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REACTION OF ERGOSTERYL ACETATE WITH ACRYLONITRILE AND α-ACETOXYACRYLONITRILE

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Abstract—Ergosteryl acetate reacted with acrylonitrile to give three adducts, formed by Alder ene reactions involving the Δ^7 double bond. α-Acetoxyacrylonitrile and ergosteryl acetate formed two adducts by the ene reaction. No 1,4 adducts were detected. In both cases addition occurred from the α side of the steroid, with bond formation between C-7 and the α-C atom of the unsymmetrical olefin, and concomitant abstraction of either the 9α or 14α allylic hydrogen.

STEROIDAL-5,7-DIENES react with symmetrically substituted olefins to give, predominantly, products of an Alder ene synthesis[1] involving the Δ^7 double bond and the allylic 9α or 14α hydrogens, and not the expected Diels–Alder 1,4 adducts. For example, methyl esters of diazodicarboxylic[2] and acetylene dicarboxylic acid[3] react with steroidal-5,7-dienes to give respectively 7α-(1′,2′-dicarbomethoxyhydrazo) and 7α-(1′,2′-*cis*-dicarbomethoxyvinyl) derivatives of steroidal-5,8- and -5,8(14)-dienes, but no 'normal' Diels–Alder adducts were formed. Maleic anhydride does form a 1,4 Diels–Alder adduct with ergosteryl acetate,[4] but this is accompanied by three other adducts formed by the 'ene' synthesis at the Δ^7 double bond. The presence of a $\Delta^{9(11)}$ double bond enhances the reactivity of the cholesta-5,7-diene system toward 1,4 addition of the above dienophiles,[5] and this was ascribed to increased polarizability of the diene, and to the reduction in steric interaction associated with the absence of the 9α hydrogen. We required 5α,8α Diels–Alder adducts of ergosteryl acetate for synthetic purposes, and we investigated the reactions of ergosteryl acetate with acrylonitrile and α-acetoxy acrylonitrile in order to determine if the smaller steric requirements and unsymmetrical nature of these dienophiles would result in appreciable 1,4 addition.

Ergosteryl acetate reacted with acrylonitrile at 130° to give three one-to-one adducts I (23%), II (29%) and III (10%). I was obtained by crystallization of the mixture from methanol, whilst II and III were obtained by preparative TLC. The structures are given at this point to simplify further discussion.

The NMR spectra of the acetoxy nitriles (I and II) were almost identical, and displayed signals at τ 4·58 (one-proton doublet; J = 3 c/s; C-6 vinyl proton) and τ 4·77 (two proton multiplet; side chain vinyl protons), consistently with the proposed structures, and not for Diels–Alder adducts (such as IV) which have four vinyl protons. The doublet C-6 proton resonance is consonant with the presence of only one C-7 proton, and the coupling constant is in agreement with the observed dihedral angle (Dreiding models) of 55° between the C-6 and a C-7 proton.[6] This evidence pointed to the presence of a C-7 substituent, which was later confirmed chemically. The 3α proton resonated at τ 5·44 ($W\frac{1}{2}$ = 15 c/s) whilst the acetate Me group gave

rise to a singlet at τ 8·03. The NMR spectrum of III also revealed the presence of three vinyl protons, one at τ 4·54 (doublet, J = 5 c/s; C-6 vinyl proton) and two at τ 4·80 (multiplet; side chain vinyl protons). The 3α proton resonated at τ 5·40 ($W_{\frac{1}{2}}$ = 17 c/s) whilst a sharp singlet at τ 8·03 was attributed to the 3β acetate Me group.

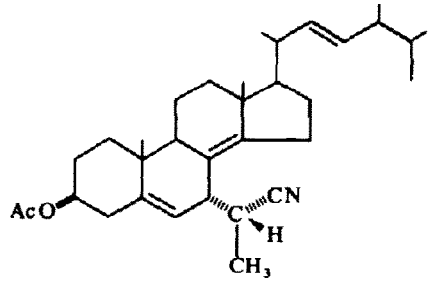
The NMR spectrum of III differed significantly from that of I and II in the region τ 8–10. Whereas in the spectra of I and II the C-18 and C-19 Me group signals coincided at τ 9·1, the C-18 and C-19 Me groups in III resonated at τ 9·32 and 8·76 respectively. The coincidence of the C-18 and C-19 Me signals in both I and II reveals the presence of a $\Delta^{8(14)}$ double bond, which causes an upfield shift in the C-19 Me resonance, and a downfield shift in the C-18 Me resonance (relative to their position in 5α-androstane)[7] to extents such that they coincide. Similar phenomena have been observed previously in two adducts of maleic anhydride and ergosteryl acetate,[4] and an adduct of dimethyl acetylene–dicarboxylate with 7-dehydrocholesteryl acetate,[3] all of which had structures analogous to I and II. The separation of 33·6 c/s in the C-18 Me and C-19 Me signals in III, compared with a separation of 18·5 c/s in androst-5-ene, indicates the presence of a $\Delta^{8(9)}$ double bond, which exerts a deshielding effect on the C-19 Me group, and a shielding effect on the C-18 Me group.[7] The presence of a $\Delta^{8(9)}$ double bond in an adduct of maleic anhydride with ergosteryl acetate,[4] and an adduct of dimethyl acetylene–dicarboxylate with 7-dehydrocholesterol[3] has been demonstrated by similar means. The adducts I and II both displayed a doublet at τ 8·7 (J = 7 c/s) attributed to the C-2′ Me group, whilst III similarly displayed a doublet at τ 8·67 (J = 7·5 c/s) due to the same group. Therefore all the adducts are steroidal 1′-cyanoethane derivatives.

The acetoxy nitriles I and II differed only in the configuration of the cyanide group, since they were both converted to the same mixture of I and II on treatment in sequence with refluxing aqueous glycollic potash for 28 hr, and acetic anhydride in pyridine. The epimerization confirmed that the cyanide was attached to a chiral centre. Evidently epimerization occurred more rapidly than hydrolysis of the nitrile group, which proceeded to completion after 60 hr in refluxing aqueous glycollic potash.

