Participation of the 19-Substituent in the Conversion of 19-Hydroxyandrost-4-ene-3,17-dione into the Corresponding 4,5-Diosphenol

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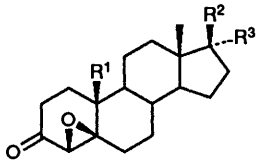
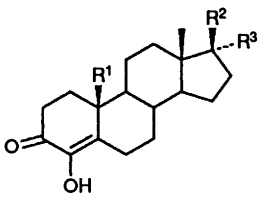
The synthesis of 4,19-dihydroxyandrost-4-ene-3,17-dione from 19-hydroxy-4β,5-epoxy-5β-androstane-3,17-dione and from 4β,5,19-trihydroxy-5β-androstane-3,17-dione is described. Under various reaction conditions other products are obtained as a result of participation of the 19-hydroxy group to form cyclic ethers. The formation of two of these products, 4α,5-isopropylidene-3α-hydroxy-3β,19-epoxy-5α-androstan-17-one and 4α-hydroxy-4β,19-epoxy-5α-androstane-3,17-dione, can be avoided by treatment of the aforementioned trihydroxy dione with acetic acid in the presence of HCl.

Studies on the active site of a 17β-hydroxy steroid dehydrogenase isolated from female rabbit liver[1] required 4,19-dihydroxyandrost-4-ene-3,17-dione (**11**) as a substrate for affinity labelling experiments. The synthesis of the C-19 unsubstituted diosphenol (**9**) has been achieved[2,3] by acid-catalysed opening of a mixture of the 4β,5β-epoxide (**1**) and the corresponding 4α,5α-epoxide, and these results have been confirmed in our laboratory.[4] Similarly, the 4β,5β-epoxide of testosterone acetate, compound (**2**), and its 4α,5α-isomer, when treated with sulphuric acid in acetic acid, have been shown[5] to give the diosphenol (**10**) in low yield. There are few reports on acid-catalysed reactions of 19-substituted 4,5-epoxy-3-ketones. Hosoda and Fishman[6] obtained the 2α-hydroxy compound (**14**) in 20% yield on aqueous sulphuric acid treatment of the 19-hydroxy-4β,5β-epoxide (**3**) (α′ mode of ring opening).[7] This same reaction was also found[8] to produce estrone (**21**) along with the 2α-hydroxy compound (**14**).

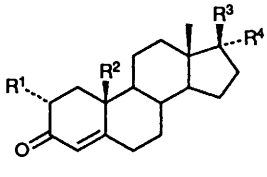
The reaction of the 19-alcohol (**4**) and the 19-aldehyde (**8**) with acid under oxidative conditions [dimethyl sulphoxide(DMSO)] has been described by Morisawa and Tanabe.[9] Diosphenol (**12**) and the 2,19-epoxide (**19**) were obtained in low yields from the 19-alcohol (**4**) while compound (**18**) was obtained in low yield from both the 19-alcohol (**4**) and the 19-aldehyde (**8**). The major product (**17**) obtained from both substrates (**4**) and (**8**) was suggested to arise *via* the initial acid-catalysed opening (α′ mode) of the epoxide by DMSO. However, under these conditions, an epoxide should normally be converted into an α-hydroxy ketone.[10] Mastalerz and Morand[11] found that when the 4β,5β-epoxy aldehyde (**7**) was treated with conc. perchloric acid in tetrahydrofuran (THF), up to 49% of the diosphenol (**13**) was formed along with other products. Since the nature of the solvent affects the mode of epoxide opening, we decided to study in more detail the influence of the reaction conditions and of the 19-hydroxymethyl group on the outcome of epoxide opening. In order to improve the yield of the desired diosphenol (**11**), an alternative route involving dehydration of the 4,5-diol derived from the appropriate 4,5-unsaturated precursor[12] was also investigated. In this paper, we report on the influence of the 19-hydroxy group under different conditions of acid-catalysed opening of 19-substituted 4β,5β-epoxides and of acid-catalysed dehydration of 19-substituted 4,5-diols.



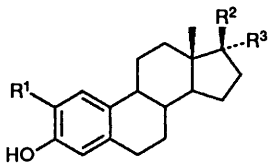
(1) R^1 = Me, R^2R^3 = O
(2) R^1 = Me, R^2 = OAc, R^3 = H
(3) R^1 = CH_2OH, R^2R^3 = O
(4) R^1 = CH_2OH, R^2 = OAc, R^3 = H
(5) R^1 = CH_2OAc, R^2R^3 = O
(6) R^1 = CH_2OAc, R^2 = OAc, R^3 = H
(7) R^1 = CHO, R^2R^3 = O
(8) R^1 = CHO, R^2 = OAc, R^3 = H



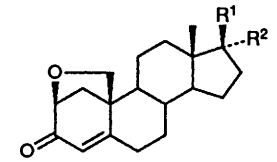
(9) R^1 = Me, R^2R^3 = O
(10) R^1 = Me, R^2 = OH, R^3 = H
(11) R^1 = CH_2OH, R^2R^3 = O
(12) R^1 = CH_2OH, R^2 = OAc, R^3 = H
(13) R^1 = CHO, R^2R^3 = O



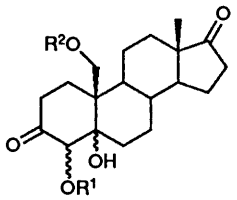
(14) R^1 = OH, R^2 = CH_2OH, R^3R^4 = O
(15) R^1 = OAc, R^2 = CH_2OAc, R^3R^4 = O
(16) R^1 = R^3 = OAc, R^2 = CH_2OAc, R^4 = H
(23) R^1 = H, R^2 = CH_2OH, R^3R^4 = O



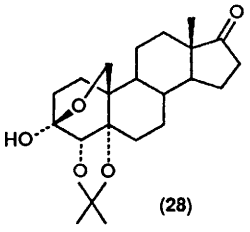
(17) R^1 = OH, R^2 = OAc, R^3 = H
(18) R^1 = R^3 = H, R^2 = OAc
(20) R^1 = OH, R^2R^3 = O
(21) R^1 = H, R^2R^3 = O



