GLUCOSYLATION OF SOME STEROIDAL 17-HYDROXY DERIVATIVES*

Ivan ČERNÝ, Vladimír POUZAR, Pavel DRAŠAR and Miroslav HAVEL

Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague 6

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Eight 17-monoglucosides derived from androst-5-ene-3,17-diol, 14β-androst-5-ene-3,17-diol, 5β-androstane-3,17-diol and estradiol derivatives differing in configuration in the positions 17 and 3, have been prepared. The silver silicate — catalyzed glycosylation with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide gave 43—72% of the corresponding peracetylated β-D-glucopyranosides. The starting selectively protected 5β-androstane-3,17-diol derivatives were synthesized by a procedure utilizing orthogonality of the pivalate, acetate and nitrate protecting groups.

In our recent studies[1-3] we investigated the silver silicate catalyzed glycosylation of steroidal hydroxy derivatives with glycosyl halides. In most cases, this method affords very good yields of the corresponding glucosides or galactosides. With peracetylated halogenoses containing participating group on C-2 the reaction leads exclusively to β-D-glycosides. As seen from the results obtained thus far with steroidal 3-hydroxy derivatives, neither the annelation of the rings A and B nor the presence of double bond in position 5 seem to affect seriously the yields.

In the present paper we investigate glycosylations of steroidal 17-hydroxy derivatives in order to compare their reactivity in the glycosylation reaction with the reactivity of the 3-hydroxy derivatives studied so far. As hydroxy derivatives served the epimeric androst-5-ene-3β,17β-diol and androst-5-ene-3β,17α-diol 3-acetates (*I* and *II*) and the analogous 14β-androstane derivatives[4] *III* and *IV*; of the 5β-androstane series we prepared the nitrates epimeric at C-3, i.e. 5β-androstane-3α, 17β-diol and 5β-androstane-3β,17β-diol 3-nitrates (*V* and *VI*). For comparison with the already published results the estradiol derivatives[5,6] *VII* and *VIII* were also studied.

The synthesis of 5β-androstane derivatives (Scheme 1) starts from the acetate *I* which was converted via the acetate pivalate *IX* into the pivalate *X* with free hydroxyl in position 3. In the next step, a modified Oppenauer reaction[7], utilizing oxidation with 1-methyl-4-piperidone in the presence of aluminium isopropoxide in toluene,

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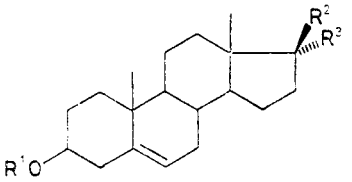
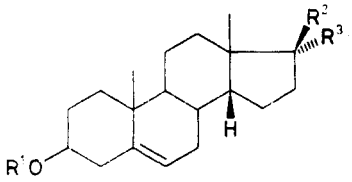
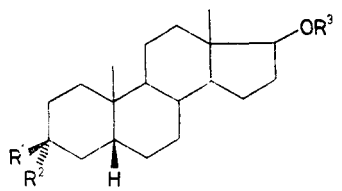


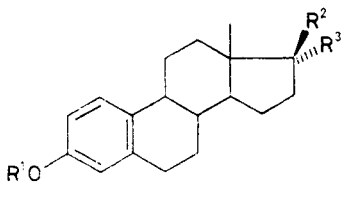
I, R^1= Ac ; R^2= OH ; R^3= H
II, R^1= Ac ; R^2= H ; R^3= OH
XIX, R^1= Ac ; R^2= OGlcA ; R^3= H
XX, R^1= Ac ; R^2= H ; R^3= OGlcA
XXIX, R^1= H ; R^2= OGlc ; R^3= H
XXX, R^1= H ; R^2= H ; R^3= OGlc



III, R^1= Ac ; R^2= OH ; R^3= H
IV, R^1= Ac ; R^2= H ; R^3= OH
XXI, R^1= Ac ; R^2= OGlcA ; R^3= H
XXII, R^1= Ac ; R^2= H ; R^3= OGlcA
XXXI, R^1= H ; R^2= OGlc ; R^3= H
XXXII, R^1= H ; R^2= H ; R^3= OGlc



V, R^1= H ; R^2= ONO_2 ; R^3= H
VI, R^1= ONO_2 ; R^2= H ; R^3= H
XXIII, R^1= H ; R^2= ONO_2 ; R^3= GlcA
XXIV, R^1= ONO_2 ; R^2= H ; R^3= GlcA
XXVII, R^1= H ; R^2= OH ; R^3= GlcA
XXVIII, R^1= OH ; R^2= H ; R^3= GlcA
XXXIII, R^1= H ; R^2= OH ; R^3= Glc
XXXIV, R^1= OH ; R^2= H ; R^3= Glc



VII, R^1= Ac ; R^2= OH ; R^3= H
VIII, R^1= Ac ; R^2= H ; R^3= OH
XXV, R^1= Ac ; R^2= OGlcA ; R^3= H
XXVI, R^1= Ac ; R^2= H ; R^3= OGlcA
XXXV, R^1= H ; R^2= OGlc ; R^3= H
XXXVI, R^1= H ; R^2= H ; R^3= OGlc

