A Nuclear Magnetic Resonance Study of the Conversion of 4β-Acetoxy-3β-hydroxy-Δ⁵-steroids into 3β,6β-Diacetoxy-Δ⁴-steroids

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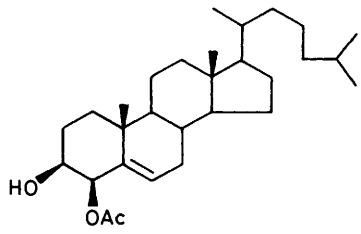
^{2}H,^{13}C, and ^{14}C Labelling studies have shown that the reaction of 4β-acetoxy-3β-hydroxy-Δ⁵- and 3β-acetoxy-4β-hydroxy-Δ⁵-steroids with acetic acid to form 3β,6β-diacetoxy-Δ⁴-steroids involves an intramolecular rearrangement of the C-4 acetate to C-3 proceeding *via* a 3β,4β-dioxolanylium ion accompanied by S_Ni attack at C-6β by the incoming acetate.

The allylic rearrangement and acetylation of 4β-acetoxy-3β-hydroxycholest-5-ene (**1**) to form 3β,6β-diacetoxycholest-4-ene (**3**) on brief treatment with refluxing acetic acid has been known for many years.[1] Although there is relatively little evidence for this, much of the discussion has centred on the probable intervention of a 3β,4β-dioxolanylium ion (**2**).[1-4] Similar intermediates have been invoked to account for the equilibration of 3,6-diacetoxycholest-4-enes at C-3 in sulphuric acid–acetic acid–acetic anhydride mixtures and the acetolysis of 6β-substituted-3β-acetoxycholest-4-enes[3,4] which proceeds either at C-6 with retention of configuration or in some instances leads to substitution at C-4. The migration of an acetate from C-3 to C-4 during the addition of hypobromous acid to 3β,17β-diacetoxyestr-4-ene[5] probably involves a similar intermediate. Recently we have provided some evidence for the involvement of an intermediate of this type in allylic acetoxylation of Δ⁵-steroids at C-4.[6] In this paper[7] we now present some evidence which supports the intervention of a 3β,4β-dioxolanylium ion in the rearrangement reaction.

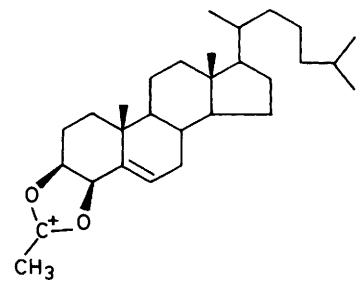
Whilst both 4β-acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) and 3β-acetoxy-4β-hydroxyandrost-5-en-17-one (**5**) yield 3β,6β-diacetoxyandrost-4-en-17-one (**10**)[8] on treatment with refluxing acetic acid for 15 min, the corresponding 3β,4β-diacetate (**6**) was recovered unchanged after 2 h. The structure of the 3β,6β-diacetate (**10**) was confirmed by its ^{1}H n.m.r. spectrum (determined at 360 MHz) which showed signals at δ 5.24 (octet, *J* 10, 6.2, and 1.8 Hz, 3α-H), 5.34 (t, *J* 2.8 Hz, 6α-H), and 5.65 (d, *J* 1.8 Hz, 4-H). Irradiation at δ_H 5.65 collapsed the octet at δ 5.24 (3α-H) to a quartet (*J* 10 and 6.2 Hz). The 3β-hydroxy group of dehydroisoandrosterone (**3**), which lacks a 4β-acetoxy group, is only acetylated to a minor extent (*ca.* 5%) in refluxing acetic acid for 15 min. The diol (**8**) is also unreactive. Treatment of the diacetate (**6**) with a trace of sulphuric acid or hydrobromic acid in glacial acetic acid leads to aromatization of ring A.[9] Thus a free hydroxy group and an acetoxy group are required for the allylic rearrangement to proceed.