(19) R^1 = OAc, R^2 =H
(22) R^1R^2 =O



(24) R^1 = R^2 = H (4β, 5β)
(25) R^1 = R^2 = H (4α, 5α)
(26) R^1 = R^2 = Ac (4β, 5β)
(27) R^1 = R^2 = Ac (4α, 5α)



(28)

Results and Discussion

Reaction of 19-*Acetoxy*-4β,5β-*epoxides* (**5**) *and* (**6**) *with*

Sulphuric Acid in Acetic Acid.—Treatment of 4β,5β-epoxide (**5**) with sulphuric acid in acetic acid afforded only one product, in 44% yield. This compound was identified as 2α,19-diacetoxyandrost-4-ene-3,17-dione* (**15**) arising from the α′ mode of ring opening. This result is similar to that obtained by Hosoda and Fishman[6] on treatment of the 19-hydroxy-4β,5β-epoxide (**3**) with aq. sulphuric acid in acetone. The 2α,19-diacetate (**15**) was hydrolysed with ethanolic potassium carbonate to the known[6] 2α,19-diol (**14**).

We found that a 2α-acetoxy compound (**16**) was also obtained when 17β,19-diacetoxy-4β,5-epoxy-5β-androstan-3-one† (**6**) was treated with sulphuric acid in acetic acid. The ^{1}H NMR spectrum showed resonances at δ 2.02, 2.05, and 2.14 (2α, 17β, and 19-OAc) and a quartet integrating for one proton centred at δ 5.75 (2β-H). This last reaction indicates that, with sulphuric acid in acetic acid, the α′ mode of opening of a C-19 substituted steroid occurs whether there is a carbonyl group or an acetoxy group at C-17.

Reaction of 19-*Hydroxy*-4β,5-*epoxy*-5β-*androstane*-3,17-*dione* (**3**) *with Sulphuric Acid in DMSO.*—The sulphuric acid-catalysed reaction of Morisawa and Tanabe[9] on the 17-acetoxy-4β,5β-epoxide (**4**) was repeated with the corresponding 17-ketone (**3**). None of the diosphenol [*cf.* (**12**)] was obtained and only 16% of the 2,3-dihydroxy compound (**20**)[13] was isolated. Instead, a small amount (8%) of the 2α,19-diol (**14**)[6] was obtained as well as estrone (**21**) and the cyclic ether (**22**) in yields of 21 and 25%, respectively.

In the ^{1}H NMR spectrum of compound (**20**), the resonances at δ 4.95 and 4.98, both integrating for one proton and exchanging with D_2O, are assigned to the C-2 and C-3 hydroxy groups. Two singlets integrating for one proton each at δ 6.58 and 6.79 were assigned to C-4 and C-1 hydrogen atoms, respectively.[3,14] Compound (**14**) was identical (^{1}H NMR) with authentic material previously obtained by sulphuric acid opening of the 19-acetoxy-4β,5β-epoxide (**5**). 2β,19-Epoxyandrost-4-ene-3,17-dione (**22**) has a ^{1}H NMR spectrum comparable to that of 17β-acetoxy-2β,19-epoxyandrost-4-ene-3-one (**19**), and the other spectroscopic data (see Experimental section) support the structure (**22**) assigned to this compound.

Reaction of 19-*Hydroxy*-4β,5-*epoxy*-5β-*androstane*-3,17-*dione* (**3**) *with Perchloric Acid in THF.*—This reaction was performed in order to compare the behaviour of the 19-hydroxy-4β,5β-epoxide (**3**) with that of the 10-formyl-4β,5β-epoxide (**7**) under the same reaction conditions since, with the latter compound, a reasonable yield (49%) of the diosphenol (**13**) was obtained.[11] Treatment of the 19-hydroxy compound (**3**) with perchloric acid in THF afforded a mixture of compounds. The diosphenol (**11**) was isolated in 35% yield. This compound (**11**) shows the characteristic UV absorption of diosphenols at 280 nm.[5,14] The proton on the 4-hydroxy group resonates at δ 6.31 and is exchanged with D_2O. Two other compounds, estrone (**21**) and the cyclic ether (**22**), were isolated and identified by comparison with authentic material. At least four other compounds were present in the reaction mixture but could not be purified. Because this method requires long and tedious chromatographic separation to obtain the pure diosphenol (**11**) in relatively low yield, it was decided to explore an alternative route for the synthesis of this compound.

Reaction of 19-*Hydroxyandrost*-4-*ene*-3,17-*dione* (**23**) *with Osmic Acid.*—Treatment of the 19-hydroxy compound (**23**) with osmic acid in pyridine resulted in the formation of a mixture of the triols (**24**) and (**25**). In order to assign the configurations at C-4 and C-5, it was necessary to purify each diastereoisomer. The mixture of alcohols was acetylated, in the hope that the R_f-values of the acetates formed would be distinct, thus facilitating their purification. However, the mixture of acetates (**26**) and (**27**) was homogeneous on the basis of TLC, but ^{1}H NMR analysis indicated a 1:2.3 mixture. Column chromatography of the triol mixture gave a small amount of starting material, and two other substances, assigned the 4β,5β- and 4α,5α-configuration, respectively, on the basis of CD measurements. It was observed by Djerassi and Closson[15] that inversion of configuration at C-5 in the steroid skeleton is accompanied by a significant change in the Cotton effect[16] associated with the 3-ketone group. A 3-oxo-5α-steroid (A/B-*trans*) displays a Cotton effect which is more positive than that observed for the corresponding 3-oxo-5β-steroid (A/B-*cis*). This observation has been used frequently for establishing the stereochemistry at C-5 in 3-oxo steroids.[17] The CD spectrum of the less polar compound eluted from the column showed a maximum at 300 nm with Δε +5.7 whereas the CD spectrum of the more polar compound indicates a maximum at 295 nm with Δε +8.3. Therefore the first compound was assigned the 4β,5β-configuration (**24**) and the more polar compound was assigned the 4α,5α-configuration (**25**). Similar differences in the maxima of absorption (ORD spectra) were reported by Kubota *et al.*[18] for 4,5-dihydroxycholestan-3-ones. As seen below, this assignment is consistent with the chemical behaviour of these compounds.