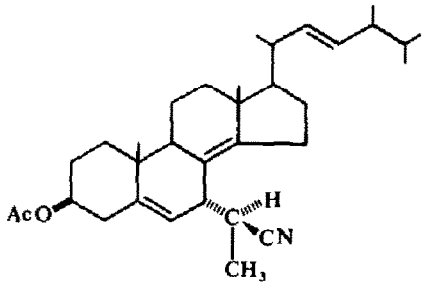
The depicted formulation of adducts I, II and III followed from the above spectroscopic and chemical evidence except for the orientation at C-7 and the chirality at C-1′. The concerted, four centre, cyclic mechanism proposed for the Alder ene reaction requires the optical asymmetry at C-14 or C-9 (14α or 9α hydrogen abstracted) to be transferred to C-7,[8] and the cyanoethane residue was therefore allocated the 7α configuration. Adduct II, formed in slight excess, was assigned the (S) configuration at C-1′ because orbital symmetry considerations indicate that endoid addition should predominate in Alder ene reactions.[9] Accordingly, adduct I has the (R) configuration at C-1′. Models indicate that the steric interactions between the cyano group and steroid molecule in the transition states leading to the adducts are possibly slightly in favour of the exoid adduct, but not sufficiently so to reverse the predictions of orbital symmetry. However the difference between the percentage yield of I (23%) and II (29%) is small, so that the assignment of chirality at C-1′ is only tentative. No diasteromers at C-1′ of adduct III was obtained, and examination of models of the transition states leading to the two possible diastereomers showed that both steric factors and orbital symmetry factors favoured endoid addition, the transition state for exoid addition being relatively destabilized by steric interaction between

the cyano group and the 15α hydrogen. The consequent assignment of (S) configuration at C-1′ therefore seems relatively secure.

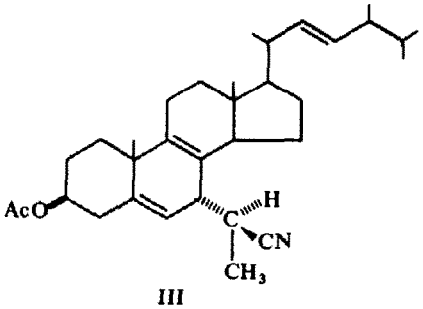
We considered that chemical evidence supporting the spectroscopically derived structures I, II and III was necessary, and the presence of a Δ^5 double bond in the adducts I and II was confirmed in the following manner. Mild hydrolysis of the



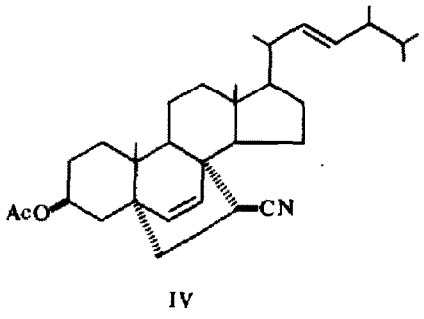
I



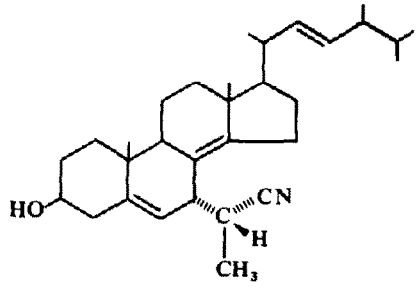
II



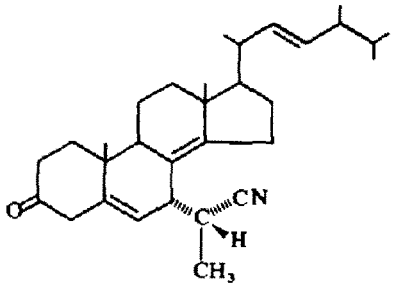
III



IV



V

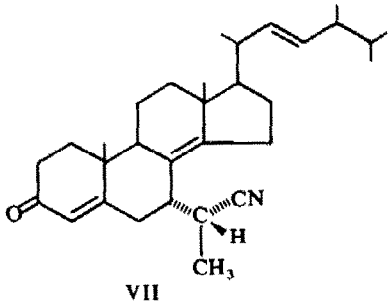


VI

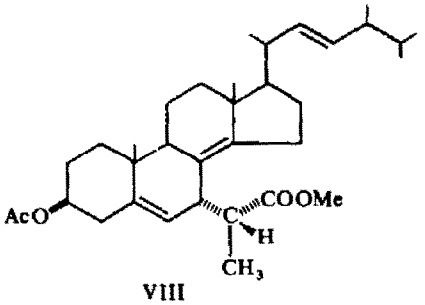
acetoxy nitrile I gave the corresponding hydroxy nitrile V, which was oxidized by the Jones reagent to a mixture containing a nonconjugated unsaturated ketone VI (ν_{max} 1730 cm^{-1}) and a conjugated unsaturated ketone VII (ν_{max} 1675 cm^{-1}). The presence of two products was indicated by TLC, but only the conjugated unsaturated ketone VII separated on crystallization. TLC confirmed that VI was rapidly converted into VII under mild acidic or basic conditions, behaviour analogous to the conversion of cholest-5-en-3-one into cholest-4-en-3-one.[10] The NMR spectrum of VII was consistent with the structure depicted, having a signal at τ 4·21 (one-proton singlet, C-4 vinyl proton), and at τ 4·78 (two-proton multiplet, side chain vinyl

protons). The C-19 Me group resonated at τ 8·86, the downfield shift of 14·4 c/s relative to its position in I being consistent with the introduction of a Δ^4-3-oxo moiety. This provides evidence that the 3β-acetoxy-Δ^5 system remained intact during the formation of adduct I, and since adducts I and II differ only in the orientation of the cyanide group adduct II also contains the 3β-acetoxy-Δ^5 system.

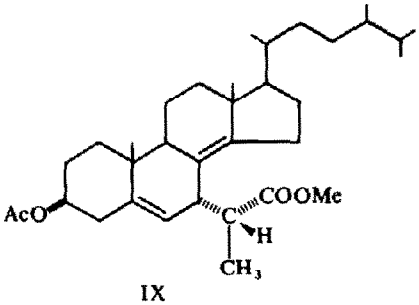
Evidence for the α configuration of the C-7 cyanoethane residue, and for the positions of the double bonds, was obtained in the following manner. Hydrolysis of adducts I and II severally by prolonged heating with aqueous glycollic potash, followed by treatment in sequence with acetic anhydride in pyridine, and ethereal diazomethane, gave the same acetoxy Me ester VIII.* Partial hydrogenation of VIII furnished the 22,23-dihydro acetoxy methyl ester IX, having in its NMR spectrum a signal due to only one vinyl proton at τ 4·77 (doublet, J = 4 c/s) attributed to the C-6 proton coupled with the lone C-7 proton. Hydrolysis of the dihydro acetoxy ester IX gave the dihydro hydroxy acid X, which with bromine in chloroform gave a



