Me) 9·10 (C-18 and C-19 Me groups). Band 3 furnished a gum (1·59 g) which crystallized from MeOH to give XI as needles, m.p. 116–118°, $[\alpha]_D$ −175° (*c*, 0·6), ν_{max} 1744, 1738, 1246 cm^{-1}, λ_{max} 211 mμ (ε, 11,200), NMR spectrum (in CCl_4) τ 4·81 (3H, multiplet, C-6, C-22 and C-23 vinyl protons), 5·48 (1H, singlet, 3α proton), 6·46 and 6·48 (6H, overlapping singlets, methoxycarbonyl Me groups) 7·01 (3H, multiplet, C-1′ and C-2′ protons), 8·06 (3H, singlet, acetate Me), 9·11 (C-18 and C-19 Me's). (Found: C, 74·1; H, 9·6%.)

3β-Acetoxy-5α,8α-ethanoergost-1′β,2′β-dicarboxylic acid anhydride (XV). The Inhoffen acetoxy anhydride[2] (XIII) (396 mg) in AcOEt (25 ml) containing 4 drops of perchloric acid was hydrogenated in the presence of PtO_2 (100 mg). After 3 days the soln was filtered through Hyflosupercel, and the filtrate evaporated to give a colourless gum (393 mg). Crystallization from MeOH gave XV as needles m.p. 183–186°, $[\alpha]_D$ −36° (*c*, 0·5) (lit.[2] m.p. 187°, $[\alpha]_D$ −25°), ν_{max} 1843, 1781, 1737, 1256 cm^{-1}, NMR spectrum (in $CDCl_3$) τ 4·92 (1H, multiplet, 3α proton), an AB system τ_A 6·50, τ_B 7·19, J_{AB} = 9·5 c/s (C-1′ and C-2′ protons), 7·99 (singlet, acetate Me) 8·87 (C-19 Me) and 9·24 (C-18 Me). (Found: C, 75·8; H, 9·6. Calc. for $C_{34}H_{52}O_5$, C, 75·6; H, 9·6%.)

The anhydride (XIII), prepared by the method previously described,[2,7] had m.p. 217–220°, $[\alpha]_D$ −20°, (lit., m.p. 216–218°, $[\alpha]_D$ −19°) NMR spectrum (in CCl_4) an AB system, τ_A 3·77, τ_B 4·20, J_{AB} = 8·5 c/s. (C-6 and C-7 vinyl protons), 4·81 (2H, multiplet, C-22 and C-23 vinyl protons), 5·01 (1H, multiplet, 3α proton), an AB system τ_A 6·43, τ_B 7·15, J_{AB} = 8·5 c/s (C-1′ and C-2′ protons), 8·00 (singlet, acetate Me), 9·04 (C-19 Me) and 9·26 (C-18 Me).

Compound XIV, prepared by partial hydrogenation of the adduct (XIII) in the manner previously described,[7] had m.p. 172–173°, NMR spectrum (in $CDCl_3$) an AB system τ_A 3·75, τ_B 4·20, J_{AB} = 8·5 c/s (C-6 and C-7 vinyl protons), 4·78 (1H, broad multiplet, 3α proton), an AB system τ_A 6·42, τ_B 7·06, J_{AB} = 8·5 c/s (C-1′ and C-2′ protons), 7·96 (singlet, acetate Me), 9·06 (C-19 Me) 9·25 (C-18 Me). Further hydrogenation of XIV in the presence of perchloric acid in the above manner gave XV.

Treatment of 3β-hydroxy-5α,8α-ethanoergost-6-ene-1′β,2′β-dicarboxylic acid (XVI) *with bromine.* Compound XVI (1·43 g) prepared by hydrolysis of XIV as described,[7] was dissolved in $CHCl_3$ (1·4 l.) and treated with Br_2 (0·9 ml). After 5 days ether and water were added and the organic layer was washed with water, dried and evaporated to give a brown gum. TLC on a preparative scale with ether–benzene (7:3) as developer gave *3β-hydroxy-5α,8α-ethanoergost-6-ene-1′β,2′β-dicarboxylic acid anhydride* (XVII; 273 mg) as needles (from MeOH) m.p. 196–198°, $[\alpha]_D$ −28° (*c*, 1·5), ν_{max} 1843, 1776 cm^{-1}, NMR spectrum (in $CDCl_3$) an AB system τ_A 3·74, τ_B 4·20, J_{AB} = 8·5 c/s (C-6 and C-7 vinyl protons), 5·83 (1H, multiplet, 3α proton), an AB system τ_A 6·53, τ_B 7·13, J_{AB} = 8 c/s (C-1′ and C-2′ protons), 9·06 (C-19 Me), 9·25 (C-18 Me). (Found: C, 77·6; H, 9·9. $C_{32}H_{48}O_4$ requires: C, 77·4; H, 9·7%.)