In the light of this several reaction pathways can be envisaged for the rearrangement (see Scheme, a and b). One pathway (a) involves the formation of a 3β,4β-dioxolanylium ion and its subsequent displacement by an incoming acetate at the relatively hindered C-6β position. Another pathway (b) involves an initial intramolecular rearrangement of the C-4 acetate to C-6β, the formation of a 5β,6β-dioxolanylium ion, and finally *syn* attack at the less hindered 3β-position. A distinction between these may be made in terms of the origin of the acetate units. This was done using ^{2}H,^{13}C, and ^{14}C labelling.

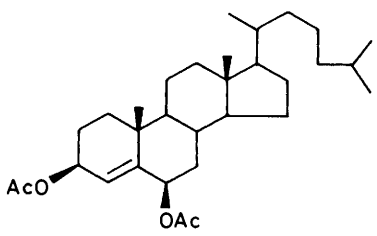
The ^{1}H and ^{13}C n.m.r. signals associated with the 3β- and 6β-acetates were assigned as follows. 3β-Acetoxy-5,6α-epoxy-5α-androstan-17-one (**12**) was hydrolysed to the 3β-acetoxy-5α,6β-diol (**13**)[10] with periodic acid, and the diol was then acetylated with [$^{2}H_6$]acetic anhydride in pyridine to afford the diacetate (**14**). Alternatively the 5α,6α-epoxide was hydrolysed



(**1**)



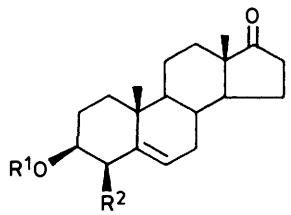
(**2**)



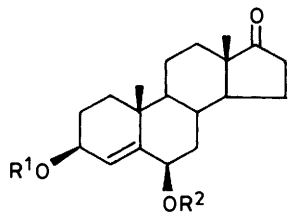
(**3**)

with [1-^{13}C]acetic acid (8.3% enriched) to form the ^{13}C-labelled diacetate (**14**) directly. In both instances the 5α-hydroxy group was then dehydrated with thionyl chloride[11] to afford 3β,6β-diacetoxyandrost-4-en-17-one (**10**) in which the C-6 acetoxy group was labelled. The ^{1}H and ^{13}C n.m.r. spectra were then examined at 360 and 90 MHz respectively. The ^{13}C n.m.r. signal at δ_C 169.81 p.p.m. was absent from the ^{2}H-labelled material as was the ^{1}H n.m.r. signal at δ_H 2.057. On the other hand the ^{13}C n.m.r. signal at δ_C 21.64 p.p.m. was enhanced in the sample prepared using [1-^{13}C]acetic acid. Consequently these signals were assigned to the 6β-acetate and those at δ_C 170.77 and 21.31 p.p.m. and δ_H 2.067 to the 3β-acetate (see Table).

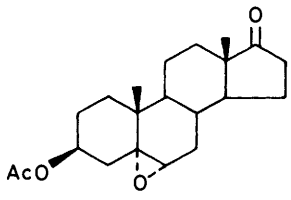
4β-(1′-^{14}C)Acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) was prepared by treatment of 3β-[(1-^{14}C)acetoxy]androst-5-en-17-one with bromine and silver acetate in pyridine at −60 °C.[6] The 4β-acetate (**4**) (2.29×10^6 dpm mmol^{-1}) was subjected to the rearrangement. The resultant 3β,6β-diacetoxyandrost-4-en-17-one (**10**) (2.01×10^6 dpm mmol^{-1}) retained 87.8% of the



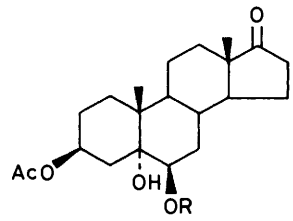
(4) R^1 = H, R^2 = OAc
(5) R^1 = Ac, R^2 = OH
(6) R^1 = Ac, R^2 = OAc
(7) R^1 = OH, R^2 = H
(8) R^1 = H, R^2 = OH
(9) R^1 = CO_2Et, R^2 = OH



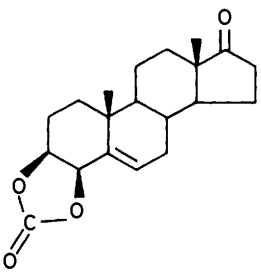
(10) R^1 = R^2 = Ac
(11) R^1 = H, R^2 = Ac