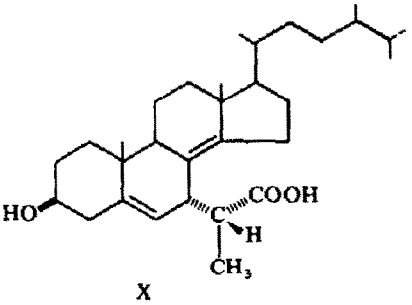
VII



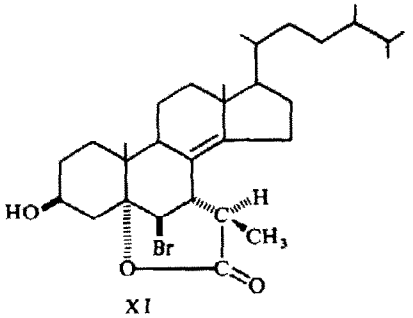
VIII



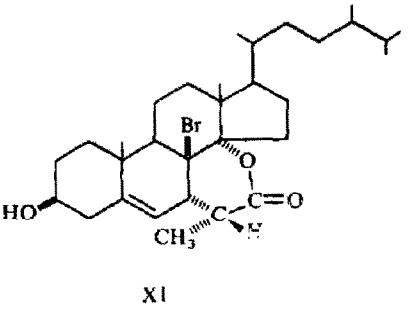
IX



X



XI



XI

* The C-1′ chiral centre in VIII was tentatively considered to be of (R) configuration, which appeared to be (from models) the more stable on steric grounds, and therefore expected as predominant product under the equilibrating conditions of the hydrolysis.

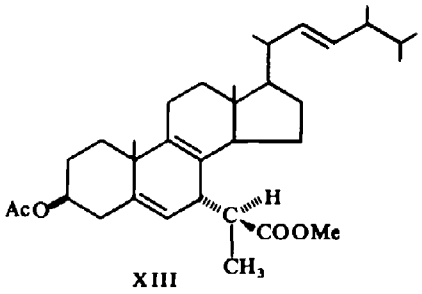
mixture of the two bromolactones XI and XII, formulated as δ lactones because they showed infrared maxima at 1728 and 1735 cm^{-1} respectively. Treatment of the bromolactones XI and XII with zinc in boiling acetic acid regenerated the hydroxy acid X, indicating that no skeletal rearrangement had occurred during bromolactonization.

The bromolactone XI had no vinyl proton signal in the NMR spectrum, and the intensity (ε 5,600) of the UV absorption at 213 mμ was consistent with the presence of a $\Delta^{8(14)}$ double bond.[11] The 6α proton geminal to bromine appeared at τ 5·62 as a broad singlet ($W\frac{1}{2} = 5$ c/s); the lack of appreciable coupling with the C-7 proton is consistent with the presence of a geminal electronegative substituent.[12] The 3α proton geminal to OH resonated at τ 5·81 ($W\frac{1}{2} = 20$ c/s). The pronounced downfield shift from the usual position near τ 6·4 in 3β-hydroxy-5α-steroids[13] is consistent with the presence of a 5α oxygen function,[14] and therefore with lactone ring closure to the 5α position.

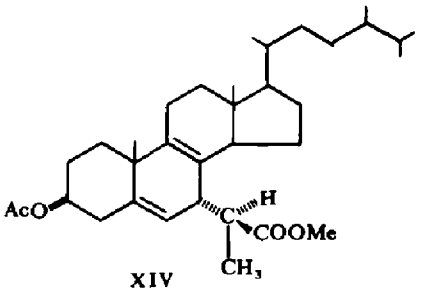
The NMR spectrum of the bromolactone XII had a one-proton signal at τ 4·38 (doublet; $J = 6$ c/s) attributed to the C-6 vinyl proton coupled with the C-7 proton. Thus lactonization had occurred to the $\Delta^{8(14)}$ double bond. Consistently, the UV spectrum had λ_{max} 210 mμ (ε 1900) indicating the absence of a $\Delta^{8(14)}$ double bond, and consonant with the presence of a Δ^5 double bond. An intense signal at τ 8·7 in the NMR was attributed to the C-19 Me group, which is no longer shielded by the $\Delta^{8(14)}$ double bond, but is deshielded by the 8β Br atom. This may be compared with known deshielding effect of a 2β and 6β Br atom,[7] which also have a 1,3-diaxial relationship with the C-19 Me group. The intensity of the signal suggests that the C-18 Me group may also be resonating at τ 8·7; this would be strongly deshielded by the 8β Br atom held rigidly in a 1,3-diaxial relationship with it. An 8β Br atom is expected on mechanistic grounds if lactonization occurs at the 14α position. Examination of Dreiding models shows that lactone formation between a C-7 2-propanoic acid residue and a Δ^5 or $\Delta^{8(14)}$ double bond is possible only if the C-7 residue is α-orientated. This provides convincing evidence for the 7α orientation of the cyanoethyl group in the adducts I and II.

A similar series of reactions established the presence of a 7α cyanoethyl residue in III. Hydrolysis of the acetoxy nitrile III with boiling aqueous glycollic potash, followed by acetylation (acetic anhydride–pyridine) and methylation (ethereal diazomethane) gave the acetoxy methyl ester XIII, which was partially hydrogenated to give the 22,23-dihydro acetoxy methyl ester XIV. The NMR spectrum of XIV displayed a one-proton signal at τ 4·89 (doublet; $J = 3$ c/s) attributed to the C-6 proton coupled with the C-7 proton. Hydrolysis of XIV afforded the dihydro hydroxy acid XV, which was treated with bromine in chloroform to give the bromolactone XVI. Lactonization had occurred to the Δ^5 double bond, since the NMR spectrum of XVI contained no vinyl proton signal. The 5α-position of the lactone oxygen was indicated by the downfield shift of the 3α-proton resonance to τ 5·85 ($W\frac{1}{2} = 25$ c/s). The 6α proton geminal to bromine resonated at τ 5·61 ($W\frac{1}{2} = 4$ c/s), the coupling with the 7β proton being reduced by the presence of the bromine,[12] as observed for the bromolactone XI. The UV spectrum had λ_{max} 212 mμ (ε 3900) consistently with the structure XVI, but the IR maximum at 1711 cm^{-1} was at abnormally low wavenumber for a δ-lactonic CO group.

