PREPARATION OF UNLABELLED AND [^{3}H]-LABELLED EPITESTOSTERONE AND ITS METABOLITES*

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Cold as well as [^{3}H]-labelled substrates and metabolites *IX* – *XI*, *XV*, *XVI*, *XX* – *XXII*, *XXIV*, *XXV* and *XXVIII* were prepared by catalytic hydrogenation of epitestosterone (*VIII*) and Δ^1-dehydroepitestosterone (*XIII*). The key step in the preparation of compound *XXVIII* was reaction of 3β-tosylates *XXVI* and *XXX* with potassium nitrite in dimethyl sulfoxide.

Diseases of androgen-dependent tissues are recently treated only with cyproterone acetate[1], flutamide[2] and its hydroxy derivative[3], and anandrone[4]. Because these compounds are not completely free from side-effects, there is a continuing interest in other antiandrogens which could possibly substitute the above-mentioned compounds[5]. One such potential candidate is 17α-hydroxy-4-androsten-3-one (*VIII*, epitestosterone), a 17,20-lyase and 17-hydroxylase inhibitor[6]. As an endogenic antiandrogen[7], this compound is well accepted by the organism; moreover, its ability of inhibiting 5α-reductase[7] contributes by a concurrent mechanism to its overall antiandrogenic effect[8,9].

In order to be able to follow the fate of this compound in the body, as well as the activity and functions of its metabolites, we decided first to prepare the compound and its metabolites both in the cold and radioactive forms.

Starting from the commercially available 17-ketone *I*, we prepared the known[10] 17β-hydroxy derivative *II* the tosylate of which (*III*, ref.[11]) was solvolyzed with sodium nitrite in dimethyl sulfoxide[12,13]. Beside the desired 17α-hydroxy derivative *IV* we also isolated products of elimination, the 17-ketone *I* and the corresponding 16-olefin. The alcohol *IV* was benzoylated to give compound *V* (ref.[14]), obtained previously by fract-

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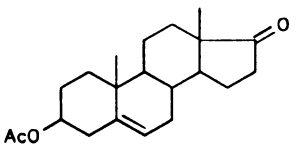
ional crystallization of product of nonstereospecific catalytic hydrogenation of 17-keto derivatives. After hydrolysis of the acetate *V*, we obtained alcohol *VI* (ref.[14]). This compound had been oxidized by Ruzicka with chromium trioxide after protection of the double bond by bromine; we oxidized the compound to epitestosterone benzoate *VII* (ref.[14]) using the Oppenauer method. Most of these compounds had been prepared by other procedures so long ago that not only characteristic NMR parameters but often also specific rotation values and sometimes even the melting points are not given. At that time an "archaic" steroid nomenclature had been used (e.g. the 17α-alcohols were named[14] "17-cis-hydroxy derivatives") and later nomenclature changes and graphical distinction of the α- and β-bonds introduced many errors that persisted in the literature: thus, e.g. under the name and formula of 17α-hydroxy and 17α-acyloxy derivatives the properties of the corresponding 17β-epimers were described and vice versa (e.g., in Chemical Abstracts (ref.[15]) in 1949 and in Pouvoir Rotatoir[16] in 1965). Therefore, we give the ^{1}H NMR spectra not only for the new compounds but also for all unlabelled compounds of the whole series *I* – *XXX* (see Table I).

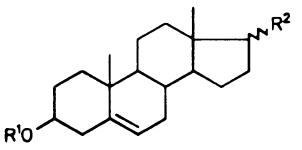
Since we intended to perform the labelling by catalytic tritiation, we first dehydrogenated epitestosterone benzoate (*VII*, see Scheme 1) with DDQ to give dienone *XII* in which the protecting ester group was removed by hydrolysis under formation of compound *XIII*.

The selective catalytic tritiation of dienone *XIII* in the presence of tris(triphenylphosphine)rhodium(I) chloride[16,17] took place preferentially at the Δ^1-double bond leading thus to 1α,2α-labelled epitestosterone (*IX*) in the yield of 51%.

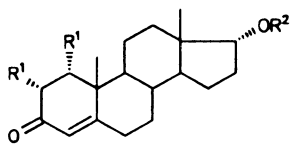
As the first of the metabolites of epitestosterone[18] we prepared its 5α-dihydro derivative *XV*. In the cold form it was prepared by hydrogenation of epitestosterone (*VIII*) on palladium catalyst, but unfortunately only as a minor component, the 5β-isomer *XXIV* arising as the principal product. Also hydrogenation of benzoate *VII* on platinum in acetic acid afforded predominantly the 5β-derivative (in this case the 3α-hydroxy derivative *XVII*) in addition to the minor isomer of the 5α-series (*XVIII*). The predominant formation of 5β-dihydro derivatives in the catalytic hydrogenation of Δ^4-3-ketones containing the 17α-hydroxyl group, particularly in an alkaline medium, had been observed already earlier[19,20] but no similar effect of 17α-benzoyloxy group in this reaction had been described. An almost exclusive formation of compounds of the 5α-series is usually guaranteed by catalytic hydrogenation of Δ^5-olefins, however, in the hydrogenation of compound *VI* the predominance of the 5α-product was not so marked. Nevertheless, the optimal approach to dihydroepitestosterone (*XV*) included hydrogenation of Δ^5-unsaturated benzoate *VI* on platinum which led predominantly to 17α-cyclohexanecarboxylate *XVIII* (ref.[21]); the hydrogenation was followed by oxidation to ketone *XIV* and then hydrolysis. For obtaining the radioactive form of dihydroepitestosterone (*XVI*) we have chosen the palladium-catalyzed reduction of dienone *XIII* with tritium[22], a low-yield but one-step method. At the same time, we also obtained the