Reaction of Mixture of 4,5,19-*Trihydroxyandrostane*-3,17-*diones* (**24**) *and* (**25**) *with HCl.*—We first tried to dehydrate the mixture of 4β,5β,19- and 4α,5α,19-triol (**24**) and (**25**) by dissolution of the compounds in acetone in the presence of traces of aq. HCl. The reaction was followed by TLC for a period of 30 minutes and only one product, less polar than the starting mixture and UV-negative, was formed. After 23 h, the starting mixture of alcohols (**24**) and (**25**) had been completely converted into two products. The first product was isolated in 56% yield and the mass spectrum indicated a molecular ion at *m/z* 318, in agreement with the loss of one molecule of water. Lack of UV absorption in the 275 nm region[14] and of a ^{1}H NMR signal near δ 6.3, exchangeable with D_2O, eliminated the possibility of the desired diosphenol (**11**) as the structure for this compound. The ^{1}H NMR spectrum indicates that the C-19 methylene group is probably in a ring since its coupling constant is 7.6 Hz, which is similar to the value found for the cyclic ether (**22**). Structure (**29**) is proposed for this compound: the ^{13}C NMR resonance at δ_C 205.7 is attributed to the C-3 ketone and the resonance at δ_C 103.6 is assigned to C-4 (see Table 1). The stereochemistry at C-5 must be α, based on examination of Dreiding molecular models.

Formation of the lactol (**29**) from the triols (**24**) and (**25**) can be explained by protonation of the C-5 hydroxy group, loss of a molecule of water, and cyclization of the tautomer (**30**) of the diosphenol (**11**). When the reaction was repeated using more starting material (240 mg instead of 80 mg), it was possible to isolate 4% of the diosphenol (**11**), which was identical with an authentic sample. Had cyclization occurred by path 'b' rather than path 'a' (see below), the resulting lactol (**31**) would be expected to exhibit a ^{13}C NMR signal for C-5 (methine group adjacent to a carbonyl group) at δ_C *ca.* 59[19] rather than at δ_C 48.1, as observed.

The second product obtained upon treatment of triols (**24**) and (**25**) with aq. HCl in acetone was isolated in 19% yield. The mass spectrum showed a molecular ion at *m/z* 376 corresponding to an increase of 40 mass units compared with the mass of the starting material. The ^{1}H NMR spectrum with two resonances at δ 1.40 and δ 1.47, both integrating for three

* 3,17-Dioxoandrost-4-ene-2α,19-diyl diacetate.

† 3-oxo-4β,5-epoxy-5β-androstane-17β,19-diyl diacetate.

Table 1. ^{13}C NMR chemical shifts.[a,b]

Carbon Atom	(9)	(14)	(15)	(16)	(20)
1	22.7	42.7	39.7	39.8	[115.5]
2	37.8	69.6	70.9	71.1	[141.6]
3	193.4	200.1	193.6	193.7	[141.5]
4	139.0	123.5	125.1	125.0	[112.5]
5	141.3	167.9	164.4	165.0	[129.0]
6	31.7	32.9	32.1	32.3	28.7
7	31.3	31.5	31.4	31.6	26.6
8	34.7	35.5	35.3	35.6	38.2
9	54.2	54.9	54.9	54.8	44.0
10	35.7	46.2	44.2	42.4	[131.3]
11	20.2	20.5	20.5	20.8	26.0
12	29.8	30.8	30.8	36.7	31.5
13	47.4	47.5	47.3	44.2	48.1
14	50.9	51.0	51.0	50.5	50.4
15	21.7	21.6	21.5	23.2	21.6
16	34.6	35.6	35.5	27.3	35.9
17	220.6	220.0	219.5	82.1	221.6
18	13.7	13.7	13.7	12.1	13.8
19	17.1	65.7	66.3	66.6	
CO*Me*			20.8	20.9	
CO*Me*			20.9	20.9	
CO*Me*				21.1	
*C*OMe			170.1	170.2	
*C*OMe			170.6	170.7	
*C*OMe				171.1	

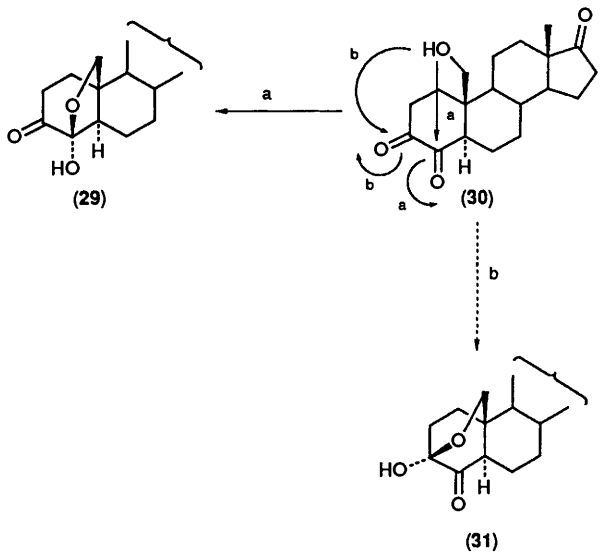
	(22)	(24)	(29)	(28)
1	42.3	[30.8]	[35.2]	[23.3]
2	80.9	[34.0]	[32.9]	[33.7]
3	196.8	209.4	205.7	109.7
4	123.4	73.6	103.6	83.3
5	167.3	83.3	48.1*	97.2
6	[28.1]	25.8	[22.4]	25.4
7	[28.1]	27.6	[28.1]	26.6
8	37.7	34.0	36.0	34.9
9	44.5	43.6	49.9*	43.4
10	50.6	43.9	46.6	39.7
11	21.0	20.8	21.1	21.0
12	[31.4]	31.6	31.7	31.5
13	47.4	47.5	47.9	47.6
14	51.3	52.1	50.5	50.8
15	21.7	21.8	21.8	21.5
16	35.7	35.7	35.8	35.7
17	219.3	219.6	220.0	220.6
18	13.8	13.8	14.1	13.7
19	69.5	63.6	70.1	67.6
Me				27.2
Me				26.8