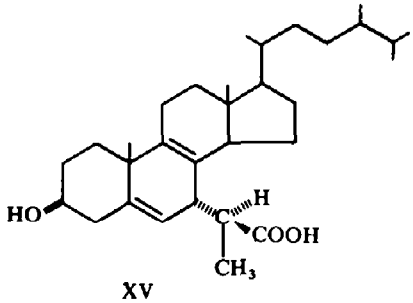
The dihydro acetoxy methyl ester IX with peroxylauric acid in petroleum ether



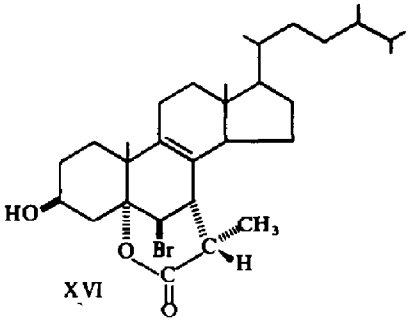
XIII



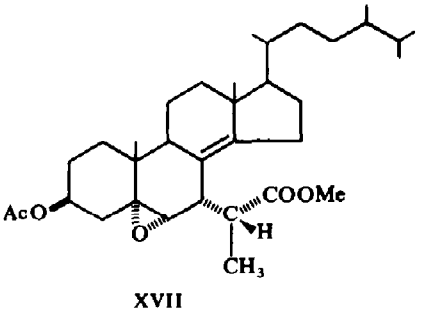
XIV



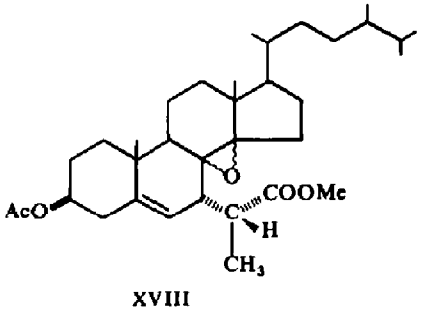
XV



XVI



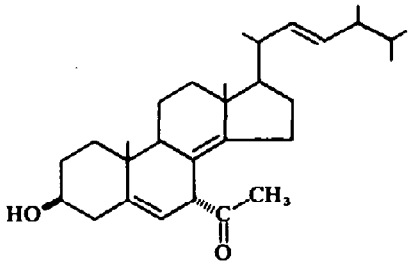
XVII



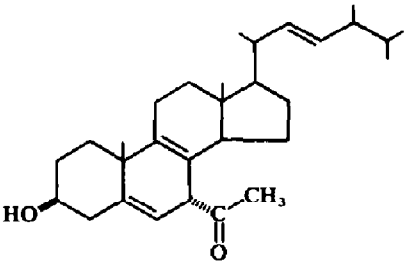
XVIII

gave two oxides XVII (56%) and XVIII (11%). The oxide XVII showed no vinyl proton in its NMR spectrum, and the 3α proton resonated at τ 5·2 ($W\frac{1}{2}$ = 16 c/s); the downfield shift from the usual position is consistent with the presence of a 5α, 6α epoxide.[14] Further evidence for the presence of an α epoxide was provided by the resonance of the 6β proton, which occurred at τ 7·1 ($W\frac{1}{2}$ = 4 c/s). Cross has demonstrated that the C-6 proton resonates near τ 6·9 in 5β,6β epoxides and near τ 7·1 in 5α,6α epoxides.[15] The UV spectrum, λ_{max} 213 mμ (ε 6800) was as expected for a $\Delta^{8(14)}$ olefin.[11] The oxide XVIII displayed a signal at τ 4·69 (doublet; J = 4·5 c/s) attributed to the C-6 proton, and the C-19 Me group resonated at τ 8·9. The C-19 Me group in IX resonated at τ 9·12 and the paramagnetic shift of 13·2 c/s which accompanied epoxidation is consistent with the removal of the shielding effect of the $\Delta^{8(14)}$ double bond. The UV spectrum, λ_{max} 209 mμ (ε 2100) confirmed the absence of a $\Delta^{8(14)}$ double bond, and the presence of a Δ^5 double bond.[11] The available spectroscopic evidence did not permit an assignment of configuration to the 8(14)

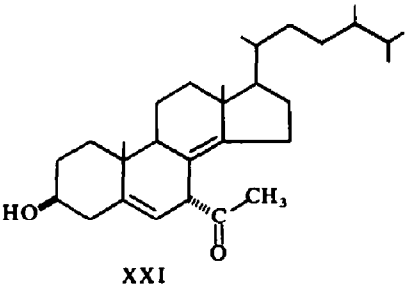
epoxide, and it was not possible to allocate configuration by consideration of steric effects in the transition state leading to its formation. Peroxyacids usually attack steroidal double bonds predominantly from the α side,[16] because of steric compression of the β side by the C-18 Me and C-19 Me groups. However the presence of the 7α-methyl propionate residue in IX would appear (from models) to hinder α attack, and the relative importance of various steric factors in determining the predominant direction of attack is difficult to assess.



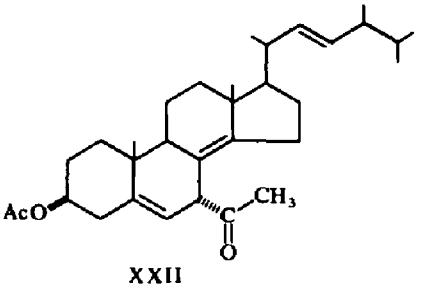
XIX



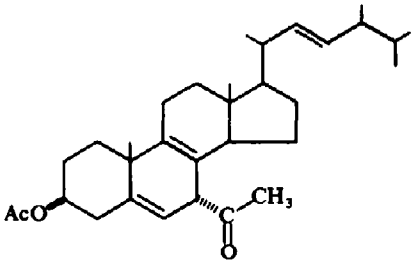
XX



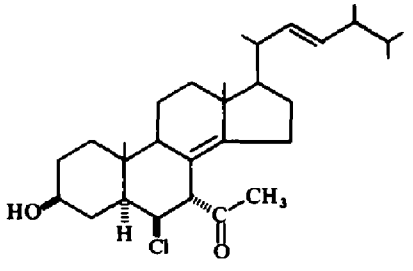
XXI



XXII



XXIII



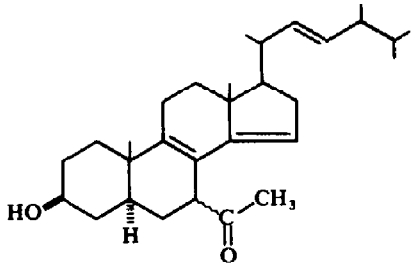
XXIV

Ergosteryl acetate reacted with α-acetoxy acrylonitrile at 190° in the absence of solvent to give a mixture of adducts, which were hydrolysed with aqueous methanolic potassium bicarbonate to a mixture of the hydroxy ketones XIX and XX. They could not be separated by fractional crystallization, and were chromatographically very similar. Separation was therefore difficult, and was aggravated by autoxidation to a mixture of unknown products during chromatography and in the non-crystalline state. The hydroxy ketone XIX had a three proton signal at τ 4·75 (multiplet) in its NMR spectrum, assigned to the overlapping C-6 and side chain vinyl protons,

