PREPARATION AND PROPERTIES OF SOME NEW STEROID β-D-GLUCOPYRANOSIDES, β-D-GLUCOPYRANOSIDURONIC ACIDS, AND DERIVATIVES

JOHN J. SCHNEIDER
Department of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania 19107 (U. S. A.)
(Received August 25th, 1970)

ABSTRACT

Twelve β-D-glucopyranoside tetraacetates and three tri-*O*-acetyl-β-D-glucopyranosiduronic methyl esters in the androstane and pregnane series, all of them new or characterized adequately for the first time, were prepared by a standardized, simplified Koenigs–Knorr synthesis in an average yield of 51%. The configuration at the anomeric center of the new derivatives was confirmed in each case by comparing observed M_D values with those obtained earlier for 21 anomeric pairs of steroid tri-*O*-acetyl-D-glucopyranosiduronic methyl esters. Examination of their i.r. spectra confirmed an earlier observation that steroid D-glucopyranoside tetraacetates and tri-*O*-acetyl-D-glucopyranosiduronic methyl esters can be distinguished by the relative position of a band in the region of 900 cm^{-1}. Saponification of the new derivatives provided the corresponding free β-D-glucopyranosides and β-D-glucopyranosiduronic acids (or salts). The present report includes the preparation and characterization of the 3β-yl and 17β-yl β-D-glucopyranosides (and their pentaacetates), and the 3β-yl and 17β-yl β-D-glucopyranosiduronic acids (and their methyl ester tetraacetates) of androst-5-ene-3β,17β-diol.

INTRODUCTION

In an earlier paper[1], the preparation and differentiation of 21 anomeric pairs of steroid tri-*O*-acetyl-D-glucopyranosiduronic methyl esters and 7 pairs of D-glucopyranoside tetraacetates was presented. We have since extended this work to the synthesis of a series of twelve β-D-glucopyranosides and their tetraacetates in the androstane and pregnane series, both because very few D-glucosides of these classes have been prepared (or described adequately in the literature), and because of a growing interest in the metabolism of such conjugates[2]. The preparation and characterization of the 3β-yl and 17β-yl β-D-glucopyranosides and the 3β-yl and 17β-yl β-D-glucopyranosiduronic acids (and derivatives) of androst-5-ene-3β,17β-diol (androstenediol) was initiated earlier, in the course of a biochemical study concerned in part with the D-glucosidation *in vitro* of various steroids by surviving plant tissues[3].

METHODS, RESULTS, AND DISCUSSION

Preparation of the β-D-glucopyranoside tetraacetates and tri-O-acetyl-β-D-glucopyranosiduronic methyl esters. — Fifteen of the derivatives listed in Table I*, namely compounds **1–12**, **15**, **16**, and **18**, were prepared by the standardized, simplified Koenigs–Knorr synthesis described in an earlier publication[4], and illustrated in the present article by the preparation of **18**. Within the present series, yields of these derivatives varied from 39 to 63%, averaging 51% of the theoretical**. All of these compounds, as well as the remaining derivatives in this Table, crystallize readily and completely (invariably from methanol or methanol–ethyl acetate), melt sharply, can be freed of solvent without difficulty, and are ideally suited for chromatography on silica gel[6].

The free β-D-glucopyranosides and β-D-glucopyranosiduronic acids (or salts) (Table II). — Hydrolysis of the 14 β-D-glucopyranoside peracetates listed in Table I was effected by treating each with a catalytic amount of sodium methoxide in dry methanol for 12 to 24 h at room temperature. The reaction mixture was percolated through a small column of the cation-exchange resin Amberlite IR-120 (H^+), and the effluent and washings were evaporated to dryness. The free D-glucosides were crystallized from methanol or aqueous methanol. Hydrolysis of the four methyl (steroid tri-*O*-acetyl-β-D-glucopyranosid)uronates was conducted in the same way, except that an excess of aqueous sodium hydroxide was added to the system at the conclusion of the methanolysis step; 24 hours later, the reaction mixture was processed as just described. For the isosapogenin derivatives, the sodium salts **20a** and **21a** began to crystallize shortly after addition of the aqueous alkali. Twelve hours later, the salts were recovered by filtration, and recrystallized from aqueous ethanol.

