Boar Taint Steroid Derivatives for Immunological Studies. Synthesis of 11α-Hydroxy-5α-androst-16-en-3-one and its Hemisuccinate

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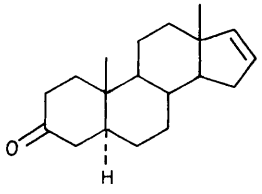
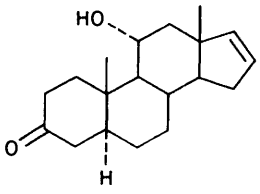
5α-Androstane-3,11,17-trione has been converted into the title alcohol in five steps in an overall yield of 42%. Selective acetalization at C-3 allowed the use of the vinyl iodide route for introduction of the double bond at C-16,17 by transformation of the 17-oxo group. Reduction of the intermediate 17-iodo-16-ene with sodium in ethanol took place with concomitant reduction of the 11-oxo group to the 11α-alcohol. Esterification with succinic anhydride in pyridine gave the 11α-yl hemisuccinate.

The off-flavour of cooked boar meat, often referred to as 'boar taint,' is thought to be due mainly to the presence of 5α-androst-16-en-3- one [(**1**), the 'boar taint steroid'].[1] Without the problem of boar taint, pig farmers would be able to raise boars for meat production, rather than sows or castrates, since the male animal provides a much leaner carcase. The availability of boar meat of acceptable flavour would improve bacon considerably from a dietary point of view, by limiting its saturated fat content. As part of a project aimed at the reduction of boar taint steroid levels in entire male pigs by auto-immunization,[2] a derivative of androstenone having a side-chain terminating in a carboxylic group was required. In androstenone (**1**) the functional groups are located in rings A and D, at the extremities of the molecule, so that protein conjugates linked through rings B or C should be



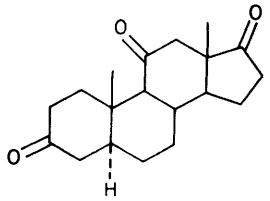
(**1**)



(**2**)

capable of eliciting the optimum immune response. Accordingly, we undertook the synthesis of 11α-hydroxyandrostenone (**2**) and the preparation of its hemisuccinyl derivative to provide a steroid hapten suitable for immunological studies.†

The key to the synthesis is the exploitation of the differences in reactivity of the three carbonyl groups of 5α-androstane-3,11,17-trione (**3**), which are subject to differing degrees of steric hindrance. The triketone (**3**) was obtained from the readily available adrenosterone (**4**) by catalytic hydrogenation over



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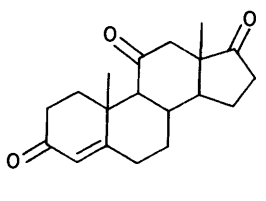


(**4**)

palladium. A series of experiments with varying amounts of catalyst showed that use of less catalyst resulted in increased yields of the α-isomer (Figure 1). Optimum conditions allowed an isolated yield of over 60% of the 5α-isomer (**3**).

† Preliminary communication: *Chem. Ind.* (*London*), 1987, 337.

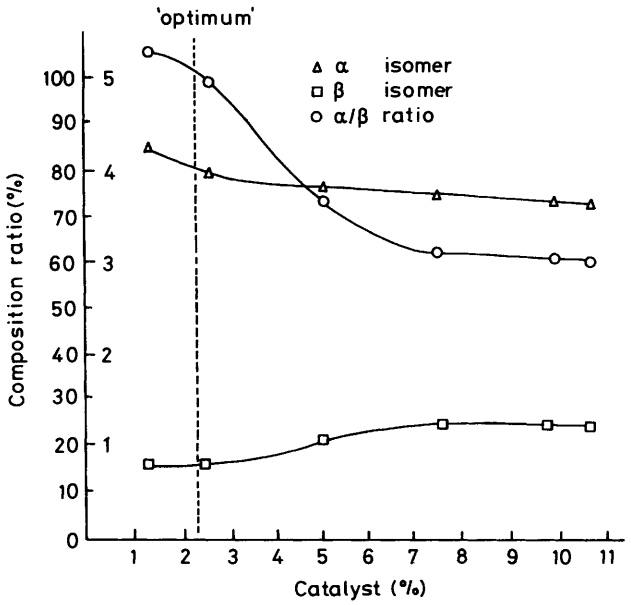


Figure 1. Effect of variation of catalyst amount on product composition for hydrogenation of adrenosterone