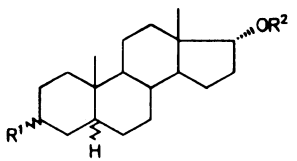
I



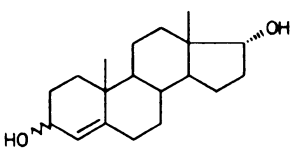
II, R^1 = Ac; R^2 = β–OH
III, R^1 = Ac; R^2 = β–OTos
IV, R^1 = Ac; R^2 = α–OH
V, R^1 = Ac; R^2 = α–OBz
VI, R^1 = H; R^2 = α–OBz



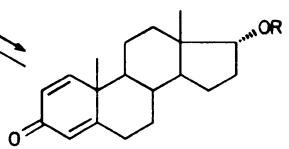
VII, R^1 = H; R^2 = Bz
VIII, R^1 = R^2 = H
IX, R^1 = ^{3}H; R^2 = H



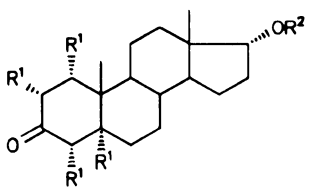
XVII, 5β–H; R^1 = α–OH; R^2 = $C_6H_{11}CO$
XVIII, 5α–H; R^1 = β–OH; R^2 = $C_6H_{11}CO$
XIX, 5β–H; R^1 = β–OH; R^2 = $C_6H_{11}CO$
XX, 5α–H; R^1 = β–OH; R^2 = H
XXI, 5β–H; R^1 = β–OH; R^2 = H
XXII, 5β–H; R^1 = α–OH, R^2 = H



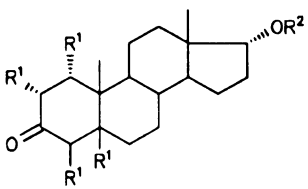
X, α–OH
XI, β–OH



XII, R = Bz
XIII, R = H



XIV, R^1 = H; R^2 = $C_6H_{11}CO$
XV, R^1 = R^2 = H
XVI, R^1 = ^{3}H; R^2 = H



XXIII, R^1 = H; R^2 = $C_6H_{11}CO$
XXIV, R^1 = R^2 = H
XXV, R^1 = ^{3}H; R^2 = H

SCHEME 1

5β-isomer *XXV* (its cold analog *XXIV* is reported as the principal metabolite of epitestosterone[23,24]).

Catalytic hydrogenation of the 3-ketone *XXIV* afforded in one step both completely saturated metabolites[24] of the 5β-series, diols *XXII* and *XXI*.

The corresponding 4-unsaturated 3β- and 3α-hydroxy derivatives *X* and *XI* are supposed to be epitestosterone metabolites which, although mostly reduced further under physiological conditions, have been found[24] in the product of reduction of

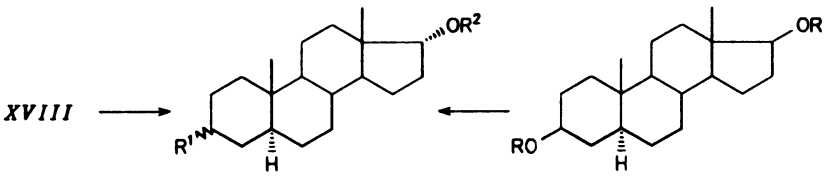
TABLE I
Characteristic parameters of ^{1}H NMR spectra (δ, ppm; *J*, Hz)