The yields of free conjugates were lower than anticipated, ranging from 38 to 75%, and averaging 55% of the theoretical. This appeared to be due less to incomplete hydrolysis or side reactions than to the appreciable solubility of many of them in water or aqueous alcohol, and the consequent greater difficulty in recovering them completely in crystalline form. (The free conjugates compare poorly with their derivatives in other respects: their melting points are commonly far higher, and are so

*All *derivatives* are designated by bold-face, arabic numerals, and appear in Table I; corresponding *free conjugates* bear the same number plus a bold-face letter "**a**", and are listed in Table II.

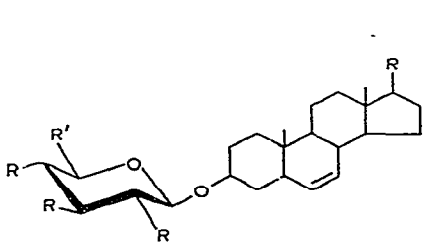
**Yields recorded earlier[1,4] for the preparation of 16 steroid tri-*O*-acetyl-β-D-glucopyranosiduronic methyl esters by the same method ranged from 38 to 86%, and averaged 56% of the theoretical. It is common practice to effect this synthesis under mildly forcing conditions, such as may be gained by the simultaneous addition of benzene to and its distillation from the reaction mixture, the use of internal desiccants, or the periodical addition of fresh halogen acceptor or glycosyl halide (see ref. 5, pp. 116–121). However, yields obtained under the more vigorous conditions (considered without regard to the various substrates and modifications employed) do not exceed, and are generally less than, those we have recorded. This result suggests that such manoeuvres do not favorably direct the course of the reaction, and that the yields actually reflect the influence of factors that cannot be controlled. These factors do not appear to include limitations imposed by steric features of the aglycon itself; we have been unable to demonstrate any consistent relationship between the structure of the aglycon and the yield of product.

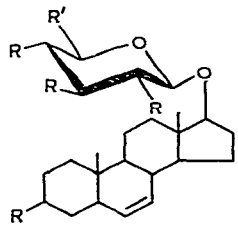
frequently accompanied by decomposition as to limit their usefulness. Moreover, they tend to retain various proportions of water, even after extended drying, thus complicating their elemental analysis.)

In the course of the biochemical study already referred to[3], all of the free β-D-glucopyranosides were incubated with emulsin, and the free β-D-glucopyranosiduronic acids with β-glucuronidase. It was observed that, although all were hydrolyzed (in the qualitative sense), rates with emulsin were strongly influenced by the configuration at C-3 of the steroid moiety.

In addition, all of the derivatives were examined by t.l.c., and the free conjugates by both t.l.c. and paper chromatography in order to verify purity and establish mobility relationships. The results of this study will be presented at a later date.

Preparation of the androstenediol β-D-glucopyranosides, β-D-glucopyranosiduronic acids, and derivatives. — It was earlier found[3] that, when androstenediol was incubated with surviving potato slices, D-glucosidation occurred only at C-3; this required the preparation of the 3β-yl β-D-glucopyranoside pentaacetate of the diol **13** by chemical means. Subsequently, it also seemed of interest to prepare the corresponding 17β-yl derivative **14**, as well as the 3β-yl and 17β-yl β-D-glucopyranosiduronic





13 R′=CH_2OAc; R=OAc
13a R′=CH_2OH; R=OH

14 R′=CH_2OAc; R=OAc
14a R′=CH_2OH; R=OH

17 R′=CO_2Me; R=OAc
17a R′=CO_2Na; R=OH

18 R′=CO_2Me; R=OAc
18a R′=CO_2H; R=OH

acids (**17a** and **18a**, respectively), and their derivatives. Details of each preparation, with the exception of that of **13**, are given in the Experimental section.