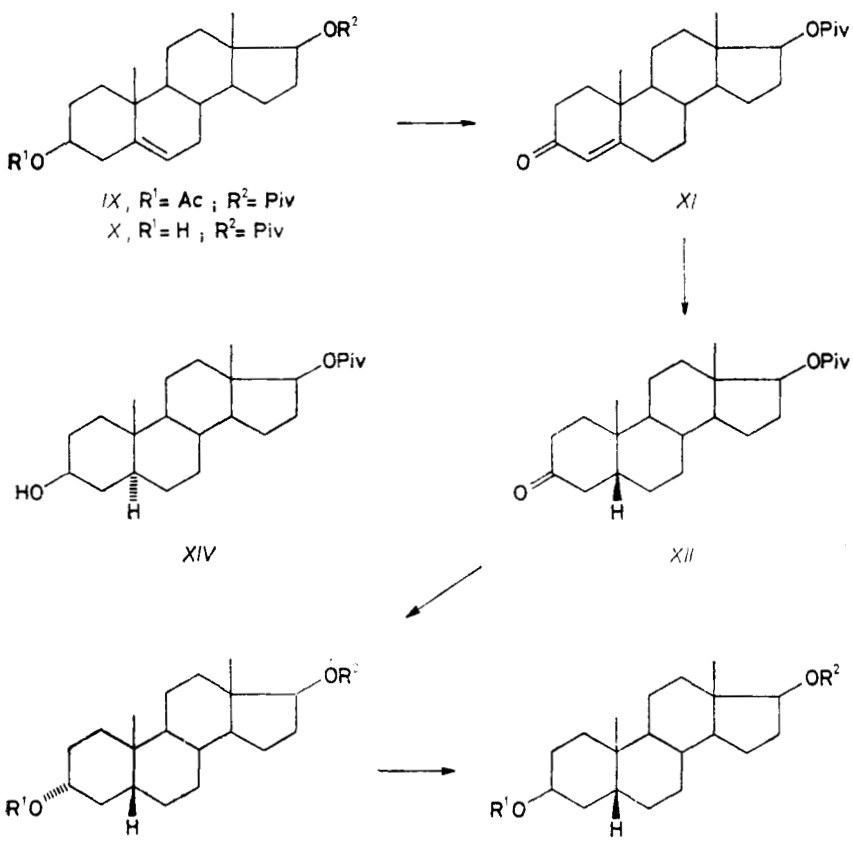
GlcA = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl
Glc = β-D-glucopyranosyl

afforded the 4-unsaturated 3-ketone *XI*. Hydrogenation on palladium on charcoal in ethanol in the presence of sodium hydroxide gave ketone *XII* which, without further purification, was reduced with sodium borohydride to give 3α-hydroxy-5β--androstan-17β-yl pivalate (*XIII*). We did not find the alternative product *XIV* corresponding to the attack by hydrogen from the α-side of the steroid skeleton. An authentic sample of *XIV*, prepared from derivative *IX* by hydrogenation of the double bond in position 5, differs from the pivalate *XIII* both in TLC behaviour and in the ^{1}H NMR spectrum. Epimerization of the 3-hydroxy group was performed by reaction of the tosyl derivative *XV* with sodium nitrite in hexamethylphosphoramide[4]. Both the formed 3β-hydroxy-5β-androstan-17β-yl pivalate (*XVI*) and the

starting isomeric 3α-hydroxy derivative *XIII* were oxidized with pyridinium chlorochromate to give ketone *XII* which represents an additonal proof of their structure.

The further reaction path involved protection of the 3-hydroxy group as nitrate (derivatives *XVII* and *XVIII*) and deblocking the 17β-hydroxyl by hydrolysis with potassium hydroxide in benzene–ethanol which afforded the desired 17-hydroxy derivatives *V* and *VI*.

The glycosylation reactions were performed under the same conditions as in our previous studies[1-3] in 1,2-dichloroethane in the presence of silver silicate on silica gel and of molecular sieve 4A with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide



IX, R^1= Ac ; R^2= Piv
X, R^1= H ; R^2= Piv

XI

XIV

XII

XIII, R^1= H ; R^2= Piv
XV, R^1= Tos ; R^2= Piv
XVII, R^1= NO_2 ; R^2= Piv

XVI, R^1= H ; R^2= Piv
XVIII, R^1= NO_2 ; R^2= Piv

Ac = acetyl
Piv = 2,2-dimethylpropionyl (pivaloyl)
Tos = *p*-toluenesulfonyl

in about 1·2–1·5 molar excess. The yields (Table I) ranged from 43% to 72%. In the C-17 epimeric pairs the higher yield was obtained with the 17β-isomers even when the C/D annelation was *cis*. Average yields did not differ much from those of glycosylations of steroidal 3-hydroxy derivatives performed under similar conditions. In contrast to glycosylations with silver carbonate in benzene[6], the estradiol derivatives gave no perceptible amounts of the corresponding orthoesters; this is reflected in the somewhat higher yield.

The structure of peracetylated glucosides *XIX–XXVI* was proved by their ^{1}H NMR spectra (Table II), the β-configuration of the anomeric center follows from the values of coupling constants $J(1, 2) = 7{\cdot}0-7{\cdot}8$. All the spectral parameters correspond (with small scatter) to those found earlier for analogous derivatives[2].

The free glycosides were obtained by deacetylation with sodium methoxide in methanol which, in case of nitrates *XXIII* and *XXIV*, was preceded by reductive removal of the nitro group with zinc in a mixture of tetrahydrofuran, acetic acid and water and led to 3-hydroxy derivatives *XXVII* and *XXVIII*. The free glycosides *XXIX–XXXVI* were prepared in yields of 71–90%.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured at 25°C on a Perkin–Elmer 141 MC polarimeter. Infrared spectra were recorded on a Perkin–Elmer PE 580 spectrometer (wavenumbers in cm^{-1}). ^{1}H NMR spectra were taken on a Tesla BS-497 (FT mode, 100 MHz) and on Varian XL-200 (FT mode, 200·057 MHz) instruments at 23°C in deuteriochloroform with tetramethylsilane as internal