Compound	H-18[a]	H-19[a]	H-3	H-17	Other signals
I	0.89	1.05	4.61[b]	–	5.41[c]
II	0.76	1.04	4.61[b]	3.66[d]	2.04[a], 5.37[c]
III	0.81	1.00	4.57[b]	4.26[d]	2.03[a], 2.45[a], 5.33[c], 7.32[e], 7.78[e]
IV	0.68	1.03	4.61[b]	3.75[f]	2.04[a], 5.39[c]
V	0.84	1.04	4.60[b]	5.06[f]	2.04[a], 5.41[c], 7.47[e], 8.04[e]
VI	0.84	1.03	3.53[b]	5.06[f]	5.38[c], 7.50[e], 8.03[e]
VII	0.89	1.22	–	5.05[f]	5.75[g], 7.48[e], 8.04[e]
VIII	0.71	1.19	–	3.76[f]	5.73[g]
X	0.69	1.00	4.10[i]	3.77[f]	5.51[h]
XI	0.68	1.06	4.14[k]	3.74[f]	5.28[j]
XII	0.90	1.25	–	5.10[f]	6.09[l], 6.21[m], 7.05[n], 7.43[e], 7.99[e]
XIII	0.74	1.24	–	3.76[f]	6.07[l], 6.23[m], 7.07[n]
XIV	0.76	1.02	–	4.79[f]	
XV	0.68	1.02	–	3.74[f]	
XVII	0.72	0.93	3.67[b]	4.76[f]	
XVIII	0.73	0.81	3.59[b]	4.77[f]	
XIX	0.73	0.97	4.13[o]	4.77[f]	
XX	0.66	0.82	3.58[b]	3.71[f]	
XXI	0.65	0.97	4.11[o]	3.73[f]	
XXII	0.65	0.93	3.63[b]	3.73[f]	
XXIII	0.76	1.03	–	4.77[f]	
XXIV	0.68	1.03	–	3.76[f]	
XXVI	0.71	0.78	4.42[b]	4.77[f]	2.44[a], 7.33[e], 7.79[e]
XXVII	0.73	0.79	4.04[o]	4.77[f]	
XXVIII	0.65	0.78	4.04[o]	3.72[f]	
XXIX	0.73	0.82	3.60[p]	3.60[p]	
XXX	0.78	0.76	4.39[b]	4.22[d]	2.44[a], 7.34[e], 7.76[e]

[a] s, methyl group. [b] m, $W_{1/2}$ = 38. [c] d, *J* = 4.88, H-6. [d] dd, *J* = *J'* = 8, H-17. [e] Aromatic protons. [f] d, *J* = 5.5. [g] m, $W_{1/2}$ = 3.3, H-4. [h] d, *J* = 3, H-4. [i] m, $W_{1/2}$ = 5. [j] s, $W_{1/2}$ = 2, H-4. [k] m, $W_{1/2}$ = 8. [l] bs, H-4. [m] dd, *J* = 10, *J'* = 2.4, H-2. [n] d, *J* = 10, H-1. [o] m, $W_{1/2}$ = 15. [p] Overlapping signals.

epitestosterone with NADH, catalyzed with enzymatic systems from rat or human liver microsomal preparations. We prepared both compounds from ketone *VIII* by reduction with sodium borohydride[25] in the presence of cerium chloride[26].

More common metabolites[27] of epitestosterone are 3ξ,17α-diols *XX* and *XXVIII*. The



XXVI, R^1 = β–OTos; R^2 = $C_6H_{11}CO$

XXVII, R^1 = α–OH; R^2 = $C_6H_{11}CO$

XXVIII, R^1 = α–OH; R^2 = H

XXIX, R = H

XXX, R = Tos

SCHEME 2

3β,17α-diol *XX* was prepared long time ago by Ruzicka[14] who hydrogenated the corresponding Δ^5-unsaturated diol. We obtained this compound by hydrolysis of diester *XVIII*. In a rather nonspecific way, the derivative *XX* was also obtained by reduction of epitestosterone benzoate (*VII*) with sodium in alcohol[28]. The 3α,17α-diol *XXVIII* was first prepared[21] by catalytic hydrogenation of 5α-dihydroepitestosterone (*XV*) in the presence of hydrobromic acid (at that time, it was named "3-trans,17-cis-diol" but the C(17α)–OH bond was drawn as a full line, the broken line being used for the "17-trans", i.e. 17β-alcohols). We prepared this diol from compound *XVIII*, in which the 3β-hydroxyl group was inverted using the above-mentioned solvolysis of 3β-tosyloxy derivative *XXVI* with sodium nitrite[13] in DMSO (see Scheme 2); the subsequent removal of the ester functionality in compound *XXVII* was accomplished by reaction with lithium aluminium hydride under formation of diol *XXVIII*. An unequivocal proof of its structure, and thus of all other products of this series, was obtained by correlation of this compound with a sample prepared from an authentic[29] 5α-androstane-3β,17β-diol (*XXIX*). The corresponding 3β,17β-ditosylate *XXX* reacted with sodium nitrite in dimethyl sulfoxide to give a product of double inversion that was identical with compound *XXVIII*: the 5α-configuration is given by the structure of the starting compound, the configuration in positions 3 and 17 follows from the NMR spectra (see Table I).