* Assignment within vertical column may be reversed. [ ] Tentative assignments. [a] Data are in ppm in $CDCl_3$ with $\delta(CDCl_3)$ 77.0. [b] The assignment of ^{13}C NMR peaks in terms of number of attached hydrogen atoms has been made using the pulse sequence DEPT[23] (Distortionless Enhancement of NMR signal by Polarization Transfer).

protons, showed that one molecule of acetone had been added by formation of an isopropylidene derivative. Furthermore, the ^1H NMR spectrum suggested that the 19-methylene group is in a ring since the coupling constant for the quartet integrating for two protons and resonating at δ 3.94 was 9.3 Hz, as opposed to 11–12 Hz for non-cyclic C-19 methylene protons. That only one ketone was present was confirmed by the ^{13}C NMR spectrum, where only one signal, at δ_C 220.6 (17-carbonyl), was observed. Therefore structure (**28**) is assigned to the second product obtained by treatment of the mixture of triols (**24**) and (**25**) with aq. HCl in acetone. The stereochemistry of the 4,5-isopropylidene group must be α since a β-arrangement does not allow cyclization of the 19-alcohol at C-3 (Dreiding models).

Table 2. Acid-catalysed reactions of 4β,5,19-trihydroxy-5β-androstane-3,17-dione (**24**) and 4α,5,19-trihydroxy-5α-androstane-3,17-dione (**25**).

		Products yields (%)		
		(28)	(11)	(29)
(24), (25)	Acetone–HCl, 20 h	22	4	36
(24), (25)	Acetone–HCl, 23 h	19		56
(24), (25)	Methanol–HCl, 22h			20
(24), (25)	THF–HCl, 21 h			35
(24)	Acetic acid–HCl, 16 h		100	



In order to eliminate the formation of the isopropylidene derivative (**28**) and possibly favour formation of the diosphenol (**11**), different solvents were used in the presence of HCl to effect the dehydration of the triols (**24**) and (**25**). The results are summarized in Table 2. When the reaction was carried out in methanol or THF the cyclized compound (**29**) was formed and some unchanged starting material remained but none of the diosphenol (**11**) was present when the reaction was terminated. However, when the reaction was performed in acetic acid, with the 4β,5β,19-trihydroxy compound (**24**), the 4,19-dihydroxy derivative (**11**) was obtained in 100% yield.

Experimental

M.p.s were determined with a Hoover Uni-melt apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter for solutions in chloroform. CD spectra were obtained with 95% ethanol solutions on a Cary 61 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 783 IR spectrophotometer for chloroform solutions unless otherwise specified. ^1H NMR spectra were obtained with a Varian XL-300 instrument (with deuteriochloroform as solvent and internal standard). ^{13}C NMR spectra were recorded with a Varian FT-80 instrument or on a Bruker AM-500 instrument (with deuteriochloroform as solvent and internal standard). The high-resolution and electron-impact mass spectra were determined with a V.G. 7070-E double-focusing instrument. Microanalyses were carried out by M-H-W Laboratories, Phoenix, AZ 85018, U.S.A. The reactions were monitored by TLC on Merck pre-coated silica gel 60F-254 plates. For column chromatography Terochem Silica gel 1918 (equivalent to Merck 9385, 20–45 μm) was used unless

otherwise specified. Some chromatographic separations were performed on a preparative, centrifugally accelerated, radial, thin-layer chromatograph (Chromatotron Model 7924, Harrison Research Inc.). Work-up involved pouring of the reaction mixture into water, extraction with methylene dichloride, and washing of the extracts with water (with 5% HCl if pyridine was present). After the extracts were dried (Na_2SO_4), the solvents were removed under reduced pressure. Light petroleum refers to the fraction boiling in the range 30–60 °C.

4-Hydroxyandrost-4-ene-3,17-dione (**9**).—To a solution of androst-4-ene-3,17-dione (1.00 g) in methanol (40 ml), cooled in an ice–water-bath, were added ice-cold, 20% aq. sodium hydroxide (2 ml) and 30% hydrogen peroxide (3.4 ml). The mixture was stirred for 30 min at 0–4 °C and left for 22 h at 5–7 °C. The reaction mixture was added to ice-cold water and, after 15 min, the product was filtered off and washed with water. The product was a mixture of 4β,5-epoxy-5β- and 4α,5-epoxy-5α-androstane-3,17-dione (*ca.* 4:1, by ^{1}H NMR) (0.60 g, 60%), δ_H 0.88 (0.89) (3 H, s, 18-H_3), 1.17 (3 H, s, 19-H_3), [2.98 (3.04) 1 H, s, 4-H^{β}(4-H^{α})]; *m/z* 302 (M^+, 37%), 287 (6), 284 (33), 274 (44), 273 (12), and 230 (100). The mixture of epoxides was then dissolved in a cooled solution of sulphuric acid in acetic acid (2:98) and the reaction mixture was stirred at room temperature. The reaction was terminated after 45 min when the mixture was poured into ice-cold water, and the product was filtered off and dried. Recrystallization in ethyl acetate was not successful in removing the more polar impurities. Column chromatography (Davisil silica gel) with 1:1 hexane–diethyl ether as eluant afforded 4-hydroxyandrost-4-ene-3,17-dione) (**9**) (0.18 g, 27%), m.p. 202 °C (lit.,[2] 203.5–206 °C; lit.,[3] 199–202 °C); δ_H 0.90 (3 H, s, 18-H_3), 1.18 (3 H, s, 19-H_3), and 6.07 (1 H, s, 4-OH); *m/z* 302 (M^+, 78%), 287 (15), 274 (8), 260 (20), and 147 (100) (Found: M^+, 302.1870. Calc. for $C_{19}H_{26}O_3$: *M*, 302.1875).