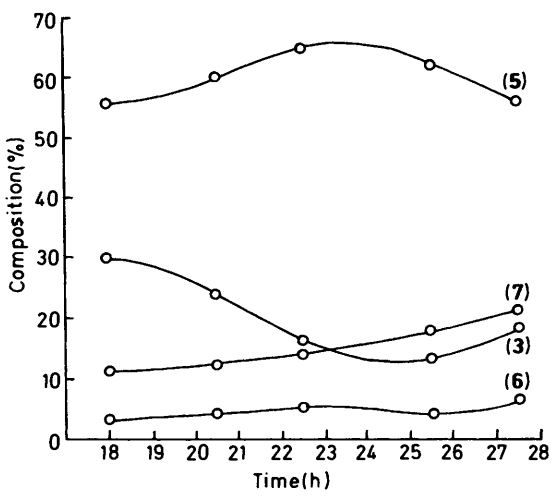
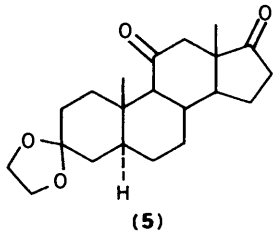
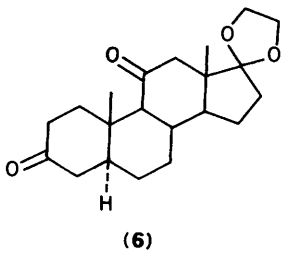


Figure 2. Acetalization of triketone (**3**)

Acetalization of the triketone (**3**) to the 3-monoacetal (**5**) was most selective using 1.15 equiv. of ethylene glycol in the presence of toluene-*p*-sulphonic acid. The composition of the reaction mixture during optimization was conveniently monitored by g.c. analysis. Higher proportions of the 17-monoacetal (**6**), the 3,17-bis-acetal (**7**), and starting triketone (**3**) were obtained when long reaction times were employed (Figure 2). This was ascribed to the existence of an equilibrium between all the possible acetals, causing a build-up of the more hindered 17-acetals under the conditions of thermodynamic control. Although acetals of cyclopentanones are reportedly[3] more reactive than those of cyclohexanones, the sterically hindered nature of the 17-acetals is thought to prevent their ready hydrolysis in this instance. The crude 3-monoacetal (**5**) could be



(**5**)



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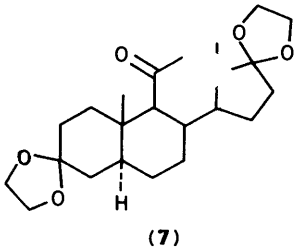
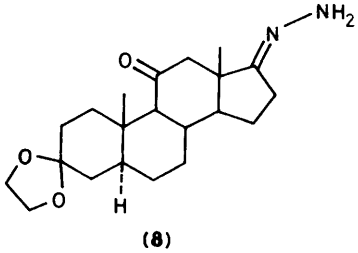


(**7**)

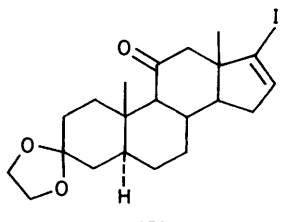


(**8**)

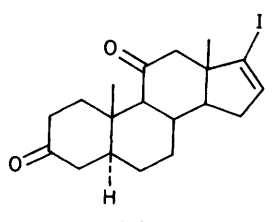
used for the next stage, since the main impurity was the bisacetal (**7**), which could be removed later in the sequence. A sample of the 3-monoacetal (**5**) was purified by preparative t.l.c. on alumina. The base peak in its mass spectrum appeared at *m/z* 99 (acetal directed fragmentation[4]), as was also the case with the two subsequent 3-monoacetals in the reaction sequence.