TABLE I
Yields of glycosidations of steroids *I–VIII*

Steroid	Yield[a] %	Configuration 3	5	14	17	Product
I	61	β	Δ	α	β	*XIX*
II	58	β	Δ	α	α	*XX*
III	54	β	Δ	β	β	*XXI*
IV	45	β	Δ	β	α	*XXII*
V	65	α	β	α	β	*XXIII*
VI	60	β	β	α	β	*XXIV*
VII	72	—	—	α	β	*XXV*
VIII	43	—	—	α	α	*XXVI*

[a] Preparative yields after chromatography.

standard. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and bandwidths (W) in Hz. All values were obtained by the first order analysis. Column chromatography was performed on silica gel (Service Laboratories of this Institute, 60–120 μm) or alumina (Reanal, activity II), thin-layer chromatography on silica gel G according to Stahl (ICN Biochemicals). Spots were detected by spraying with sulfuric acid followed by heating. Prior to evaporation in vacuo (about 2 kPa), solutions in organic solvents were dried over anhydrous sodium sulfate.

TABLE II

Proton NMR spectral data for β-D-glucoside peracetates *XIX*–*XXVI* in $CDCl_3$

Parameter	*XIX*[a]	*XX*[a]	*XXI*[a]	*XXII*[a]	*XXIII*[b]	*XXIV*[b]	*XXV*[b]	*XXVI*[b]
H-3	4·62 m	4·60 m	4·59 m	4·60 m	4·93 m	5·24 m	—	—
H-6	5·36 bd	5·36 bd	5·38 bt	5·37 bd	[c]	[c]	2·83 m	2·84 m
H-17	3·56 bt	3·71 d	3·48 bd	3·78 bt	3·55 bt	3·54 bt	3·63 bt	3·76 d
H-18[d]	0·73 s	0·69 s	0·96 s	0·99 s	0·70 s	0·71 s	0·78 s	0·70 s
H-19[d]	1·02 s	1·01 s	0·99 s	1·04 s	0·96 s	0·97 s	—	—
H-1′	4·53 d	4·46 d	4·50 d	4·45 d	4·52 d	4·52 d	4·56 d	4·50 d
H-2′	4·99 dd	4·94 dd	5·00 dd	4·96 dd	4·98 dd	4·97 dd	4·99 dd	4·96 dd
H-3′	5·20 t	5·20 t	5·19 t	5·20 t	5·22 t	5·21 t	5·23 t	5·23 t
H-4′	5·05 t	5·07 t	5·04 t	5·06 t	5·06 t	5·04 t	5·05 t	5·07 t
H-5′	3·66 ddd	3·66 ddd	3·66 ddd	3·66 ddd	3·64 m	3·65 ddd	3·66 m	3·66 ddd
H-6′a	4·25 dd	4·25 dd	4·26 dd	4·25 dd	4·26 dd	4·27 dd	4·28 dd	4·29 dd
H-6′b	4·12 dd	4·12 dd	4·11 dd	4·12 dd	4·10 dd	4·10 dd	4·11 dd	4·12 dd
J(6, 7)	4·6	5·0	≈3	3·8	[c]	[c]	[c]	[c]
J(16, 17)	8·5	5·5	5·4	8·4	≈8	8·5	≈8	≈5
J(1′, 2′)	7·8	7·8	7·7	7·8	7·5	7·5	7·5	7·5
J(2′, 3′)	9·5	9·3	9·4	9·4	≈9	≈9	≈9	≈9
J(3′, 4′)	9·3	9·3	9·3	9·3	≈9	≈9	≈9	≈9
J(4′, 5′)	9·5	9·5	9·5	9·5	≈9	≈9	≈9	≈9
J(5′, 6′a)	4·9	4·9	5·0	5·0	5·0	5·0	5·0	4·5
J(5′, 6′b)	2·8	2·8	2·8	2·6	3·0	3·0	2·5	2·6
J(6′a, 6′b)	12·2	12·2	12·2	12·2	12·0	12·0	12·0	12·0

[a] 200 MHz spectral parameters, [b] 100 MHz spectral parameters. [c] Undeterminable value. [d] Three proton singlets. Other signals: *XIX* – 2·082 s, 3 H, 2·045 s, 3 H, 2·029 s, 3 H, 2·017 s, 3 H, 2·003 s, 3 H, (CH_3CO); *XX* – 2·081 s, 3 H, 2·028 s, 3 H, 2·018 s, 6 H, 2·006 s, 3 H, (CH_3CO); *XXI* – 2·079 s, 3 H, 2·026 s, 3 H, 2·018 s, 3 H, 2·014 s, 3 H, 1·999 s, 3 H, (CH_3CO); *XXII* – 2·077 s, 3 H, 2·032 s, 3 H, 2·028 s, 3 H, 2·019 s, 3 H, 2·002 s, 3 H, (CH_3CO); *XXIII* – 2·08 s, 3 H, 2·04 s, 3 H, 2·02 s, 3 H, 2·00 s, 3 H, (CH_3CO), *XXIV* – 2·07 s, 3 H, 2·04 s, 3 H, 2·01 s, 3 H, 2·00 s, 3 H, (CH_3CO); *XXIV* – 2·08 s, 3 H, 2·04 s, 3 H, 2·02 s, 3 H, 2·00 s, 3 H, (CH_3CO); *XXV* – 6·78–7·30 m, 3 H (H-1, H-2, H-3), 2·27 s, 3 H, 2·08 s, 3 H, 2·04 s, 3 H, 2·02 s, 6 H, (CH_3CO); *XXVI* – 6·78–7·34 m, 3 H (H-1, H-2, H-3), 2·27 s, 3 H, 2·09 s, 3 H, 2·03 s, 6 H, 2·00 s, 3 H, (CH_3CO).