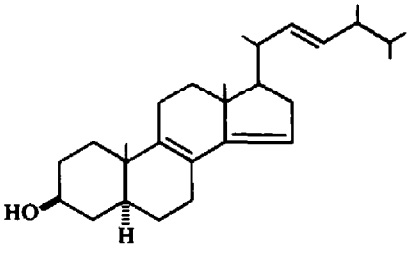
and a sharp singlet at τ 7·96 attributed to the C-2′ Me group. A two-proton signal at τ 6·52 ($W_{\frac{1}{2}} = 9$ c/s) was attributed to the overlapping 3α- and 7β-proton resonances. Partial hydrogenation of XIX gave the dihydro hydroxy ketone XXI, which contained in its NMR spectrum a one-proton doublet at τ 4·75 ($J = 3{\cdot}5$ c/s) attributed to the C-6 proton coupled with the C-7 proton, and a two-proton signal at τ 6·51 ($W_{\frac{1}{2}} =$ 9 c/s) due to the overlapping 3α and 7β protons. The C-2′ Me group resonated at τ 7·96. Acetylation of the mixture of hydroxy ketones gave the corresponding acetoxy ketones XXII and XXIII which were more easily separated by chromatography, and less readily autoxidized than the hydroxy ketones. The acetates had similar NMR spectra below τ 8·7, each having a 3-proton multiplet at τ 4·75 (overlapping C-6 and side chain vinyl protons), with signals at τ 7·97 (in XXII) and τ 8·0 (in XXIII) due to the C-2′ Me group, and at τ 8·05 (in XXII) and τ 8·04 (in XXIII) due to the 3β acetate Me group. In both compounds the 3α-proton resonated at τ 5·52 (one-proton multiplet, $W_{\frac{1}{2}} = 16$ c/s) and the 7β proton resonated at τ 6·52 (one-proton, $W_{\frac{1}{2}} = 8$ c/s). It appears that the C-7 proton undergoes long range coupling which obscures the doublet expected by coupling with the C-6 proton. A significant feature of the NMR spectrum of the acetoxy ketone XXII was its identity in the region τ 8·8–9·5 with that of the acetoxy nitriles I and II. Similarly the NMR spectrum of the acetoxy ketone XXIII was identical in the region τ 8·75–9·5 with that of the acetoxy nitrile III. This indicated that the relative dispositions of the double bonds and angular Me groups in the acetoxy ketone XXII and the acetoxy nitriles I and II were the same. Similarly this NMR evidence indicated the same disposition of double bonds in III as in XXIII. The UV spectra of the acetoxy ketones XXII and XXIII, with λ_{max} 212 mμ (ε 8900) and λ_{max} 207 mμ (ε 5300) respectively were in accord with these assignments.

Conjugation of the double bonds with the keto group in the hydroxy ketones XIX and XX could not be effected in refluxing ether containing a few drops of perchloric acid, the crude product having ν_{max} 1730 cm^{-1}, and no band attributable to an αβ unsaturated ketone. However hydrolysis of the acetoxy ketones XXII and XXIII with 10% methanolic potash gave a mixture of two substances having an IR maximum at 1690 cm^{-1}, diagnostic of the presence of an α,β unsaturated ketone. Further investigation was prevented by rapid autoxidation of the products. Treatment of a mixture of the ketones XIX and XX with hydrogen chloride in chloroform at 0° gave the chloroketone XXIV and the dienone XXV, the structures of which were established spectroscopically. Under these conditions the pure hydroxy ketone XX was recovered unchanged, so it appears that both products were derived from XIX. The infrared and UV spectra of XXIV had ν_{max} 1710 cm^{-1}, and λ_{max} 209 mμ (ε 8500) respectively, which indicated the absence of a conjugated diene and enone function. However the intensity of UV absorption was consistent with the presence of a $\Delta^{8(14)}$ double bond.[11] XXIV showed a 2-proton multiplet at τ 4·75 in its NMR spectrum, attributed to the side chain vinyl protons, and a one-proton signal at τ 5·5 ($W_{\frac{1}{2}} = 4$ c/s) attributed to the C-6 proton geminal to chlorine. A signal at τ 6·33 ($W_{\frac{1}{2}} = 3$ c/s) was attributed to the C-7 proton, the insignificant coupling with the C-6 proton being consistent with the presence of the electronegative C-6 chlorine and C-7 acetyl groups.[12] The spectroscopic evidence does not permit an unequivocal assignment of configuration at C-6 and C-7. However, the narrow band width of the C-6 proton resonance indicates that a 6α configuration for the chloride is unlikely,

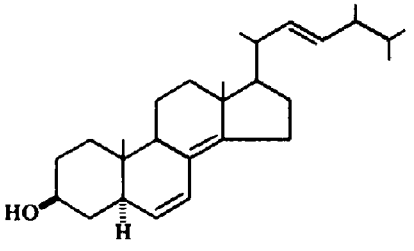
since the *trans* diaxial arrangement of 6β and 5α protons (in a 6α-chloro-7α-acetyl compound) and 6β proton with both 5α and 7α protons (in a 6α-chloro-7β-acetyl compound) should lead to appreciable coupling despite the presence of electronegative geminal substituents.[6] The narrow ($W\frac{1}{2} = 3$ c/s) C-7 proton signal is further evidence for the lack of a diaxial relationship between C-6 and C-7 protons. The C-2′ Me group resonated at τ 7·96, as in the parent compound XIX, and the coincidence of these signals is taken (tentatively) as evidence for the same configuration at C-7 in both compounds. Anti-Markownikoff addition of hydrogen chloride to the Δ^5 double bond is a logical consequence of the presence of a C-7 acetyl group. Transient formation of the conjugated Δ^6-C-1′ enone system under acid catalysis would lead to conjugative nucleophilic addition by the chloride ion at C-6.