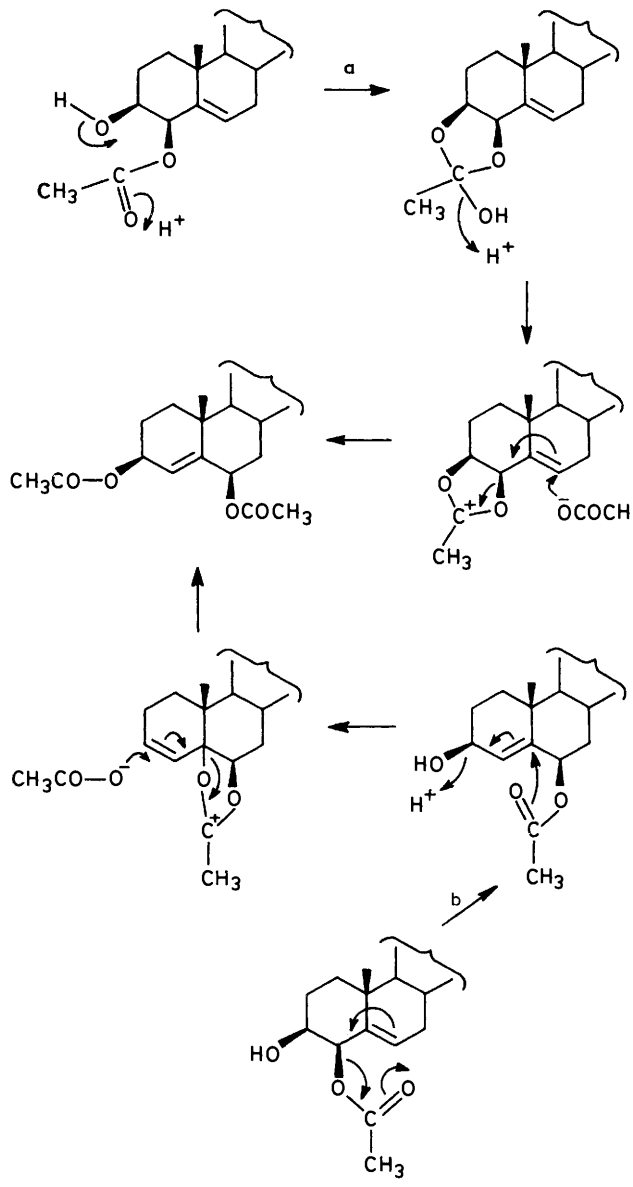
(12)



(13) R = H
(14) R = Ac



(15)



Scheme.

Table. ^{13}C N.m.r. assignments for 3β,6β-diacetoxyandrost-4-en-17-one (**10**) (determined at 90 MHz in $CDCl_3$)

Carbon atom	δ_C	Carbon atom	δ_C
1	36.6	13	47.7
2	24.7	14	51.0
3	70.2	15	21.75
4	128.2	16	35.7
5	143.5	17	220.2
6	74.7	18	13.9
7	36.1	19	20.6
8	30.6	3-OAc	21.31,
9	54.0		170.77
10	36.8	6-OAc	21.64,
11	20.1		169.81
12	31.4		

radioactivity thus establishing that the reaction was intramolecular. Mild hydrolysis with methanolic potassium carbonate afforded 6β-acetoxy-3β-hydroxyandrost-4-en-17-one (**11**) [$\nu_{max.}$ 3 460 and 1 735 cm^{-1}; δ_H 2.06 (3 H, s, OAc), 4.15 (1 H, m, $w_{\frac{1}{2}}$ 12 Hz, 3α-H), 5.30 (1 H, t, *J* 3 Hz, 6α-H), and 5.70 (1 H, br s, 4-H)] as a gum. To establish that only the 3β-acetoxy group was hydrolysed, the mono-ol was re-acetylated with [$^2H_6$]acetic anhydride. The resultant crystalline 3β-($^2H_3$)-acetoxy-6β-acetoxyandrost-4-en-17-one (**10**) (1.73×10^4 dpm $mmol^{-1}$) lacked the ^{13}C n.m.r. signal at δ_C 21.31, which had been assigned to the 3β-acetoxy group, and had lost 99.14% of the radioactivity of the diacetate. Hence the 4β-acetoxy group had migrated to C-3.