Having protected the most reactive of the three carbonyl groups, it was possible to transform the 17-oxo group by the vinyl iodide route.[5] The monoacetal (**5**) reacted with hydrazine in refluxing ethanol in the presence of triethylamine giving good yields of the 17-hydrazone (**8**). The carbonyl group at C-11 is not affected under these conditions owing to steric hindrance. Overall yields for the two stages were in the range 67—84% (kinetic control) and 43—64% (thermodynamic control).

Reaction of the hydrazone (**8**) with iodine[5] in THF in the presence of base gave the vinyl iodide (**9**) in yields of up to 90%. This compound was readily hydrolysed to the diketone (**10**) with acid.[6] When the vinyl iodide (**9**) was treated with sodium in ethanol to remove the iodine atom at C-17,[5] the product showed no carbonyl absorption in its i.r. spectrum. Instead, strong hydroxy absorption was present, and other

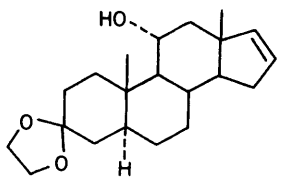


(**9**)

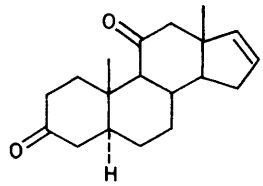


(**10**)

spectral data confirmed that concomitant reduction of the 11-oxo group had occurred to give the 16-en-11α-ol (**11**). Typical yields for the double reduction were in the range 70—80% after preparative h.p.l.c. [In syntheses carried through without purification of the intermediates (**5**), (**8**), and (**9**), the major impurity which had to be removed in the final chromatographic purification was the alcohol (**13**) derived from the bisacetal (**7**) by reduction with sodium in ethanol.] The configuration of the 11-hydroxy group in (**11**) was assigned on the basis of n.m.r. data. The 11-proton has both peaks shape and



(**11**)



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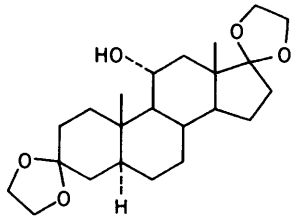


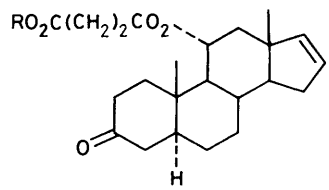
(**13**)

chemical shift typical of an 11β-proton in an 11α-alcohol,[7] and the additivity rules[8] for the resonance positions of the angular methyl groups, which are unaffected by removal of the iodine atom at C-17, confirm that the hydroxy group has the equatorial 11α-configuration (Table 1).

The (M − 18) ion was of low intensity (< 5%) in the mass spectrum of the alcohol (**11**), and its derivatives, indicating that the elements of water were not readily eliminated. This behaviour is typical of equatorial alcohols. Formation of the thermodynamically favoured equatorial alcohol is expected in this type of reduction, even in the case of severely hindered ketones.[9]

Hydrolysis of the acetal (**11**) occurred rapidly in boiling methanolic HCl to give the desired 3-ketone (**2**) in high yield. No migration of the olefinic bond was observed under these conditions. The alcohol (**2**) was readily oxidised to the diketone (**12**) by sodium dichromate in acetic acid. This diketone (**12**) was also obtained by chromic acid oxidation of the alcohol (**11**), with concomitant hydrolysis of the acetal function.