17β-Hydroxy-5β-androstan-3α-yl Nitrate (*V*)

A solution of potassium hydroxide (2·69 g, 48 mmol) in ethanol (60 ml) was added to a solution of diester *XVII* (1·26 g, 3·0 mmol) in benzene (15 ml). After heating to reflux for 15 h, the reaction mixture was evaporated in vacuo. The dry residue was partitioned between dichloromethane and water and the aqueous phase was extracted with dichloromethane. The combined organic phases were washed with water (3×) and dried over anhydrous sodium sulfate. The residue after evaporation was chromatographed on a column of silica gel (100 g). Elution with light petroleum–benzene–ether (50 : 40 : 10) afforded 575 mg (57%) of hydroxy derivative *V*, m.p. 106–109°C, $[\alpha]_D$ +30° (*c* 0·3, chloroform). IR spectrum (chloroform): 3 615, 3 460, (O—H); 1 623, 1 278 (ONO_2). ^{1}H NMR spectrum: 4·92 m, 1 H (H-3β, W = 36); 3·65 bt, 1 H (H-17α, J = 8); 0·97 s, 3 H (3 × H-19); 0·72 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 337 (M^+). For $C_{19}H_{31}NO_4$ (337·5) calculated: 67·63% C, 9·26% H, 4·15% N; found: 67·73% C, 9·13% H, 3·89% N.

17β-Hydroxy-5β-androstan-3β-yl Nitrate (*VI*)

The title compound was prepared from diester *XVIII* (1·26 g, 3·0 mmol) using the procedure described for the hydroxy derivative *V*. The residue after evaporation was chromatographed on a column of silica gel (100 g). Elution with light petroleum–acetone (95 : 5) afforded 535 mg (53%) of hydroxy derivative *VI*, m.p. 99–101°C (light petroleum), $[\alpha]_D$ +14° (*c* 0·3, chloroform). IR spectrum (chloroform): 3 612, 3 455 (O—H); 1 622, 1 280 (ONO_2). ^{1}H NMR spectrum: 5·25 m, 1 H (H-3α, W = 14); 3·64 bt, 1 H (H-17α, J = 8); 0·99 s, 3 H (3 × H-19); 0·73 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 337 (M^+). For $C_{19}H_{31}NO_4$ (337·5) calculated: 67·63% C, 9·26% H, 4·15% N; found: 67·85% C, 9·11% H, 3·99% N.

Androst-5-ene-3β,17β-diyl 3-Acetate 17-Pivalate (*IX*)

Pivaloyl chloride (29·6 ml, 240 mmol) was added dropwise to a solution of hydroxy derivative *I* (ref.[8], 19·95 g, 60 mmol) in pyridine (40 ml), precooled to 0°C. After standing for 12 h at room temperature, the mixture was poured on ice, the product was extracted with ether, the ethereal extract was washed with dilute hydrochloric acid (3×), water, saturated solution of potassium hydrogen carbonate (3×) and water, and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was chromatographed on a column of alumina (1 kg). Elution with light petroleum–benzene–ether (50 : 49 : 1) afforded 23·75 g (95%) of product *IX* which was used further without purification. An analytical sample was obtained by crystallization from light petroleum; m.p. 163–164°C; $[\alpha]_D$ −55° (*c* 0·2, chloroform) (reported[9] m.p. 162–163·5°C, $[\alpha]_D$ −60°). IR spectrum (tetrachloromethane): 1 740 (C=O); 1 668 (C=C); 1 244 (C—O acetate); 1 164 (C—O pivalate). ^{1}H NMR spectrum: 5·39 bd, 1 H (H-6, J = 4·5); 4·59 m, 2 H (H-3α and H-17α); 2·02 s, 3 H (CH_3COO); 1·19 s, 9 H ($OOCC(CH_3)_3$); 1·03 s, 3 H (3 × H-19); 0·82 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 356 (M − CH_3COOH).

3β-Hydroxyandrost-5-en-17β-yl Pivalate (*X*)

A solution of potassium hydroxide (5·38 g, 96 mmol) in methanol (100 ml) was added to a solution of diester *IX* (26·66 g, 64 mmol) in a mixture of benzene (200 ml) and methanol (200 ml). After stirring at room temperature for 3 h, acetic acid (6 ml) was added and the solvents were evaporated in vacuo. The residue was partitioned between water and ether and the aqueous phase was extracted with ether. The combined organic phases were washed with saturated potassium hydrogen carbonate solution (5×) and water and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was chromatographed on a column of alumina (1 kg).

Elution with light petroleum–benzene–ether (50 : 40 : 10) afforded 19·9 g (83%) of product *X*, m.p. 172–174°C (light petroleum); $[\alpha]_D$ −57° (*c* 0·3, chloroform). Reported[9] m.p. 172–174·5°C; $[\alpha]_D$ −60°. IR spectrum (chloroform): 3 615, 3 490 (O—H); 1 718, 1 175 (COOR); 1 044, 1 035 sh (C—O). ^{1}H NMR spectrum: 5·37 bd, 1 H (H-6, J = 4·5); 4·58 dd, 1 H (H-17α, J = 7; J' = 9). 3·57 m, 1 H (H-3α, W = 36); 1·20 s, 9 H ($OOCC(CH_3)_3$); 1·02 s, 3 H (3 × H-19); 0·82 s, 3 H; (3 × H-18). Mass spectrum, *m/z*: 374 (M^+).