The following is an outline of the method of preparation. Reduction of 17-oxoandrost-5-en-3β-yl tetra-*O*-acetyl-β-D-glucopyranoside (**1**) with a large excess of sodium borohydride, followed by re-acetylation of the crude product, provided 17β-acetoxyandrost-5-en-3β-yl tetra-*O*-acetyl-β-D-glucopyranoside (**13**) in a yield[3] of 76%. Hydrolysis of **13**, by the method already indicated, gave the corresponding free β-D-glucopyranoside, namely, 17β-hydroxyandrost-5-en-3β-yl β-D-glucopyranoside (**13a**). It was not possible to prepare the analogous derivative in the D-glucosiduronic acid series, namely **17a**, by similarly reducing methyl (17-oxoandrost-5-en-3β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**12** in Ref. 1), because extensive reduction of the methoxycarbonyl function occurred under these conditions. However, when 17-oxoandrost-5-en-3β-yl β-D-glucopyranosiduronic acid, a well known

conjugate[5,7], was reduced with a slight excess of sodium borohydride in aqueous, alkaline ethanol, the desired product, namely 17β-hydroxyandrost-5-en-3β-yl β-D-glucopyranosiduronic acid (**17a**), was isolated (as the sodium salt) in a yield of 89%. Its conversion into the free acid, followed by sequential treatment with diazomethane and acetic anhydride–pyridine, afforded methyl (17β-acetoxyandrost-5-en-3β-yl 2,3,4,-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**17**). Methyl (3β-acetoxyandrost-5-en-17β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**18**) was prepared, in 63% yield, directly from androst-5-ene-3β,17β-diol 3-acetate by the Koenigs–Knorr reaction. Hydrolysis of **18**, as already described, furnished the corresponding free acid, namely, 3β-hydroxyandrost-5-en-17β-yl β-D-glucopyranosiduronic acid (**18a**). Reduction of **18** with lithium aluminum hydride, by use of the conditions of reaction and isolation indicated earlier[1], gave 3β-acetoxyandrost-5-en-17β-yl tetra-*O*-acetyl-β-D-glucopyranoside (**14**) in a yield of 82%. Hydrolysis of **14** gave free 3β-hydroxyandrost-5-en-17β-yl β-D-glucopyranoside (**14a**).

Miscellaneous conjugates and their derivatives. — The remaining compounds in Tables I and II, are **15**, **15a**, **16**, **16a**, **19a**, **20a**, and **21a**. Sodium (17-oxo-5β-androstan-3β-yl β-D-glucopyranosid)uronate (**15a**) has not previously been prepared; its methyl ester triacetate (**15**) completes the series of 17-ketosteroid derivatives presented earlier[1]. Similarly, methyl (20-oxo-5α-pregnan-3α-yl 2,3,4-tri-*O*-acetyi-β-D-glucopyranosid)uronate (**16**) is a new compound, and completes the series of 20-ketopregnane derivatives listed earlier[1]. The corresponding free β-D-glucopyranosiduronic acid (**16a**) was used in the biochemical work already mentioned[3].

Known conjugates of (22*S*, 25*S*)-spirost-5-en-3β-ol (diosgenin) and (22*S*, 25*S*)-5α-spirostan-3β-ol (tigogenin) include only the free β-D-glucopyranoside of diosgenin ("trillin") and its tetraacetate[8]. The preparation and characterization of (22*S*, 25*S*)-5α-spirostan-3β-yl β-D-glucopyranoside tetraacetate, methyl [(22*S*, 25*S*)-spirost-5-en-3β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid]uronate, and methyl [(22*S*, 25*S*)-5α-spirostan-3β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid]uronate were given in the earlier paper[1]; the respective free β-D-glucopyranoside (**19a**), sodium [(22*S*, 25*S*)-spirost-5-en-3β-yl β-D-glucopyranosid]uronate (**20a**), and sodium [(22*S*, 25*S*)-5α-spirostan-3β-yl β-D-glucopyranosid]uronate (**21a**) are now described.