Preliminary results show[30] that all the major metabolites of epitestosterone really lack androgenic as well as antiandrogenic activity; of the compounds prepared only *VIII*, *IX* and *XIII* are bound to androgenic receptors. This finding agrees with the pre-

viously formulated hypothesis that if a structurally modified androgens should be antiandrogenically active, the substantial structural change should be concentrated at only one end of the molecule. The inhibition of 5α-reductase by the compounds prepared, as well as the results of study of the metabolism proper, will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a Boetius block. Unless stated otherwise, optical rotations and IR spectra were measured in chloroform and ^{1}H NMR spectra in deuteriochloroform solutions on Bruker IFS 88 (FT-IR) and Varian XL-200 (FT-mode, 200.058 MHz for ^{1}H, internal standard tetramethylsilane) instruments. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and halfwidths ($W_{1/2}$) in Hz. All values were obtained by first-order analysis. Column chromatography was performed on silica gel (60 – 120 μm), thin-layer chromatography on silica gel G (Woelm, layers 200 × 200 × 0.7 mm). Solutions of the compounds in organic solvents were dried over sodium sulfate, the solvents were evaporated on a rotatory evaporator in vacuo (at about 3 kPa). The identity of samples prepared by different routes was checked by TLC, comparison of their IR and ^{1}H NMR spectra and by mixture melting points. The radiochemical purity of the labelled compounds was checked by measurement of thin-layer chromatograms (TLC-scanner Berthold) and by a high-pressure chromatography isotope detector (Beckmann 171). The specific activity of the labelled substrates was assayed by measurement of aliquotes of solutions whose concentration was determined: (i) by UV spectroscopy (compound *IX*, methanol, 240 nm) on a Specord UV VIS instrument and (ii) by integration of an HPLC chromatogram (refractometric detector) (compounds *XVI* and *XXV*, Waters 410).

The checking of purity and preparative purification of the labelled compounds by thin-layer chromatography was carried out on silica gel (Merck) in systems S1 (ether), S2 (benzene–ethyl acetate 7 : 3), S3 (benzene–acetone 3 : 1), S4 (benzene–ether 2 : 1).

17β-Hydroxyandrost-5-en-3β-yl Acetate (*II*)

Sodium borohydride (4.0 g, 48.8 mmol) was added during 15 min at 0 °C to a stirred solution of ketone *I* (40.0 g, 121 mmol) in a mixture of methanol (400 ml), ethyl acetate (100 ml) and dichloromethane (120 ml). After the addition, the cooling bath was removed. After further 60 min, the reduction was completed as shown by TLC (silica gel, 30% ether in benzene). Acetic acid (25 ml) was added dropwise and the solution was concentrated in vacuo on a rotatory evaporator to one quarter of the original volume. The product was taken up in ethyl acetate, the extract was washed with potassium hydrogen carbonate solution, water, and dried by filtration through a layer of sodium sulfate. The product (40.0 g, 99%) was crystallized from acetone, m.p. 145 – 147 °C (ref.[10], m.p. 143 – 144 °C).

Androst-5-ene-3β,17β-diyl 3-Acetate 17-*p*-Toluenesulfonate (*III*)

p-Toluenesulfonyl chloride (9.0 g, 47.2 mmol) was added to a solution of azeotropically dried hydroxy derivative *II* (10.0 g, 30.1 mmol) in pyridine (40 ml). After standing at 30 °C for 72 h, the mixture was poured into a stirred mixture of ice and water and the separated product was taken up in ethyl acetate. The extract was washed with dilute hydrochloric acid, water, potassium hydrogen carbonate solution, and dried over sodium sulfate. The residue (10.0 g, 68%) was crystallized from dichloromethane (10 ml) and methanol (30 ml); m.p. 162 – 164 °C (7.5 g), $[\alpha]_D$ –59° (c 1.7) (ref.[11] reports the preparation but gives neither the m.p. nor $[\alpha]_D$). IR spectrum (CCl_4): 3 034, 1 600, 1 496, 1 306, 1 290, 1 189, 1 099, 1 011, 827

(aromatic ring); 1 734, 1 245, 1 031 (acetate); 1 345, 1 178, 973 (*p*-toluenesulfonate). For $C_{28}H_{38}O_5S$ (486.7) calculated: 69.10% C, 7.87% H; found: 68.85% C, 7.96% H.

17α-Hydroxyandrost-5-en-3β-yl 3-Acetate (*IV*)

A mixture of tosylate *III* (14.5 g, 29.8 mmol), sodium nitrite (31.0 g, 449 mmol) and dimethyl sulfoxide (220 ml) was heated at 135 °C for 1 h under stirring. After cooling, the mixture was poured into a solution of sodium chloride, the product was taken up in ethyl acetate and the extract was washed with water and dried.

The solvent was evaporated and the product chromatographed on silica gel (700 ml) in ether–toluene (1 : 20). Successively were eluted: androsta-5,16-dien-3β-yl acetate (2.44 g, 26%), ketone *I* (1.69 g, 17%) and finally 17α-alcohol *IV* (4.36 g, 44%), m.p. 110 – 115 °C. For $C_{21}H_{32}O_3$ (332.5) calculated: 75.86% C, 9.70% H; found: 75.62% C, 9.82% H.