4,17β-Dihydroxyandrost-4-en-3-one (**10**).—17β-Hydroxyandrost-4-en-3-one (1.00 g) was epoxidized with 30% hydrogen peroxide in alkaline methanol as previously described for androst-4-ene-3,17-dione. The resulting product was a mixture of 17β-hydroxy-4β,5-epoxy-5β- and 17β-hydroxy-4α,5-epoxy-5α-androstan-3-one (*ca.* 6.8:1, by ^{1}H NMR) (0.875 g, 83%), δ_H 0.75 (0.76) (3 H, s, 18-H_3), 1.14 (3 H, s, 19-H_3), 2.96 (3.02) [1 H, s, 4-H^{β} (4-H^{α})], and 3.63 (1 H, t, 17-H^{α}); *m/z* 304 (M^+, 24%), 289 (4), 286 (30), 276 (36), 270 (11), 261 (28), and 232 (100). The mixture of epoxides (0.264 g) was treated with acetic anhydride in pyridine. Work-up afforded a mixture of 17β-acetoxy-4β,5-epoxy-5β- and 17β-acetoxy-4α,5-epoxy-5α-androstan-3-one* (0.30 g), which was treated with sulphuric acid in acetic acid for 47 min, as described above, and afforded 17β-acetoxy-4-hydroxyandrost-4-en-3-one† (254 mg, 85%), m.p. 192–193 °C (lit.,[5] 194–196 °C); δ_H 0.81 (3 H, s, 18-H_3), 1.16 (3 H, s, 19-H_3), 2.03 (3 H, s, 17-OAc), 4.58 (1 H, t, 17-H^{α}), 6.05 (1 H, s, exchanges with D_2O, 4-OH); *m/z* 346 (M^+, 32%), 328 (5), 331 (4), 318 (4), 304 (29), 303 (13), 302 (15), and 147 (100).

Potassium carbonate (255 mg) was added to a solution of 17β-acetoxy-4-hydroxyandrost-4-en-3-one (99 mg) in 95% ethanol (8 ml) and the reaction mixture was stirred at room temperature for 2 days. Work-up and column chromatography, elution with methylene dichloride–ethyl acetate (80:20), afforded 4,17β-dihydroxyandrost-4-en-3-one (**10**) (14 mg, 16%), δ_H 0.77 (3 H, s, 18-H_3), 1.16 (3 H, s, 19-H_3), 3.62 (1 H, t, 17-H^{α}), and 6.05 (1 H, s, exchanges with D_2O, 4-OH); *m/z* 304 (M^+, 100%), 302 (22), 289 (15), 286 (16), 271 (10), 262 (17), 260 (15), and 258 (11).

Treatment with Sulphuric Acid in Acetic Acid of 19-Acetoxy-4β,5-epoxides (**5**) and (**6**).—19-Acetoxy-4β,5-epoxy-5β-androstane-3,17-dione (**5**)[20] (0.82 g) was treated for 10 min with a cooled mixture of sulphuric acid and acetic acid (2:98; 19 ml). Work-up and column chromatography (Davisil silica gel), elution with hexane–diethyl ether (50:50), afforded 2α,19-diacetoxyandrost-4-ene-3,17-dione‡ (**15**) (402 mg, 44%), m.p. 140–141 °C (needles from diethyl ether) (lit.,[6] 141–142 °C); δ_H 0.89 (3 H, s, 18-H_3), 2.05 (3 H, s) and 2.14 (3 H, s) (2α- and 19-OAc), 4.46 (2 H, q, δ_A 4.22, δ_B 4.71, J_{AB} 11.4 Hz, 19-H_2), 5.74 (1 H, q, *J* 66, 13.6 Hz, 2-H^{β}), and 5.93 (1 H, s, 4 H); *m/z* 402 (M^+, 21%), 360 (4), 342 (4), 329 (6), 301 (14), 300 (62), and 43 (100) (Found: M^+, 402.2043. Calc. for $C_{23}H_{30}O_6$: *M*, 402.2034).

Treatment of compound (**15**) (106 mg), dissolved in 95% ethanol (5 ml), with potassium carbonate (355 mg) for 15 h 30 min at room temperature afforded, after work-up and column chromatography with methylene dichloride–ethyl acetate (50:50) as eluant, 2α,19-dihydroxyandrost-4-ene-3,17-dione (**14**)[6] (39 mg, 47%), δ_H 0.88 (3 H, s, 18-H_3), 3.47 (1 H, br s, exchanges with D_2O, 2α-OH), 4.05 (2 H, q, δ_A 3.98, δ_B 4.12, J_{AB} 10.4 Hz, 19-H_2), 4.68 (1 H, q, *J* 6.2, *J* 13.3 Hz, 2-H^{β}), and 5.99 (1 H, s, 4-H); *m/z* 318 (M^+, 70%), 300 (7), 289 (18), 288 (80), 287 (100), 274 (30), and 270 (35) (Found: M^+, 318.1823. Calc. for $C_{19}H_{26}O_4$: *M*, 318.1824). 17β,19-Dihydroxy-5β-androstan-3-one[21] was obtained by treatment of dione (**23**) with sodium borohydride in methanol for 1 h at 0–4 °C. Epoxidation was performed with 30% hydrogen peroxide in alkaline methanol as described above. The resulting 4β,5β-epoxide was treated with acetic anhydride in pyridine. Work-up afforded 17β,19-diacetoxy-4β,5-epoxy-5β-androstan-3-one§ (**6**); δ_H 0.77 (3 H, s, 18-H_3), 2.02 (3 H, s), and 2.11 (3 H, s) (17β- and 19-OAc), 2.88 (1 H, s, 4-H), 4.20 (1 H, d, *J* 11.5 Hz, 19*H*H), and 4.56 (2 H, br d, 19-CH*H* and 17-H^{α}); *m/z* 404 (M^+, 20%), 347 (4), 344 (10), 331 (7), 316 (5), 303 (27), 289 (11), and 43 (100).