3-Oxoandrost-4-en-17β-yl Pivalate (*XI*)

1-Methyl-4-piperidone (34 ml, 276 mmol) was added under argon to a solution of hydroxy derivative *X* (18·73 g, 50 mmol) in toluene (680 ml). A part of the solvent (75 ml) was distilled off, aluminium isopropoxide in toluene (42 ml of 1M solution) was added and the mixture was refluxed with stirring under argon for 3 h. After cooling, the mixture was diluted with ether (500 ml), washed with dilute hydrochloric acid (3×), water, saturated potassium hydrogen carbonate solution, water and dried over anhydrous sodium sulfate. After evaporation, the residue was chromatographed on a column of alumina (1 kg). Elution with light petroleum–benzene–ether (50 : 45 : 5) afforded 14·53 g (78%) of product *XI*, m.p. 159–162°C (light petroleum); $[\alpha]_D$ +88° (*c* 0·3, chloroform). Reported[10] m.p. 159–160°C; $[\alpha]_D$ +90°. IR spectrum (tetrachloromethane): 1 727, 1 164 (COOR); 1 678, 1 620 (C=C—C=O). ^{1}H NMR spectrum: 5·73 bs, 1 H (H-4); 4·57 dd, 1 H (H-17α, J = 7; J' = 9); 1·20 s, 12 H ($OOCC(CH_3)_3$ and 3 × H-19); 0·85 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 372 (M^+).

3-Oxo-5β-androstan-17β-yl Pivalate (*XII*)

A) Pyridinium chlorochromate (151 mg, 0·7 mmol) was added to a solution of hydroxy derivative *XIII* (75 mg, 0·2 mmol) in dichloromethane (2 ml). After stirring for 2 h at room temperature, the mixture was diluted with ether (5 ml) and filtered through a column of alumina (5 g). The column was washed with ether and the combined organic phases were evaporated in vacuo. Crystallization of the residue from ether gave 53 mg (71%) of ketone *XII*, m.p. 132–133°C; $[\alpha]_D$ +24° (*c* 0·3, chloroform). IR spectrum (tetrachloromethane): 1 722 (C=O); 1 162 (C—O). ^{1}H NMR spectrum: 4·59 dd, 1 H (H-17α, J = 7·5; J' = 9·3); 1·20 s, 9 H ($OOCCCH_3)_3$; 1·03 s, 3 H (3 × H-19); 0·82 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 374 (M^+). For $C_{24}H_{38}O_3$ (374·6) calculated: 76·96% C, 10·23% H; found: 77·15% C, 10·24% H.

B) Hydroxy derivative *XVI* (75 mg, 0·2 mmol) was oxidized in the same manner as above (reaction time 1 h), yielding 60 mg (80%) of ketone *XII*, m.p. 130–132°C, identical with the compound prepared under *A*).

C) Fractions, containing less polar compound (see the preparation of *XVI*), were combined and evaporated; yield 380 mg (19%) of ketone *XII*, m.p. 130–132°C, identical with the compound prepared under *A*).

3α-Hydroxy-5β-androstan-17β-yl Pivalate (*XIII*)

A solution of sodium hydroxide (14 g, 350 mmol) in water (70 ml) was added to a solution of unsaturated ketone *XI* (7·45 g, 20 mmol) in ethanol (430 ml). The mixture was stirred with 10% palladium on activated carbon (2·1 g) in atmosphere of hydrogen at ambient temperature and pressure until the corresponding amount of hydrogen (480 ml, 40 mmol) was consumed (about 20 min). After addition of acetic acid (20 ml), the catalyst was filtered off and washed with ethyl acetate (100 ml). The solvents were evaporated in vacuo and the residue (containing mainly the saturated ketone *XII*, according to TLC in light petroleum–ether 1 : 1) was dissolved in

benzene (150 ml) and methanol (150 ml). Sodium borohydride (1·51 g, 40 mmol) was added to the cold (0°C) solution which was then stirred at 0°C for 2 h. Acetic acid (4·3 ml) was added and the solvents were evaporated in vacuo. The residue was partitioned between water and ether and the aqueous phase was extracted with ether. The combined organic phases were washed with saturated solution of potassium hydrogen carbonate (2×), water, and dried over anhydrous sodium sulfate. The residue after evaporation was chromatographed on a column of silica gel (500 g). Elution with light petroleum–benzene–ether (50 : 45 : 5) afforded a crude product which was further purified by crystallization from methanol–acetone–water. Yield 3·69 g (49%) of hydroxy derivative *XIII*, m.p. 173–175°C. $[\alpha]_D$ +20° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 610, 3 480 (O—H); 1 717, 1 175 (COOR). ^{1}H NMR spectrum: 4·55 dd, 1 H (H-17α, $J = 7$; $J' = 9$); 3·62 m, 1 H (H-3β, $W = 32$); 1·19 s, 9 H ($OOCC(CH_3)_3$); 0·93 s, 3 H (3 × H-19); 0·78 s, 3 H (3 × H-18). Mass spectrum, *m/z*: 358 ($M - H_2O$). For $C_{24}H_{40}O_3$ (376·6) calculated: 76·55% C, 10·71% H; found: 76·24% C, 10·63% H.

3β-Hydroxy-5α-androstan-17β-yl Pivalate (*XIV*)

Olefin *X* (375 mg, 1 mmol) was hydrogenated in ethyl acetate (23 ml) over palladium on activated carbon (10%, 40 mg) at ambient temperature and pressure until the corresponding amount of hydrogen was consumed (24 ml, 1 mmol). The catalyst was filtered off, washed with ethyl acetate and the filtrate was taken down in vacuo. Crystallization of the residue from methanol afforded 215 mg (57%) of hydroxy derivative *XIV*, m.p. 172–174°C; $[\alpha]_D$ 0° (*c* 0·1, chloroform). IR spectrum (chloroform): 3 620, 3 490 sh, 3 350 (O—H); 1 727 (COOR); 1 125, 1 035, 1 034 (C—O). ^{1}H NMR spectrum: 4·54 dd, 1 H (H-17α, $J = 7$; $J' = 9$); 3·56 m, 1 H (H-3α, $W = 36$); 1·17 s, 9 H ($OOCC(CH_3)_3$); 0·80 s, 3 H (3 × H-19); 0·78 s, 3 H (3 × H-18). For $C_{24}H_{40}O_3$ (376·6) calculated: 76·55% C, 10·71% H; found: 76·76% C, 10·99% H.