Optical rotatory studies. — In the earlier study[1], the configuration assigned at the anomeric center for each compound was confirmed by the anomerization increment, M_D α−β, which constituted a regular series within the limits of +704 to +985, and by the correspondence between observed and calculated M_D values. In the present series, assignments for the β-D-glucopyranoside peracetates in Table I were made by comparing their M_D values with those of the corresponding methyl (tri-*O*-acetyl-β-D-glucopyranosid)uronates, given in Table I (**15**, **16**, **17**, and **18**) or in Table I of the previous paper[1]. There are twelve such pairs; these are as follows [derivative number (bold face) in Table I, observed M_D for this derivative (set off by commas), and observed M_D for the corresponding methyl (tri-*O*-acetyl-β-D-glucopyranosid)uronate (in parentheses)]: **1**, −12, (−67); **2**, +136, (+106); **3**, +205, (+146); **4**, +267, (+195); **5**, +180, (+139); **6**, +52, (+44); **7**, +188, (+165); **8**, +250, (+184); **9**, +331,

(+273); **11**, −13, (−91); **13**, −271, (−319); and **14**, −325, (−396). As may be seen, the M_D values are similar, verifying the assignment of configuration at the anomeric center of the new derivatives. [This correlation is especially apparent when it is considered that M_D values for methyl (tri-*O*-acetyl-α-D-glucopyranosid)uronates are very much larger, averaging +710 (see Table I in ref. 1)]***.

For the four tri-*O*-acetyl-β-D-glucopyranosiduronic methyl esters in Table I, their M_D values (which range from +139 to −396, and average −102), are similar to those provided by the corresponding derivatives in Table I of ref. 1, which range from −822 to +273, and average −124. Finally, it is evident from a comparison of the M_D values in Tables I and II of the present paper that hydrolysis of a given derivative to its free form results in only a small, variable, rotational shift.

Infrared spectral data. — It was shown in the earlier report[1] that anomeric pairs of steroid tri-*O*-acetyl-D-glucopyranosiduronic methyl esters and D-glucopyranoside tetraacetates can be distinguished by a band in the region of 1140–1146 cm^{-1} which is displayed by α-D anomers only. Similarly, Sauer *et al.*[9] noted that anomeric steroid 2-acetamido-2-deoxy-D-glucopyranosides and their respective triacetates can be distinguished by a sharply defined, intense band between 1110 and 1125 cm^{-1} that is shown by the α anomer only. This rule was substantiated in the present study to the extent that the band at 1140–1146 cm^{-1} is either absent or very weak for the derivatives in Table I.

The previous report[1] also noted that steroid D-glucopyranoside tetraacetates can be differentiated from methyl (tri-*O*-acetyl-D-glucopyranosid)uronates by means of a band in the region of 900 cm^{-1}; this band invariably lies above 900 cm^{-1} for the former class, and below it for the latter. This rule is well supported by the results in the present study: all 14 β-D-glucopyranoside peracetates in Table I show a well isolated, strong band within the range 904–918 cm^{-1}, whereas the 4 β-D-glucopyranosiduronic acid derivatives display a prominent band in the range 890–898 cm^{-1}. It may be added, from Tables I and II of the earlier study, that the position of this band is either uninfluenced by the configuration at the anomeric center, or occurs at a *slightly* higher frequency for the α anomer. Finally it is evident, as predicted, that very little useful information of this kind can be derived from the i.r. spectra of free β-D-glucopyranosides and β-D-glucopyranosiduronic acids or their salts.