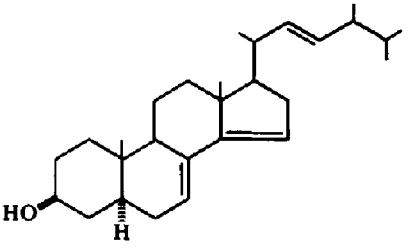
XXV



XXVI



XXVII



XXVIII

The hydroxy ketone XXV was formulated as an 8,14 diene on the basis of its UV spectrum, λ_{max} 246 mμ (ε 21,000). This spectrum may be compared with that of ergosterol B1 (XXVI), λ_{max} 250 mμ (ε 18,000), which was obtained from ergosterol by treatment with hydrogen chloride in chloroform, and with the calculated value of 244 mμ.[17] It did not contain the diene system of ergosterol B2 (XXVII; λ_{max} 252, ε 23,200) or ergosterol B3 (XXVIII; λ_{max} 242, ε 9900) (also obtained from ergosterol by treatment with hydrogen chloride)[17] as indicated by the disparity in UV data, and by the IR maximum at 1708 cm^{-1}, which confirmed that the keto group at C-1′ was not conjugated. The NMR spectrum of XXV showed a 3-proton signal at τ 4·79 (C-15 and side chain vinyl protons) and a broad 2-proton signal centered at τ 6·58 ($W\frac{1}{2} = 25$ c/s) attributed to the overlapping 3α and 7 protons. The C-7 proton signal occurred 3·6 c/s upfield from its position in the hydroxy ketone XIX, and the C-2′ Me group, which resonated at τ 8·13, occurred 11·2 c/s upfield from its position in XIX. Equilibration at C-7 during acid catalysed migration of the double bonds is reasonable, but the NMR evidence available does not permit an unequivocal assignment of configuration at C-7 in XXV because of uncertainties associated with the magnetic anisotropy of the 8,14 diene system.

EXPERIMENTAL

Rotations cited are for $CHCl_3$ solns 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 Unicam SP 700 spectrophotometer. NMR spectra were determined on a Varian A-60 spectrometer, and are recorded on the τ scale.

Acetoxy nitriles I, II and III. Ergosteryl acetate (10·75 g) and acrylonitrile (105 ml) were heated at 130° under N_2 in bomb tubes for 16 hr. The reaction mixture was extracted with boiling ether, and an insoluble ppt (polymer) filtered off. Evaporation of the filtrate gave a yellow gum (12·77 g), which was chromatographed on thin layer silica plates. Development with ether–benzene (1:19) gave 4 bands which were severally extracted with ether. Band 1 afforded ergosteryl acetate (2·22 g), whilst band 2 gave an oil (2·94 g) which crystallized from MeOH to give 3β-*acetoxy*-7α-[1′-(S)-*cyanoethano*]-*ergosta*-5,8(14),22-*triene* (II), needles, m.p. 125–127°, $[\alpha]_D$ −172°, (*c*, 0·9), ν_{max} 2243, 1741 cm^{-1}, λ_{max} 212 mμ (ε 8000), NMR (in CCl_4) τ 4·58 (1H, d, J = 3 c/s, C-6 vinyl proton), 4·77 (2H, m, side chain vinyl proton) 5·44 (1H, 3α proton), 8·03 (s, acetate Me), 9·1 (C-18 and C-19 Me), (Found: C, 80·6; H, 10·3. $C_{35}H_{49}NO_2$ requires: C, 80·6; H, 10·0%). Band 3 gave a solid 2·34 g) which crystallized from MeOH to give 3β-*acetoxy*-7α-[1′-(R)-*cyanoethano*]-*ergosta*-5,8(14),22-*triene* (I), needles, m.p. 181–182°, $[\alpha]_D$ −22° (c, 0·7), ν_{max} 2253, 1738 cm^{-1}, λ_{max} 213 mμ (ε 7800), NMR as for (II). (Found: C, 80·9; H, 10·0%), and band 4 gave a solid (1·0 g) which crystallized from MeOH to give 3β-*acetoxy*-7α-[1′-(S)-*cyanoethano*]*ergosta*-5,8,22-*triene* (III), needles, m.p. 163–165°, $[\alpha]_D$ −102° (c, 0·3), ν_{max} 2245, 1740 cm^{-1}, λ_{max} 209 mμ (ε 3000), NMR (in CCl_4) τ 4·54 (1H, d, J = 5 c/s, C-6 vinyl proton), 4·80 (2H, m, side chain vinyl protons), 5·40 (1H, 3α proton), 8·03 (s, acetate Me), 8·76 (C-19 Me), 9·32 (C-18 Me), (Found: C, 80·6; H, 10·4%).

Equilibration of the acetoxy nitriles I *and* II. The acetoxy nitrile II (199 mg) was treated with a boiling soln of KOH (0·4 g) in water (10 ml) and ethylene glycol (40 ml) for 28 hr. The reaction mixture was diluted with water, acidified, and extracted with EtOAc. The organic layer was washed with water, dried (Na_2SO_4) and separated to a solid (192 mg) which was treated with Ac_2O (0·6 ml) in pyridine (15 ml) at room temp overnight. The usual work-up procedure with ether gave an oil (202 mg) which consisted only of I and II (TLC). They were separated by preparative scale TLC with benzene as developing solvent to give I (58 mg), m.p. and mixed m.p. 180–182°, and II (72 mg) m.p. and mixed m.p. 125–127°. These were identical spectroscopically with authentic specimens. The pure acetoxy nitrile I under the same conditions also gave a mixture of I and II.

3β-*Hydroxy*-7α-[1′-(R)-*cyanoethano*]*ergosta*-5,8(14),22-*triene* (V). The acetoxy nitrile I (5·03 g) in sat $KHCO_3$ aq in aqueous MeOH (1:4, 250 ml) was boiled for 3 hr. The usual work-up gave the *product* V (4·5 g), needles double m.p. 141/150° (from MeOH), $[\alpha]_D$ −228° (*c*, 0·7), ν_{max} 2250 cm^{-1}, (Found: C, 82·5; H, 10·7. $C_{31}H_{47}NO$ requires: C, 82·8; H, 10·5%).

Oxidation of the hydroxy nitrile (V). A stirred soln of V (195 mg) in acetone (30 ml) at −30° was treated dropwise with 0·5 ml of '8N' CrO_3 in H_2SO_4aq, added over 3 min. After stirring for a further 3 min, a sat soln of SO_2 in acetone was added, and the mixture was poured into water and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and evaporated to an oil (169 mg), which was a mixture of two substances (TLC), and which showed ν_{max} (in CCl_4) 1730 and 1675 cm^{-1}. After brief treatment of a small sample of this product with ethanolic HCl or ethanolic KOH TLC revealed the presence of only one substance, with ν_{max} 1675 cm^{-1}, chromatographically identical with one of the components of the original mixture. Three crystallizations of the oily mixture from MeOH afforded 3-*keto*-7α-[1′-(R)-*cyanoethano*]*ergosta*-4,8(14),22-*triene* (VII), plates, m.p. 145–147°, $[\alpha]_D$ +21° (*c*, 0·3), ν_{max} 1675 cm^{-1}, (Found: C, 83·2; H, 10·h. $C_{31}H_{45}NO$ requires: C, 83·2; H, 10·1%).