Treatment of both 4β-acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) and 3β-acetoxy-4β-hydroxyandrost-5-en-17-one (**5**) with [$^2H_4$]acetic acid under reflux for 15 min gave 3β,6β-diacetoxyandrost-4-en-17-one (**10**). The ^1H and ^{13}C spectra of the product in each case lacked signals at δ_H 2.057 and δ_C 21.64 p.p.m., thus showing that the 6β-acetoxy group bore the label. 4β-Acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) was also treated with refluxing [1-^{13}C]acetic acid (8.3% enriched) for 15 min and, as anticipated, the signal at δ_C 169.8 p.p.m. in the product (**10**) was enhanced. Hence the 6β-acetoxy group arose from the acetic acid of the medium.

The intervention of an dioxolanylium ion implicit in this rearrangement was demonstrated by the reaction of 3β-ethoxycarbonyloxy-4β-hydroxyandrost-5-en-17-one (**9**) [prepared by partial acylation of the corresponding diol (**8**) with ethyl chloroformate in pyridine]. When this carbonate was

treated with glacial acetic acid at 80 °C for 6 h, the intermediate dioxolanylium ion was trapped as the 3β,4β-cyclic carbonate (**15**) [$\nu_{max.}$ 1 780 cm^{-1}; δ_C 155.0 p.p.m. (O-CO-O)].

In conclusion these labelling studies have established that the reaction is intramolecular and that the 4β-acetoxy-3β-hydroxy- and 3β-acetoxy-4β-hydroxy-Δ^5-steroids react *via* a 3β,4β-dioxolanylium ion which undergoes an S_Ni displacement at C-6 by the incoming acetate to form the 3β,6β-diacetate.

Experimental

^1H and ^{13}C N.m.r. spectra were determined in deuteriochloroform, on a Bruker WH 360 spectrometer, with $SiMe_4$ as internal standard. Light petroleum refers to that fraction boiling in the range 60—80 °C.

Preparation of 3β,6β-*Diacetoxyandrost*-4-*en*-17-*one* (**10**).—(a) *From* 4β-*acetoxy*-3β-*hydroxyandrost*-5-*en*-17-*one* (**4**). A solution of the steroid (**4**) (600 mg) in glacial acetic acid (10 ml) was heated under reflux for 15 min and then added dropwise to excess of aqueous sodium hydrogen carbonate. The steroid was extracted with ethyl acetate and the organic phase was dried over sodium sulphate. The solvent was evaporated off to afford a semi-crystalline residue which was chromatographed on silica. Elution with 20% ethyl acetate–light petroleum gave 3β,6β-diacetoxyandrost-4-en-17-one (**10**) (350 mg) which crystallized from acetone–light petroleum as plates, m.p. 165—167 °C (softens at 149 °C); $[\alpha]_D$ +38° (*c* 0.4 in $CHCl_3$) (lit.,[8] m.p. 163—164 °C, $[\alpha]_D$ +56°); $\nu_{max.}$ 1 745, 1 730, 1 660, and 1 235 cm^{-1}; δ 0.936 (3 H, s, 18-H_3), 1.199 (3 H, s, 19-H), 2.057 (3 H, s, 6-OAc), 2.067 (3 H, s, 3-OAc), 5.244 (1 H, octet, *J* 10.1, 6.2, and 1.8 Hz, 3α-H), 5.340 (1 H, t, *J* 2.8 Hz, 6α-H), and 5.654 (1 H, d, *J* 1.8 Hz, 4-H).

Repetition of the experiment with 4β-acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) (500 mg) in [$^2H_4$]acetic acid (3 ml) gave 3β-acetoxy-6β-[($^2H_3$)acetoxy]androst-4-en-17-one (**10**) (205 mg) which was identified by its ^1H and ^{13}C n.m.r. spectra. The experiment was also repeated with 4β-acetoxy-3β-hydroxyandrost-5-en-17-one (**4**) (500 mg) in [1-^{13}C]acetic acid (8.3% enriched) (3 ml) to afford 3β-acetoxy-6β-[(1-^{13}C)acetoxy]-androst-4-en-17-one (100 mg) which was identified by its ^{13}C n.m.r. spectrum.