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected; compounds were placed on the stage preheated to a temperature 10° below the m.p. Optical rotations were determined, with a Zeiss 0.005° photoelectric polarimeter, at a concentration of ~1.5% and a temperature of 24 ±1°. I. r. spectra

***It is to be noted that the two series of values differ to the extent that the observed M_D for a given methyl ester (the value in parentheses) is always slightly more negative than the M_D observed for the corresponding D-glucoside peracetate (the value set off by commas). It is suggested that these differences, which average −51 M_D units for the 12 examples, represent, in each instance, the difference between the rotational contribution of the methoxycarbonyl group containing C-6, and the acetylated primary hydroxyl group at C-6.

TABLE I

STEROID β-D-GLUCOPYRANOSIDE PERACETATES AND STEROID TRI-*O*-ACETYL-β-D-GLUCOPYRANOSIDURONIC METHYL ESTERS

Compound name and No.	*M.p., degrees*	$[\alpha]_D$, *degrees*[a]	M_D	*Formula*	% *C*		% *H*	
					Calc.	*Found*	*Calc.*	*Found*
Tetra-*O*-acetyl-β-D-glucopyranoside								
17-oxoandrost-5-en-3β-yl (**1**)	196–197[b]	−2	−12	$C_{33}H_{46}O_{11}$	64.06	64.29	7.49	7.53
17-oxo-5α-androstan-3α-yl (**2**)	182.5–183[c]	+22	+136	$C_{33}H_{48}O_{11}$	63.86	63.80	7.79	7.72
17-oxo-5α-androstan-3β-yl (**3**)	198–199[d]	+33	+205	$C_{33}H_{48}O_{11}$	63.86	63.48	7.79	7.84
17-oxo-5β-androstan-3α-yl (**4**)	208–209	+43	+267	$C_{33}H_{48}O_{11}$	63.86	63.90	7.79	7.85
17-oxo-5β-androstan-3β-yl (**5**)	234–235	+29	+180	$C_{33}H_{48}O_{11}$	63.86	63.63	7.79	7.56
20-oxopregn-5-en-3β-yl (**6**)	222–223	+8	+52	$C_{35}H_{50}O_{11}$	65.00	64.72	7.79	7.74
20-oxo-5α-pregnan-3α-yl (**7**)	161–162	+29	+188	$C_{35}H_{52}O_{11}$	64.79	64.82	8.08	8.05
20-oxo-5α-pregnan-3β-yl (**8**)	207–208	+39	+250	$C_{35}H_{52}O_{11}$	64.79	64.63	8.08	8.06
20-oxo-5β-pregnan-3α-yl (**9**)	168–169	+51	+331	$C_{35}H_{52}O_{11}$	64.79	64.60	8.08	8.11
20-oxo-5β-pregnan-3β-yl (**10**)	218–219	+33	+214	$C_{35}H_{52}O_{11}$	64.79	65.09	8.08	8.13
17α-hydroxy-20-oxo-5β-pregnan-3α-yl (**11**)	186–187	−2	−13	$C_{35}H_{52}O_{12}$	63.23	63.28	7.88	7.78
17α-hydroxy-20-oxo-5β-pregnan-3β-yl (**12**)	222–223	−23	−153	$C_{35}H_{52}O_{12}$	63.23	63.07	7.88	7.47
17β-acetoxyandrost-5-en-3β-yl (**13**)	181–182	−41	−271	$C_{35}H_{50}O_{12}$	63.43	63.10	7.60	7.82
3β-acetoxyandrost-5-en-17β-yl (**14**)	201–202	−49	−325	$C_{35}H_{50}O_{12}$	63.43	63.96	7.60	7.68
Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate								
17-oxo-5β-androstan-3β-yl (**15**)	162–163	+23	+139	$C_{32}H_{46}O_{11}$	63.35	63.49	7.64	7.59
20-oxo-5α-pregnan-3α-yl (**16**)	163–164	+26	+165	$C_{34}H_{50}O_{11}$	64.33	64.46	7.94	8.01
17β-acetoxyandrost-5-en-3β-yl (**17**)	186–187	−50	−319	$C_{34}H_{48}O_{12}$	62.95	62.90	7.46	7.43
3β-acetoxyandrost-5-en-17β-yl (**18**)	194–195	−62	−396	$C_{34}H_{48}O_{12}$	62.95	62.92	7.46	7.44