5β-Androstane-3α,17β-diyl 3-Tosylate 17-Pivalate (*XV*)

p-Toluenesulfonyl chloride (1·91 g, 10 mmol) was added at 0°C to a solution of hydroxy derivative *XIII* (1·88 g, 5 mmol) in pyridine (14 ml). After standing at room temperature for 24 h, the reaction mixture was poured on ice (200 g), the separated product was collected on filter and dissolved in ether–dichloromethane (2 : 1). The solution was washed successively with dilute hydrochloric acid (3×), water, saturated solution of potassium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. The residue was chromatographed on a column of alumina (150 g) in light petroleum–benzene–ether (50 : 48 : 2), yielding 2·54 g (96%) of tosylate *XV*, m.p. 160–161°C (acetone); $[\alpha]_D$ +33° (*c* 0·2, chloroform). IR spectrum (tetrachloromethane): 1 726 (C=O); 1 599 (arom. system); 1 370 (SO_2). For $C_{31}H_{46}O_5S$ (530·8) calculated: 70·15% C, 8·74% H, 6·04% S; found: 69·93% C, 9·00% H, 5·74% S.

3β-Hydroxy-5β-androstan-17β-yl Pivalate (*XVI*)

A mixture of tosylate *XV* (2·81 g, 5·3 mmol), sodium nitrite (8·28 g, 120 mmol) and hexamethylphosphoramide (65 ml) was heated to 90°C for 3 h. After cooling, the mixture was poured into water and the product was extracted with ethyl acetate. The extract was washed with 10% sulfuric acid (5×), water, saturated potassium hydrogen carbonate solution, water, and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue chromatographed on a column of silica gel (150 g) in light petroleum–benzene–ether (50 : 48 : 2) to give 380 mg (19%) of ketone *XII* (see above). Elution with light petroleum–benzene–ether (50 : 44 : 6) afforded 1·27 g (64%) of hydroxy derivative *XVI*, m.p. 171–273°C (ether–light petroleum); $[\alpha]_D$ +26° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 615, 3 490 (O—H); 1 714, 1 174 (COOR).

^{1}H NMR spectrum: 4·57 dd, 1 H (H-17α, J = 7; J' = 9); 4·12 m, 1 H (H-3α, W = 16); 1·18 s, 9 H ($OOCC(CH_3)_3$); 0·97 s, 3 H (3 × H-19); 0·79 s, 3 H (3 × H-18). Mass spectrum, m/z: 358 ($M - H_2O$). For $C_{24}H_{40}O$ (376·6) calculated: 76·55%C, 10·71% H; found: 76·32% C, 10·53% H.

5β-Androstane-3α,17β-diyl 3-Nitrate 17-Pivalate (*XVII*)

Nitric acid (65%, 1·5 ml, 2 mol) was added dropwise at −25°C to acetic anhydride (7·5 ml, 79 mmol). After stirring at −25°C for 10 min, a solution of hydroxy derivative *XIII* (1·20 g, 3·2 mmol) in dichloromethane (20 ml) was added during 20 min. The mixture was stirred at −25°C for 3 h and then poured on a mixture of ice (200 g) and concentrated aqueous ammonia (30 ml). The product was taken up in ether, the extract was washed with saturated solution of potassium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 1·29 g (96%) of nitrate *XVII* which was used without further purification. An analytical sample was obtained by crystallization from ether–light petroleum, m.p. 143–145°C; $[\alpha]_D$ +43° (*c* 0·4, chloroform). IR spectrum (tetrachloromethane): 1 725, 1 154 (COOR); 1 630, 1 280 (ONO_2). ^{1}H NMR spectrum: 4·92 m, 1 H (H-3β, W = 36); 4·57 dd, 1 H (H-17α, J = 8; J' = 9); 1·19 s, 9 H ($OOCC(CH_3)_3$); 0·97 s, 3 H (3 × H-19); 0·79 s, 3 H (3 × H-18). For $C_{24}H_{39}NO_5$ (421·6) calculated: 68·38% C, 9·32% H, 3·32% N; found: 68·58% C, 9·32% H, 3·07% N.

5β-Androstane-3β,17β-diyl 3-Nitrate 17-Pivalate (*XVIII*)

The title nitrate *XVIII* was prepared from hydroxy derivative *XVI* (1·20 g, 3·2 mmol) by a procedure described for the nitrate *XVII*; yield 1·30 g (97%) of nitrate *XVIII* which was used without further purification. An analytical sample was obtained by crystallization from ether–light petroleum, m.p. 119–121°C; $[\alpha]_D$ +17° (*c* 0·2, chloroform). IR spectrum (tetrachloromethane): 1 724, 1 162 (COOR); 1 630, 1 276, 880, 864 (ONO_2). ^{1}H NMR spectrum: 5·25 m, 1 H (H-3α, W = 14); 4·59 dd, 1 H (H-17α, J = 7; J' = 9); 1·19 s, 9 H ($COOC(CH_3)_3$); 0·98 s, 3 H (3 × H-19); 0·79 s, 3 H (3 × H-18). For $C_{24}H_{39}NO_5$ (421·6) calculated: 68·38% C, 9·32% H, 3·32% N; found: 68·60% C, 9·28% H, 3·14% N.