Androst-5-ene-3β,17α-diyl 3-Acetate 17-Benzoate (*V*)

Hydroxy derivative *IV* (17.2 g, 51.7 mmol) was benzoylated with benzoyl chloride (20.0 ml, 172 mmol) in pyridine (25 ml) at room temperature. After standing for 20 h, the solution was poured in water, the product was taken up in ether, the ethereal layer washed with 5% hydrochloric acid, water, potassium hydrogen carbonate solution, again water, and dried over sodium sulfate. The product (14.5 g, 64%) was crystallized from methanol, m.p. 130 – 132 °C (refs[31,32], m.p. 130 – 131 °C and 133 – 134 °C, respectively); $[\alpha]_D$ −112° (*c* 2).

3β-Hydroxyandrost-5-en-17α-yl Benzoate (*VI*)

Concentrated hydrochloric acid (2 ml) was added to a solution of acetate *V* (2.2 g, 5.04 mmol) in a mixture of chloroform (10 ml) and methanol (100 ml). After standing at room temperature for 64 h, the mixture was diluted with toluene (100 ml) and concentrated in vacuo to half of the original volume. The solution was washed with water, dried over sodium sulfate, the solvents were evaporated and the obtained product *VI* (1.79 g, 90%) was directly used in the next step.

3-Oxoandrost-4-en-17α-yl Benzoate (*VII*)

A solution of 3-hydroxy derivative *VI* (10.0 g, 25.3 mmol) in toluene (150 ml) was dried by distilling off a part (25 ml) of the solvent. Cyclohexanone (25 ml) was then added and a solution of aluminium isopropoxide (4.0 g) in toluene (about 40 ml) was added dropwise to the gently boiling solution. About 75 ml of distillate was collected during 45 min. After cooling, the mixture was diluted with ether, washed with 5% hydrochloric acid, water, potassium hydrogen carbonate solution and again water. The volatile material was steam-distilled, the steroidal product was extracted with chloroform, dried by filtration through a layer of sodium sulfate and the solvents were evaporated. The residue was purified by chromatography on a column of silica gel in ether–benzene (1 : 50), yield 8.9 g (89%) of benzoate *VII*. After crystallization from acetone and heptane, the product (7.5 g) melted at 138 – 139 °C (ref.[11], m.p. 136.5 – 138 °C).

17α-Hydroxyandrost-4-en-3-one (*VIII*)

The title compound was prepared by heating the benzoate *VII* (230 mg, 0.59 mmol) with 5% methanolic potassium hydroxide (7 ml) at 65 °C in an argon atmosphere. After 4 h, the mixture was concentrated in vacuo to half of the original volume, the product was precipitated by addition of saturated sodium chloride solution and taken up in chloroform. The chloroform extract was washed with water, dried, the solvent was

evaporated and the product crystallized from acetone and heptane, m.p. 220 – 221 °C (ref.[14], m.p. 220 – 221 °C); yield 136 mg (80%).

[1,2-^{3}H]17α-Hydroxy-4-androsten-3-one (*IX*)

A mixture of dienone *XIII* (3.9 mg, 13.5 mmol), tris(triphenylphosphine)rhodium chloride (4.5 mg, 4.7 mmol) and anhydrous ethyl acetate (0.4 ml) was stirred for 3 h in an atmosphere of tritium (50 – 70%) at 0.075 MPa pressure. After removal of labile radioactivity, the purity of the crude product was 89 ± 1% (TLC, in S1, S2 and S3).

The product was purified: (i) by preparative thin-layer chromatography successively in systems S1 and S2 (2 ×) and after elution with methanol (ii) by preparative HPLC on a reversed phase (C18, methanol–water 7 : 3).

Radiochemical purity of the product was 98% (TLC), 99% (HPLC), specific activity 41 Ci/mmol, total yield 278 mCi (51%).

3-Oxoandrosta-1,4-dien-17α-yl Benzoate (*XII*)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (890 mg, 3.92 mmol) was added to a solution of ketone *VII* (856 mg, 2.18 mmol) in benzene (17 ml) and the stirred mixture was refluxed for 8 h. After dilution with benzene (15 ml), the mixture was chromatographed on a silica gel column (50 g) in benzene–ether (96 : 4). The eluted product was dissolved in benzene–ether (1 : 1) and filtered through a layer of alumina (10 g). Yield 566 mg (66%) of compound *XII*, m.p. 177 – 180 °C (light petroleum–ether), $[\alpha]_D$ −26° (*c* 0.3). For $C_{26}H_{30}O_3$ (390.5) calculated: 79.97% C, 7.74% H; found: 80.23% C, 7.54% H.