[a]In chloroform. [b]K. Miescher and W. H. Fischer [*Helv. Chim. Acta*, 21 (1938) 336] gave m.p. 192–193.5°. [c]K. Miescher C. Meystre, and J. Heer [*Helv. Chim. Acta*, 24 (1941) 988] gave m.p. 179–181°. [d]M.p. 191–192° (see footnote c)

TABLE II

STEROID β-D-GLUCOPYRANOSIDES AND SODIUM SALTS OF STEROID β-D-GLUCOPYRANOSIDURONIC ACIDS

Compound name and No.	*M.p., degrees*	$[\alpha]_D$, *degrees*[a]	M_D	*Formula*	% *C*		% *H*	
					Calc.	*Found.*	*Calc.*	*Found*
β-D-Glucopyranoside								
17-oxoandrost-5-en-3β-yl (**1a**)	219–221[b]	−17	−77	$C_{25}H_{38}O_7$	66.64	66.63	8.50	8.42
17-oxo-5α-androstan-3α-yl (**2a**)	233–234[c]	+46 (M)	+208	$C_{25}H_{40}O_7$	66.34	66.31	8.91	8.46
17-oxo-5α-androstan-3β-yl (**3a**)	221–222[d]	+30	+136	$C_{25}H_{40}O_7 \cdot 1.0\ H_2O$	63.80	63.57	9.00	9.10
17-oxo-5β-androstan-3α-yl (**4a**)	[e]	+47	+213	$C_{25}H_{40}O_7 \cdot 1.5\ H_2O$	62.60	62.68	9.04	9.14
17-oxo-5β-androstan-3β-yl (**5a**)	184–185	+40	+180	$C_{25}H_{40}O_7$	66.34	66.45	8.91	8.67
20-oxopregn-5-en-3β-yl (**6a**)	258–262	−6	−301	$C_{27}H_{42}O_7$	67.75	67.76	8.85	8.87
20-oxo-5α-pregnan-3α-yl (**7a**)	205–206	+45	+216	$C_{27}H_{44}O_7 \cdot 1.0\ H_2O$	65.03	64.60	9.30	9.33
20-oxo-5α-pregnan-3β-yl (**8a**)	246–247	+36	+173	$C_{27}H_{44}O_7$	67.47	67.15	9.23	9.26
20-oxo-5β-pregnan-3α-yl (**9a**)	184–185	+58	+279	$C_{27}H_{44}O_7 \cdot 2.0\ H_2O$	62.76	63.11	9.36	9.28
20-oxo-5β-pregnan-3β-yl (**10a**)	188–189	+43	+207	$C_{27}H_{44}O_7 \cdot 2.0\ H_2O$	62.76	62.38	9.36	9.33
17α-hydroxy-20-oxo-5β-pregnan-3α-yl (**11a**)	245–246	+5	+25	$C_{27}H_{44}O_8 \cdot 1.5\ H_2O$	61.92	61.50	9.05	9.18
17α-hydroxy-20-oxo-5β-pregnan-3β-yl (**12a**)	246–247	+11 (M)	+55	$C_{27}H_{44}O_8 \cdot 0.5\ H_2O$	64.13	64.20	8.97	8.97
17β-hydroxyandrost-5-en-3β-yl (**13a**)	290–291	−71 (P)	−321	$C_{25}H_{40}O_7$	66.34	66.28	8.91	8.90
3β-hydroxyandrost-5-en-17β-yl (**14a**)	256–257	−71 (M)	−321	$C_{25}H_{40}O_7$	66.34	66.30	8.91	8.90
(22*S*,25*S*)-5α-spirostan-3β-yl (**19a**)	267–268	−76 (D)	−440	$C_{33}H_{54}O_8$	68.48	68.30	9.40	9.44
β-D-Glucopyranosiduronate								
sodium 17-oxo-5β-androstan-3β-yl (**15a**)	273–275	+11 (M)	+54	$C_{25}H_{37}NaO_8$	61.46	61.64	7.63	8.11
sodium 20-oxo-5α-pregnan-3α-yl (**16a**)	258–259	+29 (M)	+150	$C_{27}H_{41}NaO_8 \cdot 2.5\ H_2O$	57.74	57.50	8.26	8.20
sodium 17β-hydroxyandrost-5-en-3β-yl (**17a**)	310	−74 (EW)	−362	$C_{25}H_{37}NaO_8 \cdot 5.0\ H_2O$	51.89	52.07	8.19	8.21
sodium (22*S*,25*S*)-spirost-5-en-3β-yl (**20a**)	282–284	−93 (PW)	−570	$C_{33}H_{49}NaO_9 \cdot 2.0\ H_2O$	61.09	60.79	8.23	8.27
sodium (22*S*,25*S*)-5α-spirostan-3β-yl (**21a**)	279–281	−64 (PW)	−393	$C_{33}H_{51}NaO_9 \cdot 2.5\ H_2O$	60.07	59.85	8.55	8.63
3β-Hydroxyandrost-5-en-17β-yl β-D-glucopyranosiduronic acid (**18a**)	225–226	−89 (M)	−415	$C_{25}H_{38}O_8 \cdot 1.5\ H_2O$	60.83	61.21	8.37	8.01