General Procedure for Glucosylation of Derivatives *I*–*VIII*

A dry mixture of the steroid hydroxy derivative (1·0 mmol), molecular sieve 4A (0·7 g; preground and activated by heating to 300–400°C on a steel dish for 2 h) and silver silicate[11] on silica gel (1·5 g) was stirred for 1 h in vacuo (about 100 Pa) in a dark bottle closed by a septum. The flask was then filled with argon and 1,2-dichloroethane (4 ml) was added through the septum. After stirring for 30 min the mixture was cooled in an ice bath and a solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (500 mg, 1·2 mmol) in 1,2-dichloroethane (2 ml) was added through the septum. The stirring was continued at room temperature for 24 h. The solid material was filtered off on Celite which was then washed with chloroform. The filtrate was washed with a solution of potassium hydrogen carbonate and with water, dried, and the solvents were evaporated. The product was purified by HPLC on silica gel in benzene–ethyl acetate (25 : 1) and crystallized from ethanol, unless stated otherwise.

17β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)androst-5-en-3β-yl Acetate (*XIX*)

According to the above-described general procedure, hydroxy derivative *I* (516 mg, 1·55 mmol) was converted into glycoside *XIX* which was crystallized from ethyl acetate, yield 630 mg (61%), m.p. 204–206°C (subl.); $[\alpha]_D$ −42° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 755, 1 725 sh

(C=O); 1 668 (C=C); 1 254, 1 039 ($OCOCH_3$). For ^1H NMR spectrum see Table II. Reported[12] m.p. 201–202°C and $[\alpha]_D$ −49° (*c* 0·2, methanol).

17α-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)androst-5-en-3β-yl Acetate (*XX*)

According to the above-described general procedure, compound *II* (450 mg, 1·35 mmol) was converted into glycoside *XX* (522 mg, 58%), m.p. 238–239°C; $[\alpha]_D$ −76° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 755, 1 735 sh, 1 725 sh (C=O); 1 668 (C=C); 1 040 ($OCOCH_3$). For ^1H NMR spectrum see Table II. For $C_{35}H_{50}O_{12}$ (662·8) calculated: 63·43% C, 7·60% H; found: 63·68% C, 7·59% H.

17β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-14β-androst-5-en-3β-yl Acetate (*XXI*)

According to the general procedure, hydroxy derivative *III* (ref.[4], 160 mg, 0·48 mmol) was converted into glycoside *XXI* (172 mg, 54%), m.p. 230–231°C (subl.); $[\alpha]_D$ +3° (*c* 0·8, chloroform). IR spectrum (chloroform): 1 755, 1 735 sh, 1 725 sh (C=O); 1 670 sh (C=C); 1 036 ($OCOCH_3$). For ^1H NMR spectrum see Table II. For $C_{35}H_{50}O_{12}$ (662·8) calculated: 64·43% C, 7·60% H: found: 63·51% C, 7·62% H.

17α-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-14β-androst-5-en-3β-yl Acetate (*XXII*)

According to the above-described general procedure, hydroxy derivative *IV* (ref.[4], 189 mg, 0·57 mmol) was converted into glycoside *XXII* (171 mg, 45%), m.p. 236–237°C; $[\alpha]_D$ −20° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 755, 1 735 sh, 1 725 sh (C=O); 1 670 sh (C=C); 1 038 ($OCOCH_3$). For ^1H NMR spectrum see Table II. For $C_{35}H_{50}O_{12}$ (662·8) calculated: 63·43% C, 7·60% H; found: 63·51% C, 7·62% H.

17β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-5β-androstan-3α-yl Nitrate (*XXIII*)

According to the above-described general procedure, hydroxy derivative *V* (350 mg, 1·03 mmol) was converted into glycoside *XXIII* (450 mg, 65%), m.p. 146–147°C; $[\alpha]_D$ +22° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 753 (C=O); 1 624, 1 276 (NO_2); 870 (N—O). For ^1H NMR spectrum see Table II. For $C_{33}H_{49}NO_{13}$ (667·8) calculated: 59·36% C, 7·40% H, 2·10% N; found: 59·62% C, 7·35% H, 2·01% N.

17β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-5β-androstan-3β-yl Nitrate (*XXIV*)

According to the above-described general procedure, hydroxy derivative *VI* (350 mg, 1·03 mmol) was converted into glycoside *XXIV* (413 mg, 60%), m.p. 143–145°C; $[\alpha]_D$ +4° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 753 (C=O); 1 622, 1 280 (NO_2); 1 238, 1 042 (C—O); 882 (N—O). For ^1H NMR spectrum see Table II. For $C_{33}H_{49}NO_{13}$ (667·8) calculated: 59·36% C, 7·40% H, 2·10% N; found: 59·61% C, 7·33% H, 2·03% N.

17β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)estra-1,3,5(10)-trien-3-yl Acetate (*XXV*)

According to the above-described general procedure, hydroxy derivative *VII* (250 mg, 0·80 mmol)

was converted into glycoside *XXV* (367 mg, 72%), m.p. 172–173°C; $[\alpha]_D$ +16° (*c* 1·2, chloroform) (reported[6] m.p. 171–173°C, $[\alpha]_D$ +17·3°). For ^{1}H NMR see Table II.

17α-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)estra-1,3,5(10)-trien-3-yl Acetate (*XXVI*)

According to the above-described general procedure, hydroxy derivative *VIII* (250 mg, 0·80 mmol) was converted into glycoside *XXVI* (220 mg, 43%), m.p. 140–142°C; $[\alpha]_D$ −11° (*c* 0·7, chloroform) (reported[6] m.p. 140–142°C; $[\alpha]_D$ −15·0°). For ^{1}H NMR see Table II.