(b) *From* 3β-*acetoxy*-4β-*hydroxyandrost*-5-*en*-17-*one* (**5**). The steroid (**5**)[8] (500 mg) in glacial acetic acid (10 ml) was heated under reflux for 15 min. The solution was then added dropwise to aqueous sodium hydrogen carbonate and the steroids were recovered in ethyl acetate and chromatographed on silica to afford 3β,6β-diacetoxyandrost-4-en-17-one (**10**) (300 mg) which was identical (i.r. and n.m.r.) with the material described above. Repetition of the experiment with 3β-acetoxy-4β-hydroxyandrost-5-en-17-one (500 mg) in [$^2H_4$]acetic acid (3 ml) gave 3β-acetoxy-6β-[($^2H_3$)acetoxy]androst-4-en-17-one (**10**) (190 mg) which was identified by its ^1H and ^{13}C n.m.r. spectra.

(c) *From* 4β-[(1-^{14}C)*acetoxy*]-3β-*hydroxyandrost*-5-*en*-17-*one* (**4**). The steroid[6] (800 mg) (2.29 × 10^6 dpm $mmol^{-1}$) was dissolved in glacial acetic acid (20 ml) and the mixture was heated under reflux for 10 min. The product was recovered as above to afford 6β-acetoxy-3β-[(1-^{14}C)acetoxy]androst-4-en-17-one (400 mg) (2.01 × 10^6 dpm $mmol^{-1}$) which was identified by its i.r. and n.m.r. spectra.

Partial Hydrolysis of 6β-*Acetoxy*-3β-[(1-^{14}C)*acetoxy*]*androst*-4-*en*-17-*one*.—The title steroid (390 mg) in methanol (40 ml) was mixed with a solution of anhydrous potassium carbonate (270 mg) in water (5 ml), and the mixture was stirred at room temperature for 1.5 h. Acetic acid (1 ml) was added and the methanol was removed under reduced pressure. The suspension was poured into water and the steroids were recovered in ethyl acetate. The solvent was evaporated off and the residue was chromatographed on silica. Elution with 50% ethyl acetate–light petroleum gave 6β-acetoxy-3β-hydroxyandrost-4-en-17-one (**11**) (240 mg) as a gum, $\nu_{max.}$ 3 460, 1 735, 1 660, 1 250, and 1 225 cm^{-1}; δ 0.98 (3 H, s, 18-H_3), 1.24 (3 H, s, 19-H_3), 2.06 (3 H, s, 6-OAc), 4.15 (1 H, m, $w_{\frac{1}{2}}$ 3α-H) 5.30 (1 H, t, *J* 3 Hz, 6α-H), and 5.70 (1 H, br s, 4-H). Acetylation with [$^2H_6$]acetic anhydride (1 ml) in pyridine (2 ml) at room temperature for 2 d gave 6β-acetoxy-3β-[($^2H_3$)acetoxy]androst-4-en-17-one (1.73 × 10^4 dpm $mmol^{-1}$) which was identified by its ^1H and ^{13}C n.m.r. spectra.

Preparation of 3β-*Acetoxy*-6β-[($^2H_3$)*acetoxy*]*androst*-4-*en*-17-*one*.—($^2H_6$)Acetic anhydride (2 ml) was added to a solution of 3β-acetoxy-5,6β-dihydroxy-5α-androstan-17-one (**13**)[9] (900 mg) in dry pyridine (5 ml) and the mixture was stirred at room temperature for 24 h. The solution was poured into cold dil. hydrochloric acid and the steroid was recovered in ethyl acetate. The solvent was evaporated off to give a gum which was dissolved in dry pyridine (20 ml). Thionyl chloride (0.4 ml) was added at 0 °C and the solution was stirred overnight and then poured into ice-cold dil. hydrochloric acid and the steroid was recovered in ethyl acetate. The solvent was evaporated off to give a residue which was chromatographed on silica with 20% ethyl acetate–light petroleum as eluant to afford 3β-acetoxy-6β-[($^2H_3$)acetoxy]androst-4-en-17-one (**10**) (700 mg) which was identified by its i.r. and n.m.r. spectra.