[a] In chloroform, unless otherwise indicated as follows: (M), methanol; (P), pyridine; (D), *p*-dioxane; (EW), 1:1 ethanol–water; (PW), 1:1 pyridine–water. [b] Z. Prochazka [*Collect. Czech. Chem. Commun.*, 33 (1968) 4039] gave m.p. 215–217°; $[\alpha]_D$ −17° (methanol); m.p. 223–225° (ref. b, Table I). [c] M.p. 228–229° (ref. c, Table I). [d] M.p. 216–217° (ref. b, Table I). [e] Indefinite m.p., due, apparently, to solvation.

were recorded, for dispersions in potassium bromide, with a Beckman IR-8 spectrometer; bands other than those discussed in the text are referred to only where they are of utility in structural assignment. T.l.c. (ascending) was used chiefly in selecting solvent systems for column chromatography, and for confirming the homogeneity of crystallized products. It was performed on sheets of silica gel IB-F with ethanol–chloroform, ethanol–chloroform–acetic acid, or ethyl acetate–2,2,4-trimethylpentane. Columns of silica gel (Davison, grade 923) were used for recovering products from Koenigs–Knorr syntheses; they were uniformly prepared, and were developed with appropriate mixtures of ethyl acetate with 2,2,4-trimethylpentane. The yields reported are based on products twice recrystallized and then dried over anhydrous calcium chloride *in vacuo*. Specimens for elemental analysis (by August Peisker–Ritter, Brugg, Switzerland, and Alfred Bernhard, Elbach über Engelskirchen, West Germany) were dried to constant weight over phosphoric anhydride under high vacuum at an appropriate temperature.

Preparation of sodium (17β-hydroxyandrost-5-en-3β-yl β-D-glucopyranosid)uronate (**17a**). — To a solution of 17-oxoandrost-5-en-3β-yl β-D-glucopyranosiduronic acid (200 mg) in a mixture of water (2 ml), methanol (10 ml), and 1M sodium hydroxide (0.5 ml) was added solid sodium borohydride (20 mg). After 2 h at room temperature, the excess of hydride was decomposed by the addition of a few drops of acetic acid, the solution was diluted with benzene and methanol, and the solvents were removed *in vacuo*. Crystallization of the product from aqueous methanol gave needles (186 mg, 89%); ν_{max} 1610 cm^{-1} (carboxylate), carbonyl absorption absent.