3-Oxo-(1′β,2′β-dimethoxycarbonyl) 5α, 8α-ethanoergosta-6,22-diene (XX). Compound IX (408 mg) was treated with a boiling, sat $KHCO_3$ aq in 10% aqueous MeOH (25 ml) for 1 hr. The usual work-up gave a foam (379 mg) which crystallized from MeOH to give 3β-hydroxy-(1′β,2′β dimethoxycarbonyl)-5α,8α-ethanoergost-6,22-diene as cubes, m.p. 163–165° (lit.,[2,7] m.p. 163°, and m.p. 164–166°). A stirred soln of the hydroxy dimethyl ester (301 mg) in acetone (30 ml) and ether (5 ml) at −20° was treated dropwise with 0·4 ml of "8N" CrO_3 in H_2SO_4 aq, added over 2·5 min. After stirring for a further 2·5 min, a sat soln of SO_2 in acetone was added, and the mixture was poured into water and extracted with ether. The usual

washing, drying and evaporation gave a solid residue (299 mg) which crystallized from MeOH to give *3-oxo-(1'β,2'β-dimethoxycarbonyl)-5α,8α-ethanoergosta-6,22-diene* (XX) as needles m.p. 202–204°, $[\alpha]_D$ −217° (*c*, 0·5), ν_{max} 1756, 1717, 1203, 1177 cm^{-1}, NMR spectrum (in CCl_4) τ 3·92 (2H, singlet, C-6 and C-7 vinyl protons) 4·82 (2H, multiplet, C-22 and C-23 vinyl protons) 6·52 and 6·65 (6H, overlapping singlets, methoxycarbonyl Me groups), an AB system τ_A 6·89, τ_B 7·24, J_{AB} = 11 c/s (C-1' and C-2' protons), 7·20 (2H, broad singlet, C-4 methylene group). (Found: C, 75·8; H, 9·65. $C_{34}H_{50}O_5$ requires: C, 75·8; H, 9·3%.)

Equilibration of the acetoxy dimethyl esters (X *and* XI). Compound XI (897 mg) was added to a soln of Na (547 mg) in dry EtOH (50 ml) and the mixture boiled under reflux for 52 hr. Water (5 ml) and NaOH (510 mg) were added, and the mixture boiled for a further 44 hr. Isolation by ether extraction in the usual way gave a gum which was treated with ethereal diazomethane. Evaporation, and treatment with Ac_2O in pyridine overnight gave a gum (809 mg) which was chromatographed on silica (thin layer technique). Elution with ether–benzene (1:9) gave two bands which were severally collected, and the products crystallized from MeOH. Band 1 gave XI (210 mg) m.p. and mixed m.p. 116–118°, and band 2 gave X (175 mg) m.p. and mixed m.p. 78–81°. These products were identical spectroscopically and chromatographically with authentic specimens. Compound XI under the above conditions likewise gave a mixture of X and XI.

3β-Hydroxy-7α-ethanoergosta-5,8(14),22-triene-1'(R),2'-dicarboxylic acid (XXVII). Compound XI (698 mg) in a 5% KOH in 10% aqueous MeOH (95 ml) was boiled under reflux for 5 hr. The usual work-up gave a foam (609 mg) which crystallized from AcOEt–pet ether to give XXVII m.p. 168–171°, $[\alpha]_D$ −159° (acetone) (*c*, 0·6), ν_{max} 1727, 1178 cm^{-1}, NMR spectrum (in pyridine) τ 4·46, (1H, broad singlet, C-6 vinyl proton), 4·85 (2H, multiplet, C-22 and C-23 vinyl protons). (Found: C, 74·8; H, 9·65. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%.)