The alcohol (**2**) was converted into its hemisuccinate (**14**) by reaction with succinic anhydride in refluxing pyridine according



(**14**) R = H

(**15**) R = Me

to the method of Niswender,[10] as modified by Nambara.[11] Purification of the hemisuccinate was best achieved by Niswender's method[10] of dissolving in strong base and washing with organic solvent, followed by acidification and isolation

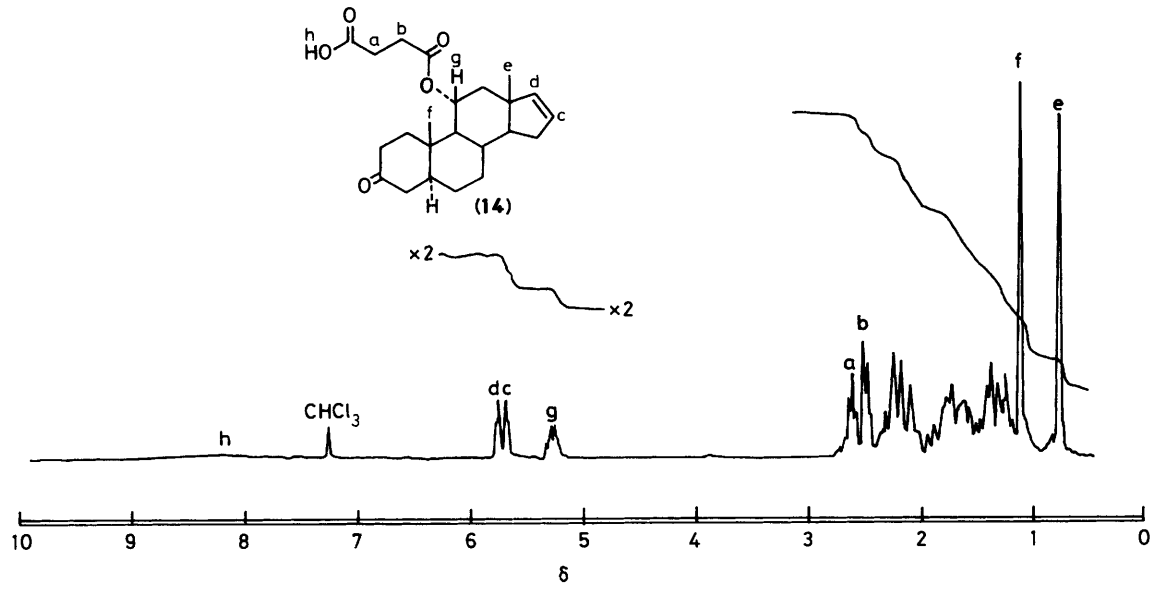


Figure 3. ^{1}H N.m.r. spectrum of compound (**14**)

Table. δ Values for angular methyl protons of (**11**)

Calculated	18-H	19-H
11α-OH	0.69	0.90
11β-OH	0.90	1.04
Found	0.69	0.92

with alkyl acetate. The methyl succinate (**15**) was prepared by reaction of the alcohol (**2**) with methyl succinoyl chloride, but, while this compound was easier to purify chromatographically than the hemisuccinate, it could not be selectively hydrolysed to the hemisuccinate.

The electron impact mass spectrum of the hemisuccinate (**14**) showed the base peak at *m/z* 270, formed by elimination of succinic acid. The intensity of the molecular ion at *m/z* 388 was less than 2% at 70 eV. Using chemical ionization the principal protonated ions appeared at *m/z* 389 (*M* + H), 289, 271 (100%), 119, and 101. The n.m.r. spectrum of the hemisuccinate (**14**) is reproduced as Figure 3, with partial assignment of protons.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 197 spectrophotometer, as KBr discs or neat films. N.m.r. spectra were determined on a Perkin-Elmer R34 spectrometer (220 MHz, ^{1}H) or a Varian CFT-20 spectrometer equipped with a Varian FT-80A dual frequency probe (80 MHz, FT, ^{13}C, or ^{1}H) for [^{2}H]chloroform solutions using TMS as internal standard. Mass spectra were determined on an AEI MS30 spectrometer operating at 70 eV.