3β-*Acetoxy*-7α-[1′(R)-*methoxycarbonylethano*]*ergosta*-5,8(14), 22-*triene* (VIII). The acetoxy nitrile I (741 mg) was treated with KOH (1·5 g) in boiling ethylene glycol (30 ml) and water (8 ml) for 60 hr. The mixture was poured into water and acidified with HCl. The ppt was collected, dried, dissolved in dry MeOH and treated with ethereal diazomethane. Evaporation gave an oily product which was treated with Ac_2O in pyridine overnight at room temp, and the gummy product obtained after the usual work-up was chromatographed on a thin layer of silica. Elution with ether-benzene gave only one strong band, which after collection and crystallization from MeOH gave *the product* VIII (395 mg), needles m.p. 103–105°, $[\alpha]_D$ −155° (*c*, 1·3), ν_{max} 1735, 1250, 970 cm^{-1}. (Found: C, 77·5; H, 10·3. $C_{34}H_{50}O_4$ requires: C, 77·8, H. 10·0%). Hydrolysis of II in the same manner gave the same acetoxy methyl ester VIII.

3β-*Acetoxy*-7α-[1′-(S)-*methoxycarbonylethano*]*ergosta*-5,8,22-*triene* (XIII). Hydrolysis of III (450 mg) in the above manner gave the product XIII (244 mg), m.p. 156–159°, $[\alpha]_D$ −111° (*c*, 1·2), ν_{max} 1755. 1257,

976 cm^{-1}. (Found: C, 77·8; H, 10·3%). For preparative purposes it was more convenient to hydrolyse the crude mixture of ergosteryl acetate–acrylonitrile adducts under the above conditions and separate VIII and XIII chromatographically. This method was quicker but less efficient than preliminary separation of the adducts and subsequent hydrolysis, 23·8 g of the crude adduct mixture giving 5·1 g of VIII and 1·1 g of XIII.

3β-*Acetoxy*-7α-[1′-(R)-*methoxycarbonylethano*] *ergosta*-5,8(14)-*diene* (IX). The methyl ester VIII (2·38 g) in EtOAc (145 ml) was hydrogenated in the presence of PtO_2 (290 mg). After 1 mole equivalent of H_2 was taken up, filtration through 'Hyflosupercel' and evaporation gave a gum (2·39 g), which crystallized from MeOH to give the *product* IX, needles, m.p. 86–88°, $[\alpha]_D$ −120° (*c*, 0·3), ν_{max} 1746, 1250 cm^{-1}. (Found: C, 77·2; H, 10·6. $C_{34}H_{54}O_4$ requires: C, 77·5; H, 10·3%).

3β-*Acetoxy*-7α-[1′-(S)-*methoxycarbonylethano*]*ergosta*-5,8-*diene* (XIV). The methyl ester XIII (1·48 g) in EtOAc (150 ml) was hydrogenated over PtO_2 as in the previous experiment to give *the product* XIV (1·45 g), needles, m.p. 170–172°, $[\alpha]_D$ −94° (*c*, 0·3), ν_{max} 1750, 1260 cm^{-1}. (Found: C, 77·7; H, 10·6%).

3β-*Hydroxy*-7α-[1′-(R)-*carboxyethano*] *ergosta*-5,8 (14)-*diene* (X). The methyl ester IX (2·09 g) and KOH (7 g) in MeOH (100 ml) and water (10 ml) was refluxed for 2 hr. The mixture was poured into water, acidified with dil HCl and extracted with EtOAc. The organic layer was washed, dried, and evaporated to small bulk, when *the product* X (1·59 g) separated, having m.p. 241–243°, $[\alpha]_D$ −122 in EtOAc (*c*, 0·2), ν_{max} 1698 cm^{-1}. (Found: C, 79·1; H, 10·7. $C_{31}H_{50}O_3$ requires: C, 79·1; H, 10·7%).

3β-*Hydroxy*-7α-[1′-(S)-*carboxyethano*] *ergosta*-5, 8-*diene* (XV). Hydrolysis of the methyl ester XIV (885 mg) in the above manner gave *the product* XV (501 mg), m.p. 246–248°, $[\alpha]_D$ −78° (*c*, 0·2), ν_{max} 1720 cm^{-1}. (Found: C, 79·3; H, 10·8%).

Treatment of the dihydro hydroxy acid (X) *with bromine*. The acid (X) (1·26 g) in $CHCl_3$ (1250 ml) was treated with 1·4 ml of a soln of Br_2 (1 ml) in $CHCl_3$ (10 ml) at room temp. After 2 hr the mixture was washed with water, dried, and evaporated to a foam (1·59 g) which gave two bands on TLC developed with chloroform. The least polar component was an oil (267 mg) which crystallized from MeOH to give the *hydroxy bromolactone* XII m.p. 112–114°, $[\alpha]_D$ −28° (*c*, 0·6), ν_{max} 1738 cm^{-1}, λ_{max} 210 mμ (ε 1900). (Found: C, 67·4; H, 9·0. $C_{31}H_{49}BrO_3$ requires: C, 67·1; H, 9·2%). The other component was the *hydroxy bromolactone* XI (332 mg) m.p. 163–165° (needles from MeOH), $[\alpha]_D$ −86° (*c*, 0·6), ν_{max} 1732 cm^{-1}, λ_{max} 213 mμ (ε 5600). (Found: C, 67·5; H, 9·5%).

Treatment of the hydroxy bromolactones XI *and* XII *with zinc in acetic acid*. The bromolactone XI (138 mg) was treated with Zn dust (500 mg) in boiling AcOH (20 ml) for 2 hr. The ppt obtained when the reaction mixture was poured into water was collected, dissolved in EtOAc and filtered free of Zn. The filtrate was washed thoroughly with water, dried and evaporated to give the acid X (80 mg), m.p. and mixed m.p. 241–243° (from EtOAc). Similar treatment of the bromolactone (XII) also regenerated the acid X.