Treatment of the 4β,5β-epoxide (**6**) with a mixture of sulphuric acid in acetic acid (2:98; 15 ml) for 50 min formed a sticky yellow precipitate after the reaction mixture was poured onto ice. Crystallization of this oily precipitate in methanol afforded 2α,17β,19-triacetoxyandrost-4-en-3-one¶ (**16**) (85 mg, 14%), m.p. 192–193 °C; $[\alpha]_D$ +98° (*c* 0.24); λ_{max} 239 nm (ε 10 190); γ_{max}(KBr) 1 735 (17β- and 19-OAc) and 1 685 cm^{-1} (3-CO); δ_H 0.81 (3 H, s, 18-H_3), 2.02 (3 H, s), 2.05 (3 H, s), 2.14 (3 H, s) (2β-, 17β-, and 19-OAc), 4.46 (2 H, q, δ_A 4.22, δ_B 4.69, J_{AB} 11.2 Hz, 19-H_2), 4.57 (1 H, t, 17-H^{α}), 5.75 (1 H, q, *J* 6.5 Hz, 2-H^{β}), and 5.91 (1 H, s, 4-H); *m/z* 446 (M^+, 8%), 404 (17), 386 (4), 344 (35), 326 (11), 318 (20), 314 (46), 302 (11), and 43 (100).

Treatment of 19-Hydroxy-4β,5-epoxy-5β-androstane-3,17-dione (**3**) *with Sulphuric Acid in DMSO.*—19-Hydroxy-4β,5-epoxy-5β-androstane-3,17-dione (**3**) (1.00 g) was dissolved in DMSO (70 ml) and conc. sulphuric acid (3 μl) was added. After being heated at 120 °C, under nitrogen for 10 h, the reaction mixture was poured into water (150 ml), the sticky precipitate was filtered off (346 mg), and the remaining aqueous phase was extracted with methylene dichloride. The extracts were combined and the solvent was first removed with a rotary evaporator, then the remaining DMSO was removed under high vacuum (at this point, it was necessary to heat the solution in a boiling water-bath in order to remove the solvent). A first chromatographic separation of the residue thus obtained, using a Chromatotron equipped with a 4 mm plate and eluted with

* 3-Oxo-4β,5-epoxy-5β- and 3-oxo-4α,5-epoxy-5α-androstan-17β-yl acetate.

† 4,17β-Dihydroxyandrost-4-en-3-one 17-acetate.

‡ 3,17-Dioxoandront-4-ene-2α,19-diyl diacetate.

§ 3-Oxo-4β,5-epoxy-5β-androstane-17β,19-diyl diacetate.

¶ 3-Oxoandrost-4-ene-2α,17β,19-triyl triacetate.

light petroleum–diethyl ether (60:40), afforded 3-hydroxy-estra-1,3,5(10)-17-one (**21**) (54 mg, 6%); δ_H 0.89 (3 H, s, 18-H_3), 4.48 (1 H, s, exchanges with D_2O, 3-OH), 6.56 (s, 1 H, 4-H), 6.62 (1 H, d, *J* 8.2 Hz, 1-H), and 7.13 (1 H, d, *J* 8.2 Hz, 2-H); *m/z* 270 (M^+, 100%), 242 (5), 226 (7), 214 (11), and 213 (20). Further elution of this plate with diethyl ether–methylene dichloride (90:10), followed by methylene dichloride, afforded 2α,19-dihydroxy-androst-4-ene-3,17-dione (**14**)[6] (84 mg, 8%) (spectroscopic characteristics identical with those described above). Further purification of the fraction obtained when the plate was eluted with light petroleum–diethyl ether (20:80) was performed using a Chromatotron equipped with a 2 mm plate. Elution with light petroleum–diethyl ether (40:60) afforded 2β,19-epoxyandrost-4-ene-3,17-dione (**22**) (238 mg, 24%), m.p. 162–163 °C (recrystallized from diethyl ether); $[\alpha]_D$ +281° (*c* 0.52); λ_{max} 242 nm (ε 16 255); ν_{max}(KBr) 1 735 (17-CO) and 1 670 cm^{-1} (3-CO); δ_H 0.90 (3 H, s, 18-H_3), 3.76 (2 H, q, δ_A 3.49, δ_B 4.04, J_{AB} 7.5 Hz, 19-H_2), 4.29 (1 H, d, *J* 1.9, *J* 6.3 Hz, 2-H$^\alpha$), and 5.78 (1 H, br s, 4-H); *m/z* 300 (M^+, 100%), 271 (15), 270 (26), 357 (14), 243 (17), and 242 (74) (Found: M^+, 300.1736. Calc. for $C_{19}H_{24}O_3$: *M*, 300.1719). The sticky precipitate first obtained was purified on a Chromatotron equipped with a 2 mm plate. Elution with light petroleum–diethyl ether (70:30) afforded estrone (**21**) (134 mg, 15%), and elution with light petroleum–diethyl ether (60:40 and 55:45) afforded 2,3-dihydroxyestra-1,3,5(10)-trien-17-one (**20**)[22] (151 mg, 16%); δ_H 0.89 (3 H, s, 18-H_3), 4.95 and 4.98 (2 H, d, exchanges with D_2O, 2- and 3-OH), and 6.58 and 6.79 (s, 2 H, 1- and 4-H); *m/z* 286 (M^+, 100%), 230 (9), 229 (10), and 201 (13).

Treatment of 19-*Hydroxy*-4β,5-*epoxy*-5β-*androstane*-3,17-*dione* (**3**) *with Perchloric Acid in THF.*—19-Hydroxy-4β,5-epoxy-5β-androstane-3,17-dione (**3**) (333 mg) was dissolved in THF (10 ml) and 35% perchloric acid (1 ml) was added. The solution was stirred at room temperature for 21 h. Work-up and column chromatography, elution successively with the following mixtures of methylene dichloride–ethyl acetate: 95:5, 90:10; 75:25; 70:30; 60:40; 50:50; 40:60, and 30:70, first afforded estrone (**21**) (23 mg, 8%) (^{1}H NMR spectrum identical with that of authentic material), and then 2β,19-epoxyandrost-4-ene-3,17-dione (**22**) was obtained (13 mg, 4%) (spectral data same as described above).