G.c. analysis was performed on a Perkin-Elmer F-11 instrument equipped for dual column operations using glass columns (1 m × 3 mm i.d.) packed with 3% silicone OV-1 on Chromosorb W-HP (100—120 mesh) at 250 °C. Retention times are quoted relative to adrenosterone (R_t = 1.00). T.l.c. was performed on aluminium oxide or silica gel plates (Merck GF_{254}) with location by u.v. illumination, exposure to iodine vapour, or charring with 3% H_2SO_4 in methanol at 100 °C. Preparative h.p.l.c. was carried out on a Waters Prep 500A chromatograph using a single silica cartridge (30 cm × 5.7 cm diameter) with hexane and ethyl acetate as eluting solvents.

Solvents. Triethylamine was freshly redistilled (b.p. 88—92 °C). Pyridine was purified by refluxing over KOH, followed by distillation, and was stored over KOH. THF was freshly distilled from $LiAlH_4$. Acetone was purified by refluxing over $KMnO_4$, followed by distillation.

Reduction of Adrenosterone.—Reduction studies were carried out on 2 g samples of adrenosterone dissolved in ethyl acetate (100 ml) containing 5% Pd/C [Koch-Light Laboratories Ltd, catalyst grade; well washed with water and dried *in vacuo*]. Each mixture was hydrogenated at atmospheric pressure until uptake of the gas had ceased, and then filtered prior to g.c. analysis. 5α- and 5β-Androstane-3,11,17-trione had R_t 0.89 and 0.80, respectively:

Catalyst (mg; %)	5α-Isomer (%)	5β-Isomer (%)	α/β Ratio
28 (1.4)	84	16	5.25
53 (2.7)	79	16	4.94
102 (5.1)	76	21	3.62
153 (7.6)	74	24	3.08
199 (9.9)	73	24	3.04
205 (10.2)	73	24	3.04
208 (10.4)	72	25	2.88
213 (10.7)	72	24	3.00

Optimum conditions. A solution of adrenosterone (4 g) in ethyl acetate (100 ml) containing Pd/C (5%; 100 mg) was shaken under hydrogen for 2 h. After filtration, the crude product, which crystallised out after evaporation of most of the solvent, was recrystallised from hexane to give 5α-androstane-3,11,17-trione (2.4 g, 60%), m.p. 177—178.5 °C (lit.,[12] 176—178 °C). [Bowers and Denot[12] reported a 40% yield of this trione using Li/NH_3 reduction of adrenosterone followed by reoxidation with chromic acid and purification by chromatography over alumina.]

3,3-*Ethylenedioxy*-5α-*androstane*-11,17-*dione* (**5**).—A solution of 5α-androstane-3,11,17-trione (267 mg), ethylene glycol (55 mg), and toluene-*p*-sulphonic acid (23 mg) in benzene (25 ml), was refluxed under a water separator for 16 h. The solvent was removed under reduced pressure and the residual gum was

dissolved in ether (50 ml). The ethereal solution was washed with saturated aqueous $NaHCO_3$ (2 × 25 ml), then with water (20 ml), and dried ($MgSO_4$). The residue left after evaporation was purified by preparative t.l.c. on alumina, developing with hexane–ethyl acetate (3:1) containing a trace of Et_3N, and eluting the band R_F 0.7 with the same solvent mixture to give the 3-*ethylene acetal* as a colourless gum (175 mg, 56%) which was crystallised from ether–hexane to m.p. 175—177 °C (Found: M^+, 346.2126. $C_{21}H_{30}O_4$ requires M, 346.2144); $\nu_{max.}$(KBr) 1 740 and 1 702 cm^{-1}; δ_H 0.78 (s, 13-Me), 1.02 (s, 10-Me), and 3.94 (m, 3-acetal); m/z 346 (M^+, 11%), 331 (3), 289 (3), 125 (25), 100 (10), 99 (100), and 86 (12).