Preparation of 3β-*Acetoxy*-6β-[(1-^{13}C)*acetoxy*]*androst*-4-*en*-17-*one*.—3β-Acetoxy-5,6α-epoxy-5α-androstan-17-one (**12**) (600 mg) was dissolved in (1-^{13}C)acetic acid (3 ml) (8.3% enriched) and the solution was heated gently under reflux for 2 h. The solution was poured into cold aqueous sodium hydrogen carbonate and the steroid was extracted with ethyl acetate. The extract was dried and the solvent was evaporated to give a gum which was dissolved in dry pyridine (10 ml). Thionyl chloride (0.3 ml) was added at 0 °C and the mixture was stirred at room temperature overnight. The mixture was poured into cold dil. hydrochloric acid and the steroid was extracted with ethyl acetate. The solvent was evaporated off and the residue was chromatographed on silica to afford 3β-acetoxy-6β-[(1-^{13}C)acetoxy]androst-4-en-17-one (250 mg) which was identified by its ^1H and ^{13}C n.m.r. spectra.

3β-*Ethoxycarbonyloxy*-4β-*hydroxyandrost*-5-*en*-17-*one* (**9**).—3β,4β-Dihydroxyandrost-5-en-17-one (**8**) (500 mg) was dissolved in dry pyridine (10 ml) and ethyl chloroformate (2.5 ml) was cautiously added at 0 °C. The solution was stirred at room temperature overnight. The mixture was poured into ice–water and the product was recovered in ethyl acetate. The extract was washed successively with aqueous copper(II) sulphate and water, and dried. The solvent was evaporated off to give a gum which was chromatographed on silica. Elution with 20% (**9**) ethyl acetate–light petroleum gave 3β-*ethoxycarbonyloxy*-4β-*hydroxyandrost*-5-*en*-17-*one* (**9**) (270 mg) which crystallized from ethyl acetate–light petroleum as prisms, m.p. 153—155 °C; $[\alpha]_D$ −20.5 (*c* 0.3 in $CHCl_3$) (Found: C, 70.4; H, 8.4. $C_{22}H_{32}O_5$ requires C, 70.2; H, 8.6%); $\nu_{max.}$ 3 440, 1 735, 1 660, and 1 275 cm^{-1}; δ 0.90 (3 H, s, 18-H_3), 1.25 (3 H, s, 19-H_3), 1.30 (3 H, t, *J* 7 Hz, OCH_2CH_3), 4.16 (2 H, q, *J* 7 Hz, OCH_2CH_3), 4.32 (1 H, d, *J* 4 Hz, 4-H), 4.5 (1 H, m, $w_{\frac{1}{2}}$ 20 Hz, 3α-H), and 5.76 (1 H, m, $w_{\frac{1}{2}}$ 6-H).

Reaction of 3β-*Ethoxycarbonyloxy*-4β-*hydroxyandrost*-5-*en*-17-*one* (**9**) *with Acetic Acid*.—A solution of 3β-ethoxycarbonyloxy-4β-hydroxyandrost-5-en-17-one (**9**) (135 mg) in glacial acetic acid (5 ml) was stirred at 80 °C for 6 h. The mixture

was then added dropwise to an excess of aqueous sodium hydrogen carbonate. The steroid was recovered in ethyl acetate. The solvent was evaporated off and the residue crystallized from ethyl acetate–light petroleum to afford 17-*oxoandrost*-5-*ene*-3β,4β-*diyl cyclic carbonate* (**15**) (100 mg) as plates, m.p. 229—232 °C; $[\alpha]_D$ +23.4 (*c* 0.4 in $CHCl_3$) (Found: C, 72.4; H, 7.7. $C_{20}H_{26}O_4$ requires C, 72.7; H, 7.9%); $\nu_{max.}$ 1 780, 1 750, and 1 665 cm^{-1}; δ 0.919 (3 H, s, 18-H_3), 1.159 (3 H, s, 19-H_3), 4.706 (1 H, m, *J* 13.1 and 7.1 Hz, 3α-H), 4.976 (1 H, d, *J* 7.1 Hz, 4α-H), and 5.969 (1 H, q, *J* 4.4 and 2.4 Hz, 6-H).

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