3β-Acetoxy-7α-ethanoergosta-5,8(14),22-triene-1'(R),2'-dicarboxylic acid anhydride (XXVIII). Compound XXVII (274 mg) was boiled with Ac_2O (12 ml) for 1 hr. The mixture was poured into water, extracted with ether, and the ethereal extract washed with $NaHCO_3$ aq. Evaporation gave a gum which crystallized from MeOH to give XXVIII, as needles, m.p. 180–182°, $[\alpha]_D$ −247° (*c*, 0·5), ν_{max} 1870, 1789, 1736, 1260 cm^{-1}. (Found: C, 76·1: H, 8·8. $C_{34}H_{48}O_5$ requires: C, 76·1; H, 9·1%.) Schubert and Bohme[4] quote m.p. 175–178°, $[\alpha]_D$ −191° for one of their acetoxy anhydrides, which was obtained from a hydroxy dicarboxylic acid of m.p. 187–189°, $[\alpha]_D$ −131°. This anhydride is most probably identical with one of m.p. 175–176°, $[\alpha]_D$ −207° isolated by Hicks *et al.*,[3] and with our anhydride (XXVIII).

3β-Acetoxy-1'(R),2'-dimethoxycarbonyl-7α-ethanoergosta-5,8(14)-diene (XXIX). Compound XI (2·61 g) in AcOEt (300 ml) was hydrogenated over PtO_2 (100 mg). After 1 mole equiv of H_2 was taken up the mixture was filtered through Hyflosupercel, and the filtrate evaporated to a gum. Crystallization from MeOH gave *the product* as needles, m.p.85–89°, $[\alpha]_D$ −99° (*c*, 1·0), ν_{max} 1748, 1741, 1241, 1168 cm^{-1}, NMR spectrum (in CCl_4) τ 4·86 (1H, broad singlet, C-6 vinyl proton), 5·45 (1H, multiplet, 3α proton) 6·46 and 6·48 (6H, overlapping singlets, methoxycarbonyl Me groups), 8·04 (singlet, acetate Me) 9·13 (C-18 and C-19 Me's). (Found: C, 74·3; H, 9·4. $C_{36}H_{56}O_6$ requires: C, 74·0; H, 9·6%.)

3β-Hydroxy-7α-ethanoergosta-5,8(14)-diene-1'(R),2'-dicarboxylic acid (XXX). Compound XXIX (2·37 g) was treated with a boiling 5% KOH in 10% aqueous MeOH (330 ml) for 6 hr. The usual work-up gave a foam which crystallized from AcOEt–pet ether to give the *hydroxy dicarboxylic acid* XXX m.p. 156–160°, $[\alpha]_D$ −63° (acetone) (*c*, 0·6) ν_{max} 1750, 1709 cm^{-1}, NMR spectrum (in pyridine) τ 4·42 (1H, doublet, *J* = 2·5 c/s, C-6 vinyl proton). (Found: C, 74·5; H, 9·8. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·7%.)

3β,5α-Dihydroxy-6β-bromo-7α-ethanoergost-8(14)-ene-2'-methoxycarbonyl-1'(R)-carboxylic acid-(5,1')-lactone (XXXI). A soln of XXX (1·3 g) in $CHCl_3$ (1300 ml) was treated with 1·5 ml of a soln of Br_2 (1 ml) in $CHCl_3$ (10 ml). After 2·5 hr in the dark, the mixture was diluted with water and ether, and the organic layer was washed with water, dried, and evaporated to give a brown gum (1·49 g) which was treated with ethereal diazomethane. Evaporation, and TLC on a preparative scale using MeOH–$CHCl_3$ (1:25) as developer gave the *bromolactone* (XXXI; 276 mg) as needles (from MeOH) m.p. 99–102°, $[\alpha]_D$ −10° (*c*, 1·0) ν_{max} 1736 cm^{-1}, λ_{max} 210 mμ (ε, 9500), NMR spectrum (in $CDCl_3$) τ 5·65 (1H, singlet, $W_{\frac{1}{2}}$ 4 c/s, 6α proton), 5·80 (1H, broad, 3α proton), 6·31 (3H, singlet, methoxycarbonyl Me group), 6·69 (2H, 2' methylene group), 7·15 (1H, 1'methylene group), 8·72 (C-19 Me), 9·07 (C-18 Me). (Found: C, 65·4; H, 8·3; Br, 12·9. $C_{33}H_{52}O_5Br$ requires: C, 65·1; H, 8·55; Br, 13·2%.)