17α-Hydroxyandrosta-1,4-dien-3-one (*XIII*)

Methanolic potassium hydroxide (2%, 20 ml) was added to a solution of benzoate *XII* (433 mg, 1.11 mmol) in methanol (20 ml). After heating to 60 °C for 2 h, the solvents were evaporated in vacuo, the residue was partitioned between dichloromethane and water and the aqueous phase was extracted with dichloromethane. The combined organic phases were washed with water, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed on 5 preparative plates of silica gel in benzene–ether (1 : 1), detection with UV light (254 nm). The main zone afforded 276 mg (87%) of compound *XIII*, m.p. 180 – 183 °C (ether), $[\alpha]_D$ −8° (*c* 0.8). IR spectrum: 3 616, 3 447 (O–H); 1 620, 1 602 (dienone); 1 048 (C–O). Mass spectrum, *m/z*: 286 (M^+); 268 (M − H_2O). For $C_{19}H_{26}O_3$ (286.5) calculated: 79.68% C, 9.15% H; found: 79.92% C, 9.00% H.

3-Oxo-5α-androstan-17α-yl Cyclohexanecarboxylate (*XIV*)

Compound *XVIII* (200 mg, 0.50 mmol) was oxidized with Jones reagent in acetone. The usual work-up procedure afforded 185 mg (93%) of ketone *XIV*, m.p. 136 – 138 °C (ethanol) (ref.[21], m.p. 137.5 – 138 °C), $[\alpha]_D$ +27° (*c* 1.1) (ref.[33], +25°).

17α-Hydroxy-5α-androstan-3-one (*XV*)

A) By hydrolysis of ester XIV. Ester *XIV* (180 mg, 0.45 mmol) was heated to the boil with a solution of potassium hydroxide in methanol (5%, 4 ml) in argon atmosphere. After 2 h, the solution was acidified with hydrochloric acid (5%, 1.5 ml) and concentrated in vacuo to a half. The product was precipitated with water, extracted into ether, the extract was washed with aqueous potassium hydrogen carbonate solution and water, dried over sodium sulfate, and the solvent was evaporated to dryness. The product (98 mg,

75%) was crystallized from acetone, m.p. 178 – 180 °C (ref.[21], m.p. 179.5 – 180 °C), [α] +12° (*c* 1.1). CD spectrum: $\Delta\varepsilon_{289}$: +1.48 (ethanol).

B) *By hydrogenation of ketone* VIII. A solution of epitestosterone (*VIII*, 180 mg, 0.62 mmol) in tetrahydrofuran (5 ml) was hydrogenated on palladium on calcium carbonate (5%, 150 mg) for 2 h. The catalyst was filtered off, washed with acetone and the solvent was evaporated. The residue was subjected to preparative thin-layer chromatography (5 plates, twofold elution with toluene–ether 1 : 1); the 5α-ketone *XV* (20 mg, 11%), m.p. 176 – 179 °C, identical with the sample prepared by procedure *A*, represented only a minor lipophilic component, the principal isomer being the 5β-ketone *XXIV* (120 mg, 66%).

[1,2,4,5-^{3}H]17α-Hydroxy-5α-androstan-3-one (*XVI*)

A mixture of dienone *XIII* (2.0 mg, 7 μmol) and palladium on barium sulfate (10%, 4.5 mg) in dry methanol (0.2 ml) was stirred in tritium (50 – 70%) atmosphere at 0.075 MPa for 2 h. After removal of labile radioactivity, the reaction mixture consisted of compound *XVI* (14%) and compound *XXV* (73%) (TLC in S2 (3 ×) or S4 (4 ×)). The products were separated by preparative thin-layer chromatography in S2 (fourfold developing). Elution of the polar zone with methanol afforded compound *XVI*; radiochemical purity 96% (TLC and HPLC), specific activity about 52 Ci/mmol, total yield 35 mCi (9.6%). After purification by HPLC (reversed phase C18; methanol–water 7 : 3), the product *XVI* was obtained in radiochemical purity 99% (TLC and HPLC).

[1,2,4,5-^{3}H]17α-Hydroxy-5β-androstan-3-one (*XXV*)

Elution of the nonpolar zone from the chromatography in the preceding experiment afforded the 5β-isomer *XXV*; radiochemical purity 99% (TLC and HPLC), specific activity about 52 Ci/mmol, total yield 190 mCi (52%).

3β-Hydroxy-5α-androstan-17α-yl Cyclohexanecarboxylate (*XVIII*)

A) *From hydroxy derivative* VI. A solution of compound *VI* (1.7 g, 4.31 mmol) in acetic acid (20 ml) was hydrogenated on Adams platinum catalyst (200 mg) at room temperature for 2 h. The hydrogen was then replaced with nitrogen, the catalyst was removed by filtration and washed with methanol. The filtrate was evaporated to dryness in vacuo and the remaining mixture of products was separated on a column of silica gel in ether–toluene (1 : 10). The lipophilic product (430 mg, 25%) consisted of the 5β-isomer *XIX*, m.p. 93 – 95 °C (acetone), $[\alpha]_D$ 0° (*c* 1.0). IR spectrum: 3 616, 1 030 (O–H); 1 716, 1 250, 1 175 (COO). For $C_{26}H_{42}O_3$ (402.6) calculated: 77.56% C, 10.52% H; found: 77.39% C, 10.60% H.