4,19-Dihydroxyandrost-4-ene-3,17-dione (**11**) was isolated (117 mg, 35%) m.p. 213–214 °C (needles from ethyl acetate); $[\alpha]_D$ +212° (*c* 0.25); λ_{max} 280 nm (ε 9 050); ν_{max} 3 450 (19-OH), 1 730 (17-CO), and 1 670 cm^{-1} (3-CO); δ_H 0.89 (3 H, s, 18-H_3), 3.95 (2 H, q, δ_A 3.87, δ_B 4.03, J_{AB} 10.5 Hz, 19-H_2), and 6.31 (1 H, s, exchanges with D_2O, 4-OH); *m/z* 318 (M^+, 6%), 288 (38), and 287 (100%).

Treatment of 19-*Hydroxyandrost*-4-*ene*-3,17-*dione* (**23**) *with Osmic Acid.*—19-Hydroxyandrost-4-ene-3,17-dione (**23**) (300 mg) was dissolved in a mixture of pyridine (1 ml) and benzene (5 ml). A solution of osmic acid (250 mg) in benzene (5 ml) was added to the solution. The reaction mixture was stirred at room temperature for 24 h. The colour of the solution rapidly changed from dark yellow to dark orange and finally to dark brown. The osmate ester was cleaved by addition of a solution of sodium hydrogen sulphite (1.50 g) in a mixture of water (20 ml) and pyridine (3 ml). The resulting two-phase solution was vigorously stirred for 30 min. The colour of the solution changed from dark brown to orange. Work-up and column chromatography, with methylene dichloride–ethyl acetate (63:37) as eluant, afforded starting material (**23**) (22 mg, 7% recovery). The second compound eluted was 4β,5,19-*trihydroxy*-5β-*androstane*-3,17-*dione* (**24**) (96 mg, 29%); m.p. 195–196 °C (Found: C, 67.7; H, 8.3. $C_{19}H_{28}O_5$ requires C, 67.81; H, 8.39%) (Found: M^+, 336.1912. $C_{19}H_{28}O_5$ requires *M*, 336.1929.); $[\alpha]_D$ +96° (*c* 0.20); λ_{max} 300 nm (Δε +5.7); ν_{max}(KBr) 3 440br OH) and 1 725 cm^{-1} (3- and 17-CO); δ_H 0.86 (3 H, s, 18-H_3), 2.89 (1 H, s, exchanges with D_2O, 5β-OH), 3.27 (1 H, d, exchanges with D_2O, 19-OH), 3.59 (1 H, t, becomes a d with D_2O, *J* 11.2 Hz, 19-*pro*-*R*-H), 3.76 (1 H, d, exchanges with D_2O, *J* 2.6 Hz, 4β-OH), 4.34 (1 H, d, *J* 11.5 Hz, 19-*pro*-*S*-H), and 4.44 (1 H, d, becomes a s with D_2O, *J* 2.6 Hz, 4-H$^\alpha$); *m/z* 336 (M^+, 24%), 318 (8), 306 (5), 288 (12), 287 (25), 272 (11), and 233 (100).

4β,5,19-Trihydroxy-5β-androstane-3,17-dione (**24**) (26 mg) was dissolved in pyridine and treated with acetic anhydride overnight. Work-up afforded 4β,19-diacetoxy-5-hydroxy-5β-androstane-3,17-dione* (**26**) (100%); δ_H 0.87 (3 H, s, 18-H_3), 2.207 and 2.210 (3 H, s, 4β- and 19-OAc), 4.46 (2 H, s, 19-H_2), and 5.64 (1 H, s, 4-H$^\alpha$); *m/z* 420 (M^+, 2%), 378 (14), 360 (8), 318 (6), 287 (13), and 43 (100). Further elution of the column with methylene dichloride–ethyl acetate (60:40) afforded mainly 4α,5,19-trihydroxy-5α-androstane-3,17-dione (**25**), which was contaminated with the 4β,5β,19-triol (**24**). Addition of methylene dichloride to this fraction removed all traces of 4β,5β,19-triol (**24**) and left 4α,5,19-*trihydroxy*-5α-*androstane*-3,17-*dione* (**25**) (11 mg, 3%) which was homogeneous on TLC; m.p. 223–224 °C (Found: C, 69.0; H, 8.6%; M^+, 336.1930. $C_{19}H_{28}O_5$ requires C, 67.81; H, 8.39%;† *M*, 336.1929); $[\alpha]_D$ +128° (*c* 0.165, MeOH); λ_{max} 295 nm (Δε +8.3); ν_{max}(KBr) 3 400br (OH) and 1 740 cm^{-1} (3- and 17-CO); δ_H([$^2H_5$]pyridine) 0.76 (3 H, s, 18-H_3), 3.60 (1 H, s) 3.82 (1 H, s), 4.21 (2 H, q, δ_A 4.13, δ_B 4.29, J_{AB} 9.0 Hz, 19-H_2), 4.83 (2 H, br s, exchanges with D_2O, 4α- and 5α-OH), and 5.03 (1 H, s, 4-H$^\beta$); *m/z* 336 (M^+, 14%), 318 (6), 287 (14), 272 (7), and 43 (100). Even if the triol (**25**) was obtained as a homogeneous compound (TLC) its ^{1}H NMR spectrum, as well as that obtained for its 4α,19-diacetoxy derivative (**27**) (below) (also homogeneous on TLC), indicated, besides the resonances at δ 5.47 (1 H, s, 4-H$^\beta$) and at δ 4.54 (2 H, d, 19-H_2), a resonance at δ 3.64 integrating for two protons which could not be assigned and which suggested the presence of an impurity. Even after examination of the ^{13}C NMR spectrum of the 4α,19-diacetoxy derivative (**27**) (below), we could not identify the impurity. The presence of this impurity may explain why the observed value for carbon in the elemental analysis of compound (**25**) differs significantly from the theoretical value. Acetylation of this material and work-up afforded 4α,19-diacetoxy-5α-hydroxyandrostane-3,17-dione‡ (**27**) (100%); δ_H 0.86 (3 H, s, 18-H_3), 2.12 and 2.20 (3 H, s, 4α- and 19-OAc), 3.64 (2 H, br s), 4.54 (2 H, d, *J* 6.8 Hz, 19-H_2), 5.47 (1 H, s, 4-H$^\beta$); δ_C 13.8 (C-18), 20.6 (OCO*Me*), 20.9 (C-11), 21.2 (OCO*Me*), 21.6 (C-15), 24.5 (C-6), 28.8 (C-7), 29.1 (C-1), 29.7 (CH_2), 31.7 (C-12), 34.2(C-8), 35.7 (C-16), 37.1 (CH_2), 43.9 (C-10), 45.5 (C-9), 47.6 (C-13), 51.2 (C-14), 64.3 (C-19), 70.6 (C-4), 78.5 (C-5), 79.4 (CH), 169.7 (O*C*OMe), 170.5 (O*C*OMe), 202.7 (C-3), and 220.3 (C-17); *m/z* 420 (M^+, 3%), 378 (23), 360 (11), 318 (6), 287 (12), and 43 (100).