The zone R_F 0.85 yielded 3,3;17,17-bisethylenedioxy-5α-androstan-11-one (**7**) as colourless plates (35 mg, 10%) from ethanol, m.p. 166—168 °C (lit.,[13,14] 163—165 °C, 176—177 °C).

Optimum conditions. A 0.13M solution of 5α-androstane-3,11,17-trione in benzene, containing ethylene glycol (0.15 mol) and toluene-*p*-sulphonic acid (0.001 mol), was refluxed under a water separator for 2 h. The residues (R_f 14.1) left after evaporation of the solution to dryness under reduced pressure was used directly for the next stage.

3,3′-*Ethylenedioxy*-5α-*androstane*-11,17-*dione Hydrazone* (**8**).—A solution of the crude monoacetal (1.0 g) in ethanol (10 ml) was treated with triethylamine (2 ml) and hydrazine hydrate (5 ml) and the solution was heated under reflux for 2 h. The solution was poured into cold water (150 ml) and the mixture cooled in ice. The precipitated solid was collected and washed with ether (20 ml) to give the crude hydrazone (0.87 g, 84%), m.p. 200—204 °C; $\nu_{max.}$(KBr) 1 703 cm^{-1}; m/z 360 (M^+, 11%), 345 (8), 125 (32), and 99 (100).

3,3′-*Ethylenedioxy*-17-*iodo*-5α-*androst*-16-*en*-11-*one* (**9**).—A solution of the hydrazone [(**8**); 1.35 g] in THF (30 ml) and triethylamine (6 ml) was treated with iodine until a slight excess was present (cessation of nitrogen evolution; brown colour not discharged). The mixture was poured into water (250 ml). The buff precipitate (1.51 g, 88%) was collected and recrystallised from methanol containing triethylamine to give the colourless *vinyl iodide* (**9**), m.p. 162—164 °C (Found: M^+, 45.1150. $C_{21}H_{29}IO_3$ requires M, 456.1163); $\nu_{max.}$(KBr) 1 703, 1 091, 947, and 910 cm^{-1}; δ_H 0.63 (s, 13-Me), 1.00 (s, 10-Me), 3.91 (m, 3-acetal), and 6.18 (m, 16-olefinic H); m/z 456 (M^+, 6%), 329 (M − I, 2%), 125 (23), 99 (100), and 86 (10).

17-*Iodoandrost*-16-*ene*-3,11-*dione* (**10**).—A solution of the vinyl iodide [(**9**); 1.07 g] in methanol (20 ml) and concentrated HCl (1 ml) was boiled for 10 min, diluted with water until slightly turbid, and refrigerated. The resulting needles of the *dione* (**10**) (0.53 g, 54%) were collected, m.p. 238—240 °C (Found: M^+, 412.0906. $C_{19}H_{25}IO_2$ requires M, 412.0900); $\nu_{max.}$(KBr) 1 695 cm^{-1}; δ_H 0.66 (3, 13-Me), 1.18 (s, 10-Me), and 6.18 (m, 16-olefinic H); m/z 412 (M^+, 100%), 397 (93), 285 (22), 257 (21), 206 (22), 163 (73), 124 (58), 121 (33), 120(28), 109 (35), 107 (26), 105 (28), 93 (75), 91 (40), and 79 (76).

3,3′-*Ethylenedioxy*-5α-*androst*-16-*en*-11α-*ol* (**11**).—Sodium (10.0 g) was added in portions to a solution of the vinyl iodide [(**9**); 1.20 g] in ethanol (70 ml) containing triethylamine (0.5 ml) at a rate sufficient to maintain gentle boiling of the mixture, which was then heated under reflux with vigorous stirring until all the metal had dissolved. The solution was diluted with 50% aqueous ethanol (500 ml) and cooled in ice. The colourless precipitate was collected and recrystallised from methanol containing triethylamine to give *the alkene* (**11**) (0.72 g, 83%), m.p. 155.5—157 °C (Found: M^+, 332.2347. $C_{21}H_{32}O_3$ requires M, 332.2351); $\nu_{max.}$(KBr) 3 480, 1 140, 1 090, 1 030, 950, and 715 cm^{-1}; δ_H 0.69 (3, 13-Me), 0.92 (3, 10-Me), 3.84 (m, 3-acetal), 3.95 (m, 11β-H), and 5.68 (m, 16- and 17-olefinic H); m/z 332 (M^+, 7%), 231 (10), 125 (32), and 99 (100).