The more polar major product consisted of the 5α-isomer *XVIII* (1 230 mg, 71%), m.p. 207 – 209 °C (ethanol, ref.[21], m.p. 208.5 – 209.5 °C). $[\alpha]_D$ −14° (*c* 1.9). IR spectrum: 3 610, 1 034, 1 027 (O–H); 1 716, 1 250, 1 176 (COO).

B) *From ketone* VII. A solution of compound *VII* (288 mg, 0.73 mmol) in acetic acid (5 ml) was hydrogenated on Adams catalyst (70 mg) at room temperature. After 2 h, the catalyst was filtered off, washed with acetone and the solvent was evaporated. The residue was subjected to preparative thin-layer chromatography (5 plates) to give 214 mg (72%) of 5β-isomer *XVII* as the principal product. The desired 5α-isomer *XVIII* represented only a minor component (18 mg, 6%).

5α-Androstane-3β,17α-diol (*XX*)

A) *By hydrolysis of ester* XVIII. A mixture of compound *XVIII* (200 mg, 0.50 mmol) and methanolic potassium hydroxide (5%, 4 ml) was refluxed for 2.5 h. The solution was then acidified with 5% hydrochloric acid (1.5 ml) and concentrated to a half in vacuo. The product was precipitated with sodium

chloride solution and extracted with ether. The extract was washed with water, dried over sodium sulfate and the solvent was evaporated to dryness. The product (115 mg, 78%) was crystallized from acetone, m.p. 211 – 213 °C (ref.[14], m.p. 213.5 – 214.5 °C), $[\alpha]_D$ –10° (*c* 0.9).

B) *By reduction of ketone* VIII. Sodium (2.0 g) was added in portions to a boiling solution of ketone *VIII* (4.0 g, 10.2 mmol) in amyl alcohol (200 ml). After the metal had been dissolved (about 40 min), the solution was neutralized with dilute hydrochloric acid and then concentrated to a quater of the original volume. The product was precipitated by addition of brine, filtered, and purified by thin-layer chromatography on silica gel (10 plates) in toluene–ether (3 : 1). The desired diol *XX* (221 mg, 7%) formed the most polar fraction.

5β-Androstane-3α,17α-diol (*XXII*)

3-Ketone *XXIV* (118 mg, 0.41 mmol) was hydrogenated in acetic acid (6 ml) on a platinum catalyst (50 mg) for 4 h. The catalyst was removed by filtration, the filtrate was concentrated in vacuo and the residue crystallized from acetone to give *XXII* as the main product (46 mg, 39%), m.p. 230 – 233 °C (ref.[35], m.p. 232 – 234 °C). The mother liquors were chromatographed on two thin layers of silica gel in benzene–chloroform–ether (twofold developing) to give further 24 mg (20%) of compound *XXII*.

5β-Androstane-3β,17α-diol (*XXI*)

A) *From ester* XIX. Compound *XIX* (168 mg, 0.57 mmol) was hydrolyzed as described for the preparation of compound *XV* to afford the diol *XXI* (87 mg, 70%), which after crystallization from acetone melted at 209 – 211 °C (sublimation) (ref.[35], m.p. 213.5 °C).

B) *By hydrogenation of ketone* XXIV. The side-product zone from the preceding experiment afforded on elution with ethyl acetate 23 mg of compound *XXI* (20%), identical with the sample prepared by procedure *A*.

3-Oxo-5β-androstan-17α-yl Cyclohexanecarboxylate (*XXIII*)

Compound *XIX* (120 mg, 0.30 mmol) in acetone was oxidized with Jones reagent. The usual work-up procedure gave 105 mg (88%) of ketone *XXIII*, $[\alpha]_D$ –5° (*c* 1.0). IR spectrum: 1 709 (C=O); 1 709, 1 249, 1 135 and 1 016 (COO). For $C_{26}H_{40}O_3$ (400.6) calculated: 77.95% C, 10.07% H; found: 77.60% C, 10.21% H.

17α-Hydroxy-5β-androstan-3-one (*XXIV*)

A) A mixture of ester *XXIII* (93 mg, 0.23 mmol) and 5% methanolic potassium hydroxide (4 ml) was boiled under argon for 2 h. After addition of 5% hydrochloric acid (1.5 ml), the solution was concentrated in vacuo to a half. The product was precipitated with water, taken up in ether, the extract was washed with water, dried over sodium sulfate and the solvent was evaporated to dryness. The product (60 mg, 89%) was crystallized from acetone, m.p. 158 – 159 °C, $[\alpha]_D$ +4.8° (*c* 1.1) (ref.[34], m.p. 159 – 161 °C and $[\alpha]_D$ 5.4° and 2.3°). IR spectrum: 1 707 (C=O); 3 617, 1 027 (O–H). CD spectrum: $\Delta\varepsilon_{288.5}$ –0.54 (ethanol). For $C_{19}H_{30}O_2$ (290.4) calculated: 78.57% C, 10.41% H; found: 78.39% C, 10.57% H.