Treatment of the dihydro hydroxy acid XV *with bromine*. The acid XV (680 mg) in $CHCl_3$ (700 ml) was treated with 0·8 ml of a soln of Br_2 (1 ml) in $CHCl_3$ (10 ml) at room temp. After 3 hr ether was added and the organic layer was washed, dried, and evaporated to give a brown foam (755 mg). Preparative TLC, developed with $CHCl_3$, gave as the major new component the *hydroxy bromolactone* XVI (156 mg), m.p. 166–168° (from MeOH), $[\alpha]_D$ −115° (*c*, 0·3), ν_{max} 1711 cm^{-1}, λ_{max} 212 mμ (ε 3900). (Found: C, 67·2; H, 9·0. $C_{31}H_{49}BrO_3$ requires: C, 67·1; H, 9·2%). Treatment of the hydroxy bromolactone with Zn and AcOH in the manner described previously regenerated the acid XV.

Treatment of the dihydro acetoxy methyl ester IX *with peroxylauric acid*. The compound IX (1·53 g; 2·9 mmoles) in light petroleum (15 ml) was treated with peroxylauric acid (0·6 g; 3 mmoles) at room temp overnight. The crystalline deposit (405 mg) was collected and recrystallized from methanol to give 3β-*acetoxy*-5α, 6α-*epoxy*-7α-[1′-(R)-*methoxycarbonylethano*] *ergost*-8 (14)-*ene* (XVII) m.p. 142–144°, ν_{max} 1732, 1238, 1163, 1037 cm^{-1}, λ_{max} 213 mμ (ε 6800). (Found: C, 75·0; H, 9·8. $C_{34}H_{54}O_5$ requires: C, 75·6; H, 10·0%). Evaporation of the combined petroleum ether and MeOH mother liquors gave a gum which was chromatographed on alumina (35 g). Elution with ether gave a gum (1·13 g) which was rechromatographed using preparative TLC. Development with ether–benzene (1:19) gave two bands which were severally collected. The top band gave 3β-*acetoxy*-8, 14-*epoxy*-7α-[1′-(R)-*methoxycarbonylethano*] *ergost*-5-*ene* XVIII (181 mg), m.p. 104–105° (from MeOH), ν_{max} 1732, 1721, 1245 cm^{-1}, λ_{max} 209 mμ (ε 2100). (Found: C, 75·8; H, 9·8%). The second band gave a further 482 mg of the epoxy ester XVII.

Hydroxy ketones XIX *and* XX. Ergosteryl acetate (14·2 g) and α-acetoxyacrylonitrile (20 ml) were heated at 190° under N_2 in a bomb tube for 19 hr. The black resinuous product was extracted with refluxing ether and the insoluble black residue rejected. Evaporation of the extract gave a brown solid (15 g) which was chromatographed on alumina (450 g). Elution with benzene gave a gummy mixture of ergosteryl acetate-

α-acetoxy acrylonitrile adducts, which were very similar chromatographically (TLC). A portion of this mixture of adducts (3·71 g) was hydrolysed by treatment with boiling 10% methanolic $KHCO_3$ aq (200 ml) for 2 hr. The usual work-up gave a gum (2·91 g) which crystallized from MeOH to give a mixture of XIX and XX, m.p. 127–132°, λ_{max} 1715 cm^{-1}. (Found: C, 82·1; H, 10·5. $C_{30}H_{46}O_2$ requires: C, 82·2; H, 10·5%). The mixture of hydroxy ketones (672 mg) was subjected to preparative TLC, developing with ether–benzene three times. Only one band was formed. The top part of the band gave 3β-*hydroxy-7α-acetyl-ergosta-5, 8(14), 22-triene*. XIX (123 mg), needles, m.p. 123–125° (from MeOH), ν_{max} 1700 cm^{-1}, λ_{max} 214 mμ (ε 8300). (Found: C, 82·3; H, 10·7. $C_{30}H_{46}O_2$ requires: C, 82·2; H, 10·50%). The bottom part of the band gave XX (64 mg), m.p. 150–152° (from MeOH).

Hydrogenation of XIX (120 mg) in EtOAc (20 ml) containing 10% Pd/C (52 mg) proceeded with the uptake of 1 mole equiv of H_2 in 6 hr. Filtration through 'Hyflosupercel' and evaporation gave XIX (105 mg) as an amorphous solid, m.p. 100–108°, NMR (in CCl_4) τ 4·75 (one proton doublet, J = 3·5 c/s, C-6 vinyl proton), 6·51 (two protons, $W_{\frac{1}{2}}$ = 9 c/s, 3α and 7β protons), 7·96 (sharp singlet, C-2′ Me group).

Acetoxy ketones XXII *and* XXIII. A mixture of XIX and XX (694 mg) was treated with Ac_2O (5 ml) in pyridine (15 ml) at room temp overnight. The usual work-up gave a gum (644 mg) which was subjected to preparative TLC developed by ether–benzene (3:97) twice. Two bands were obtained; the top band gave 3β-*acetoxy-7α-acetyl-ergosta-5, 8(14), 22-triene* XXII (240 mg), needles, m.p. 82–85° (from MeOH), ν_{max} 1735, 1712 cm^{-1}, λ_{max} 212 mμ (ε 8900). (Found: C, 80·2; H, 10·2. $C_{32}H_{48}O_3$ requires: C, 80·0; H, 10·0%). The other band gave 3β-*acetoxy-7α-acetyl-ergosta-5, 8, 22-triene* XXIII (108 mg), needles, m.p. 108–110° (from MeOH), ν_{max} 1733, 1718 cm^{-1}. λ_{max} 207 mμ (ε 5300). (Found: C, 79·5; H, 9·9%).

Treatment of the hydroxy ketones XIX *and* XX *with hydrogen chloride in chloroform.* A mixture (1·18 g) of XIX and XX in $CHCl_3$ at 0° was treated with dry HCl (bubbled through) for 30 min. After standing for 24 hr and evaporation of the solvent, the gum obtained (1·11 g) was subjected to preparative TLC, developed with ether–benzene (3:7) twice. Two bands were obtained. Band 1 gave 3β-*hydroxy-6β-chloro-7α-acetyl-ergosta-8(14), 22-diene* XXIV (206 mg), needles, m.p. 169–172° from MeOH, ν_{max} 1710, 743, 718 cm^{-1}, λ_{max} 209 mμ (ε 8500). (Found: C, 76·2; H, 10·0; Cl, 7·7. $C_{30}H_{47}O_2Cl$ requires: C, 75·9; H, 9·7; Cl, 7·5%). Band 2 gave 3β-*hydroxy-7β-acetyl-ergosta-8,14,22-triene* XXV (330 mg), needles, m.p. 72–75°, ν_{max} 1708 cm^{-1}, λ_{max} 246 mμ (ε 21,000). (Found: C, 82·1; H, 10·5. $C_{30}H_{46}O_2$ requires: C, 82·2; H, 10·5%).

Treatment of XX (5 mg) with HCl in the above manner afforded only unchanged starting material.

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