3β-Hydroxy-7α-ethanoergosta-5,8(14),22-triene-1'(S),2'-dicarboxylic acid (XXI). Compound X (1·93 g) and KOH (25 g) in MeOH (250 ml) containing water (30 ml) was refluxed for 6 hr. The usual work-up gave a gum (1·73 g) which crystallized from AcOEt to give XXI (1·14 g) as needles, m.p. 202–204°, $[\alpha]_D$ −145° (*c*, 0·8), NMR spectrum (in pyridine) τ 4·30 (1H, multiplet, C-6 vinyl proton), 4·77 (2H, C-22 and C-23

vinyl protons). This product was identical spectroscopically and chromatographically with the Windaus hydroxy dicarboxylic acid,[1,7] and showed no m.p. depression on admixture.

Compound XXIII prepared from XXI in the manner previously described,[1,7] had m.p. 199–201°, $[\alpha]_D$ −160° (*c*, 0·5) lit.,[7] m.p. 198–200°, $[\alpha]_D$ −164°), NMR spectrum (in CCl_4) τ 4·55 (1H, broad singlet, C-6 vinyl proton), 4·78 (2H, multiplet, C-22 and C-23 vinyl protons) 5·50 (1H, multiplet, 3α proton) 8·04 (singlet, acetate Me), 9·10 (C-18 and C-19 Me's).

3β,5α-*Dihydroxy*-6β-*bromo*-7β-*ethanoergost*-8(14)-*ene*-2′-*methoxycarbonyl*-1′(S)-*carboxylic acid*-(5,1′) *lactone* (XXIV). Compound XXI (930 mg) in AcOEt (150 ml) was hydrogenated over PtO_2 (190 mg). After 1 mole equiv of H_2 had been taken up the soln was filtered through Hyflosupercel and the filtrate was evaporated. The residual solid crystallized from AcOEt to give XVI, m.p. 193–197°, identical in all respects with the sample prepared previously.[7] Compound XVI (552 mg) in glacial AcOH (120 ml) at 20° was treated with 0·62 ml of a soln of Br_2 (1 ml) in glacial AcOH (9 ml). After 1 hr, the reaction mixture was poured into water, extracted with ether, and the ethereal extract washed several times with water and then with $NaHCO_3$aq. The solid brown residue obtained after drying and evaporation was treated with ethereal diazomethane, and the residue obtained on further evaporation was chromatographed on silica (preparative TLC). Development with ether–benzene (1:1) gave one predominant band which was extracted with ether, evaporated, and crystallized from MeOH to give the *bromolactone* (XXIV), m.p. 183–185°, $[\alpha]_D$ −43° (*c*, 0·3), ν_{max} 1730, 1738 cm^{-1}, λ_{max} 210 mμ (ε, 9100), NMR spectrum (in $CDCl_3$) τ 5·78 (1H, doublet, J = 1·5 c/s, 6β proton), 5·83 (1H, multiplet, 3α proton), 6·28 (3H, singlet, methoxycarbonyl Me group), 7·11 (3H, multiplet, C-1′ and C-2′ protons), 8·71 (C-19 Me), 9·07 (C-18 Me). (Found: C, 65·3; H, 8·3; Br, 13·4. $C_{33}H_{52}O_5Br$ requires: C, 65·1; H, 8·5; Br, 13·2%.)

3β-*Hydroxy*-7α-*ethanoergosta*-5,8,22-*triene*-1′(S),2′-*dicarboxylic acid* (XXXII). Compound XII (262 mg) was treated with a boiling 5% KOHaq in 10% aqueous MeOH (30 ml) for 4 hr. The usual work-up gave a yellow solid (200 mg) which crystallized from MeOH to give the *product* XXXII as needles, m.p. 221–223°, $[\alpha]_D$ −23° (*c*, 0·9) [lit.,[22] m.p. 221–223°, $[\alpha]_D$ −71° (acetone)], ν_{max} 1727 cm^{-1}. (Found: C, 75·0; H, 9·4. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%.)

3β-*Acetoxy*-7α-*ethanoergosta*-5,8,22-*triene*-1′(S),2′-*dicarboxylic acid anhydride* (XXXIII). Compound XXXII (100 mg) was boiled with Ac_2O for 1 hr. The residue obtained after the usual work-up crystallized from AcOH to give XXXIII, m.p. 196–198°, $[\alpha]_D$ −143° (*c*, 0·25). Schubert and Bohme[4] quote m.p. 192–198°, $[\alpha]_D$ −133° for the anhydride derived from the hydroxy dicarboxylic acid of m.p. 221–223°, $[\alpha]_D$ −71°.

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