B) The principal, and more polar, product of hydrogenation of ketone *VIII* (see preparation of *XV*) was obtained in 66% yield (120 mg), m.p. 158 – 159 °C, was identical with the ketone[34] *XXIV* prepared by procedure *A*.

5α-Androstane-3β,17α-diyl 3-*p*-Toluenesulfonate 17-Cyclohexanecarboxylate (*XXVI*)

p-Toluenesulfonyl chloride (200 mg, 1.05 mmol) was added to a solution of hydroxy ester *XVIII* (150 mg, 0.37 mmol) in pyridine (1.5 ml) and the mixture was set aside at 30 °C. After 20 h, the mixture was

poured on ice, the separated product was filtered, dissolved in chloroform and subjected to TLC on silica gel (4 plates) in toluene–ether (10 : 1). The main product *XXVI* (148 mg, 71%) had m.p. 107 – 114 °C (methanol), $[\alpha]_D$ –19° (*c* 1.1). IR spectrum: 1 718, 1 175 (COO), 1 600, 1 496 (arom.); 1 360, 1 188 (SO_2).

3α-Hydroxy-5α-androstan-17α-yl Cyclohexanecarboxylate (*XXVII*)

A mixture of tosylate *XXVI* (210 mg, 0.38 mmol) and sodium nitrite (1.5 g, 21.7 mmol) was heated in dimethyl sulfoxide (15 ml) at 130 °C. After 2 h, the mixture was concentrated in vacuo, diluted with sodium chloride solution and the precipitated product was taken up in chloroform. The extract was washed with water, the chloroform was evaporated and the residue purified by thin-layer chromatography on silica gel (4 plates) in toluene–ether (10 : 1). The main product (130 mg, 86%) melted at 59 – 64 °C (methanol), $[\alpha]_D$ –16° (*c* 1.1). IR spectrum: 3 625, 1 037, 1 016 (O–H); 1 728, 1 171 (COO). For $C_{26}H_{42}O_3$ (402.6) calculated: 77.56% C, 10.51% H; found: 77.93% C, 10.13% H.

5α-Androstane-3α,17α-diol (*XXVIII*)

A) Ester *XXVII* (120 mg, 0.30 mmol) was refluxed with a solution of lithium aluminium hydride (150 mg) in tetrahydrofuran (4 ml) for 1 h. The excess reagent was destroyed with a few drops of water, the mixture was saturated with sodium sulfate and the inorganic material was filtered off and washed with hot chloroform. The product (64 mg, 73%) was crystallized from ethyl acetate, m.p. 225 – 227 °C (ref.[21], m.p. 227 – 228 °C), $[\alpha]_D$ –12° (*c* 1.0, chloroform–methanol 1 : 1). IR spectrum: 3 614, 1 074, 1 003 (O–H).

B) A stirred mixture of ditosylate *XXX* (100 mg, 0.17 mmol), sodium nitrite (500 mg, 7.2 mmol) and dimethyl sulfoxide (4 ml) was heated at 90 °C. After 29 h, the mixture was diluted with ammonium carbonate solution, the product (60 mg) was extracted with chloroform and purified by thin-layer chromatography on silica gel (2 plates) in benzene–ether (1 : 1). The main product (19 mg, 29%) was identical with the compound prepared by procedure *A*. The lipophilic impurity consisted of the known[36] 3α-hydroxy-5α-androstan-17-one (14 mg, 29%) and 5α-androst-16-en-3α-ol (ref.[37]) (14 mg, 31%), both identical (IR spectra) with authentic specimens.

5α-Androstane-3β,17β-diol Di-*p*-toluenesulfonate (*XXX*)

A mixture of 3β,17β-diol *XXIX* (ref.[29], 7.0 g, 23.9 mmol) and *p*-toluenesulfonyl chloride (9.5 g, 49.8 mmol) in pyridine (24 ml) was allowed to stand at 28 °C for 48 h. The mixture was poured in water–ice mixture, the precipitated product filtered, dissolved in chloroform, and the solution washed with water and dried over sodium sulfate. After evaporation of the solvent, the product was crystallized from ether and methanol, yield 12.3 g (87%), m.p. 120 – 122 °C, decomposition point 137 – 142 °C, $[\alpha]_D$ –15° (*c* 1.1). IR spectrum: 1 600, 1 496 (aromatic system); 1 358, 1 189, 1 175 (SO_2). For $C_{33}H_{44}O_6S_2 \cdot 2\ H_2O$ (636.8) calculated: 62.23% C, 7.60% H; found: 62.30%C, 10.19% H.

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