When the sequence was carried through from less pure monoacetal (**5**), it was necessary to purify the product by preparative h.p.l.c., eluting with hexane–ethyl acetate (1:1). First eluted was the alkene (**11**), followed by the *bisacetal* (**13**), m.p. 164—166 °C (from methanol containing triethylamine) (Found: M^+, 392.2598. $C_{23}H_{36}O_5$ requires M, 392.2562); $\nu_{max.}$ 3 370 cm^{-1}; δ_H 0.84 (s, 13-Me), 0.94 (s, 10-Me), 3.80 (m, 17-acetal), 3.92 (s, 3-acetal), and 3.84—4.02 (m, 11-H); m/z 392 (M^+, 2%), 125 (10), and 99 (100).

11α-*Hydroxy*-5α-*androst*-16-*en*-3-*one* (**2**).—A solution of the acetal (**11**) (566 mg) in methanol (15 ml) containing concentrated HCl (0.2 ml) was boiled under reflux for 10 min. Water (50 ml) was added and the solution was extracted with ether (3 × 20 ml). The combined extracts were washed with saturated aqueous $NaHCO_3$, then with water, and dried (Na_2SO_4). The colourless solid, m.p. 102—109 °C (477 mg, 97%) obtained upon evaporation of the ether was recrystallised from hexane to give the pure *title compound* (**2**), m.p. 108—109 °C (Found: M^+, 288.2089. $C_{19}H_{28}O_2$ requires M, 288.2092); $\nu_{max.}$(KBr) 3 430, 1 700, 1 588, 1 412, 1 050, 1 023, 746, and 717 cm^{-1}; δ_H 0.78 (s, 13-Me), 1.16 (s, 10-Me), 3.98—4.12 (m, 11β-H), 5.69 (m, 16-olefinic H), and 5.82 (m, 17-olefinic H); m/z 288 (M^+, 1%), 270 (100), 255 (43), 147 (37), 145 (33), 131 (25), 119 (20), 107 (50), and 93 (62).

11α-*Hydroxy*-3-*oxo*-5α-*androst*-16-*enyl Hemisuccinate* (**14**).—A solution of the alcohol (**2**) (0.963 g) and succinic anhydride (1.037 g) in dry pyridine (10 ml) was boiled for 4.5 h under reflux. Evaporation of the pyridine under reduced pressure gave a pale yellow gum, which was triturated with ether (50 ml). The insoluble anhydride was filtered off and the filtrate was washed with water (2 × 30 ml). The hemisuccinate was extracted with 5% aqueous NaOH (2 × 20 ml) and the combined aqueous extracts were washed with ether, acidified with concentrated HCl, and extracted with ethyl acetate (3 × 30 ml). The combined extracts were washed with water (2 × 20 ml), dried ($MgSO_4$), and evaporated under reduced pressure to give the hemisuccinate as a colourless gum (0.71 g, 55%), which could not be crystallised; δ_H 0.77 (s, 13-Me), 1.11 (s, 10-Me), 2.49 (m, HO_2CCH_2), 2.61 (m, $HO_2CCH_2CH_2CO_2$), 5.20—5.36 (m, 11β-H), 5.69 (m, 16-olefinic H), and 5.78 (m, 17-olefinic H); m/z 388 (M^+, 2%) 270 (100), and 255 (50).

Acknowledgements

We thank the Agricultural and Food Research Council for financial support and the Upjohn Co. for a gift of adrenosterone.

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Received 12*th May* 1987; *Paper* 7/834