Treatment of Mixture of 4β,5,19-*Trihydroxy*-5β-*androstane*-3,17-*dione* (**24**) *and* 4α,5,19-*Trihydroxy*-5α-*androstane*-3,17-*dione* (**25**) *with HCl in Acetone.*—A mixture of triols (**24**) and (**25**) (290 mg) was dissolved in acetone (10 ml) containing conc. HCl (2 drops). The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated off under a stream of nitrogen and the residue was purified by column chromatography. Elution with methylene dichloride–ethyl acetate (85:15) afforded 4α-*hydroxy*-4β,19-*epoxy*-5α-*androstane*-3,17-*dione* (**29**) (100 mg, 36%); m.p. 188–189 °C (from Et_2O) (Found: C, 71.7; H, 8.5. $C_{19}H_{26}O_4$ requires C, 71.65; H, 8.24%); $[\alpha]_D$ +

* 4β,5,19-Trihydroxy-5β-androstane-3,17-dione 4β,19-diacetate.
† See below for a possible reason for the poor C-analysis figure.
‡ 4α,5,19-Trihydroxy-5α-androstane-3,17-dione 4,19-diacetate.

34° (*c* 0.16); ν_{max} 3 490br (OH) and 1 730 cm^{-1} (3- and 17-CO); δ_H 0.87 (3 H, s, 18-H_3), 4.03 (2 H, q, δ_A 3.80, δ_B 4.26, J_{AB} 8.3 Hz, 19-H_2), and 4.33 (1 H, s, exchanges with D_2O, 4α-OH); *m/z* 318 (M^+, 44%), 297 (9), 291 (17), 290 (84), 287 (16), 275 (39), 272 (68), and 261 (100). Further elution with methylene dichloride–ethyl acetate (80:20) afforded 3α-hydroxy-4α,5-isopropylidenedioxy-3β,19-epoxy-5α-androstan-17-one (**28**) (73 mg, 22%); m.p. 225–226 °C; $[\alpha]_D$ +70° (*c* 0.16); ν_{max} 3 580 and 3 310 (OH) and 1 730 cm^{-1} (17-CO); δ_H 0.82 (3 H, s, 18-H_3), 1.40 (3 H, s, isopropylidene-Me) and 1.47 (3 H, s, isopropylidene Me), 3.66 (1 H, d, *J* 1.6 Hz, 4β-OH), and 3.94 (2 H, q, δ_A 3.90, δ_B 3.98, J_{AB} 9.3 Hz, 19-H_2); *m/z* 376 (M^+, 69%), 361 (85), 318 (48), 301 (84), 288 (22), 283 (16), 272 (36), and 167 (100). The last compound eluted was 4,19-dihydroxyandrost-4-ene-3,17-dione (**11**) (12 mg, 4%) (^1H NMR and mass spectra identical with those described above). When the reaction was performed as described above but for 23 h with a mixture of triols (**24**) and (**25**) (80 mg), the yields for the products (**28**) and (**29**) were 19 and 56%, respectively.

Treatment of Mixture of 4β,5,19-*Trihydroxy*-5β-*androstane*-3,17-*dione* (**24**) *and* 4α,5,19-*Trihydroxy*-5α-*androstane*-3,17-*dione* (**25**) *with HCl in Methanol.*—A mixture of triols (**24**) and (**25**) (200 mg) was dissolved in methanol (6 ml) containing conc. HCl (1 drop). The solution was stirred at room temperature for 22 h. Work-up and column chromatography were as described above. 4α-Hydroxy-4β,19-epoxy-5α-androstane-3,17-dione (**29**) was isolated in 20% yield.

Treatment of Mixture of 4β,5,19-*Trihydroxy*-5β-*androstane*-3,17-*dione* (**24**) *and* 4α,5,19-*Trihydroxy*-5α-*androstane*-3,17-*dione* (**25**) *with HCl in THF.*—A mixture of triols (**24**) and (**25**) (160 mg) was dissolved in THF (6 ml) and conc. HCl (1 drop) was added. The solution was stirred at room temperature for 21 h. Work-up and column chromatography were as described above. 4α-Hydroxy-4β,19-epoxy-5α-androstane-3,17-dione (**29**) was isolated in 35% yield.

Treatment of 4β,5,19-*Trihydroxy*-5β-*androstane*-3,17-*dione* (**24**) *with HCl in Acetic Acid.*—4β,5,19-Trihydroxy-5β-androstane-3,17-dione (**24**) (33 mg) was dissolved in a mixture of acetic acid–HCl (20.0: 0.5; 5 ml). The solution was stirred at room temperature for 16 h. Work-up afforded 4,19-dihydroxyandrost-4-ene-3,17-dione (**11**) (31 mg, 100%). The ^1H NMR and mass spectra were identical with those reported above for compound (**11**).

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