General Procedure for Deacetylation of Derivatives *XIX*–*XXVI*

To a solution of the acetyl derivative in absolute methanol (10 ml) was added methanolic 1M sodium methoxide (5 drops) and the reaction mixture was allowed to stand at room temperature for 8–12 h. The reaction was monitored by TLC in chloroform–methanol (20 : 1). After all the starting compound had reacted, solid carbon dioxide (about 0·5 cm^3) was added and the reaction mixture was evaporated to dryness. The product was coevaporated with toluene and further purified by filtration through a column of silica gel (25 g) in chloroform–methanol (1 : 1) and crystallization from methanol.

17β-(β-D-Glucopyranosyloxy)androst-5-en-3β-ol (*XXIX*)

Acetyl derivative *XIX* (163 mg, 0·25 mmol) was deacetylated according to the above-described general procedure to give 100 mg (90%) of glycoside *XXIX*, m.p. 252–255°C; $[\alpha]_D$ −64° (*c* 0·2, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). Reported[12] m.p. 256–257°C; $[\alpha]_D$ −71° (methanol). For $C_{25}H_{40}O_7$ (452·6) calculated: 66·35% C, 8·91% H; found: 66·42% C, 9·07% H.

17α-(β-D-Glucopyranosyloxy)androst-5-en-3β-ol (*XXX*)

Acetyl derivative *XX* (120 mg, 0·18 mmol) was deacetylated according to the above-described general procedure to give 58 mg (71%) of glycoside *XXX*, m.p. 259–262°C; $[\alpha]_D$ −87° (*c* 0·2, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). For $C_{25}H_{40}O_7$ (452·6) calculated: 66·35% C, 8·91% H; found: 66·42% C, 9·07% H.

17β-(β-D-Glucopyranosyloxy)-14β-androst-5-en-3β-ol (*XXXI*)

Acetyl derivative *XXI* (93 mg, 0·14 mmol) was deacetylated according to the above-described general procedure to give 53 mg (83%) of glycoside *XXXI*, m.p. 210–212°C; $[\alpha]_D$ +11° (*c* 0·2, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). For $C_{25}H_{40}O_7$ (452·6) calculated: 66·35% C, 8·91% H; found: 66·37% C, 8·94% H.

17α-(β-D-Glucopyranosyloxy)-14β-androst-5-en-3β-ol (*XXXII*)

Acetyl derivative *XXII* (115 mg, 0·17 mmol) was deacetylated according to the above-described general procedure to give 59 mg (75%) of glycoside *XXXII*, m.p. 228–230°C; $[\alpha]_D$ −5° (*c* 0·2, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). For $C_{25}H_{40}O_7$ (452·6) calculated: 66·35% C, 8·91% H; found: 66·47% C, 8·85% H.

17β-(β-D-Glucopyranosyloxy)-5β-androstan-3α-ol (*XXXIII*)

Nitrate *XXIII* (330 mg, 0·49 mmol) was stirred with a mixture of tetrahydrofuran (12 ml), acetic acid (3 ml) and water (0·6 ml). Zinc powder (400 mg, 6·1 mmol) was added in the course of 30 min. After stirring for further 1 h, the solid material was filtered on Celite which was then washed with chloroform. The filtrate was washed with saturated potassium hydrogen carbonate solution (2×), dried and the solvents were evaporated. The obtained foam of the acetyl derivative *XXVII* (300 mg, 97%) was deacetylated according to the above-described general procedure to give 143 mg (78%) of glycoside *XXXIII*, m.p. 234–235°C; $[\alpha]_D$ −8° (*c* 0·2, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). For $C_{25}H_{42}O_7$ (454·6) calculated: 66·05% C, 9·31% H. found: 66·17% C, 9·22% H.

17β-(β-D-Glucopyranosyloxy)-5β-androstan-3β-ol (*XXXIV*)

Zinc powder (330 mg, 5·1 mmol) was added during 30 min to a stirred mixture of nitrate *XXIV* (270 mg, 0·40 mmol), tetrahydrofuran (10 ml), acetic acid (2·5 ml) and water (0·5 ml). After further stirring for 1 h the solid material was removed by filtration through Celite which was then washed with chloroform. The solution was washed with saturated potassium hydrogen carbonate solution (2×), dried and evaporated. The obtained foam of the acetyl derivative *XXVIII* (245 mg, 98%) was deacetylated according to the above-described general procedure affording 150 mg (84%) of glycoside *XXXIV*, m.p. 217–219°C +15° (*c* 0·3, methanol). IR spectrum (KBr pellet): 1 238, 1 042 (C—O). For $C_{25}H_{42}O_7$ (454·6) calculated: 66·05% C 9·31% H; found: 66·19% C, 9·28% H.

17β-(β-D-Glucopyranosyloxy)estra-1,3,5(10)-trien-3-ol (*XXXV*)

Acetyl derivative *XXV* (179 mg, 0·28 mmol) was deacetylated according to the above-described general procedure; yield 105 mg (87%) of glycoside *XXXV*, m.p. 202–205°C $[\alpha]_D$ +9° (*c* 0·9 methanol). For $C_{24}H_{34}O_7$ (434·5) calculated: 66·34% C, 7·89% H; found: 66·40% C, 7·87% H

17α-(β-D-Glucopyranosyloxy)estra-1,3,5(10)-trien-3β-ol (*XXXVI*)

Acetyl derivative *XXVI* (170 mg, 0·26 mmol) was deacetylated according to the above-described general procedure to give 107 mg (93%) of glycoside *XXXVI*, m.p. 146–148°C; $[\alpha]_D$ −2° (*c* 1·0, methanol) (reported[6] m.p. 149–153°C (sintering at 142–144°C); $[\alpha]_D$ −2·7° (methanol)).

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