Synthesis of methyl (3β-acetoxyandrost-5-en-17β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**18**). — To a solution of androst-5-ene-3β,17β-diol 3-acetate (1.33 g, 4 mmoles) in anhydrous benzene (70 ml) were added methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (4.8 g, 12 mmoles) and freshly prepared silver oxide (1.85 g, 8 mmoles), and the suspension was actively shaken in the dark for 24 h at room temperature. Celite was then added, the suspension was filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on a column (45 × 900 mm) of silica gel prepared and developed with a system consisting of ethyl acetate (350 ml) diluted to 1000 ml with 2,2,4-trimethylpentane. Crystallization of the product from ethyl acetate–methanol gave 1.6 g (63%) of colorless needles; ν_{max} 1745–1775 and 1220–1265 (acetate), and 897 cm^{-1} (hydroxyl absorption absent).

3β-Acetoxyandrost-5-en-17β-yl tetra-O-acetyl-β-D-glucopyranoside (**14**) *from* **18**. — To a solution of methyl (3β-acetoxyandrost-5-en-17β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (400 mg) in anhydrous ether (150 ml) was added 200 mg of solid lithium aluminum hydride in one portion. After the mixture had been boiled under reflux for 3 h, the excess of hydride was decomposed by the successive, cautious addition of ethyl acetate and water; the mixture was the processed and re-acetylated according to the procedure outlined in the footnote on p. 386 of ref. 1. Two recrystallizations of the product from ethyl acetate–methanol gave 340 mg (82%) of **14** as needles; ν_{max} 1740–1770 and 1218–1265 (acetate), and 915 cm^{-1} (hydroxyl absorption absent).

A number of analyses were present in the manuscript of the earlier paper[1], but were not included in the version published; they are listed here under their original numbers for the compounds.

(7) *Anal.* Calc. for $C_{34}H_{48}O_{11}$: C, 64.54; H, 7.65; OMe, 4.90. Found: C, 64.60; H, 7.81; OMe, 4.99.

(10) *Anal.* Calc. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.94; OMe, 4.98. Found: C, 64.01; H, 7.98; OMe, 4.60.

(12) *Anal.* Calc. for $C_{32}H_{44}O_{11}$: C, 63.56; H, 7.33; OMe, 5.13. Found: C, 63.42; H, 7.50; OMe, 5.18.

(14) *Anal.* Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OMe, 5.11. Found: C, 63.26; H, 7.60; OMe, 5.10.

(15) *Anal.* Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OMe, 5.11. Found: C, 63.30; H, 7.69; OMe, 5.17.

(16) *Anal.* Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OMe, 5.11. Found: C, 63.28; H, 7.60; OMe, 5.28.

(22) *Anal.* Calc. for $C_{41}H_{64}O_{10}$: C, 68.68; H, 9.00. Found: C, 68.46; H, 8.94.

(22a) *Anal.* Calc. for $C_{41}H_{64}O_{10}$: C, 68.68; H, 9.00. Found: C, 68.23; H, 9.11.

(23) *Anal.* Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.40; H, 9.23.

(23a) *Anal.* Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.34; H, 9.20.

(24) *Anal.* Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.70; H, 9.04.

(24a) *Anal.* Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.46; H, 9.02.

(25) *Anal.* Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.54; H, 9.08.

ACKNOWLEDGMENTS

This work was made possible by a research grant, AM01255, from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service. We are grateful to this Institute for its continued and generous support of our research. Dr. David Fukushima kindly supplied the 3β-hydroxy-5β-androstan-17-one and 3α-hydroxy-5α-pregnan-20-one used in this project.

REFERENCES

1 J. J. SCHNEIDER, *Carbohyd. Res.*, 12 (1970) 369.
2 D. G. WILLIAMSON, D. C. COLLINS, D. S. LAYNE, R. B. CONROW, AND S. BERNSTEIN, *Biochemistry*, 8 (1969) 4299.
3 J. J. SCHNEIDER, *J. Biol. Chem.*, 245 (1970) 5505.
4 J. J. SCHNEIDER AND N. S. BHACCA, *J. Org. Chem.*, 34 (1969) 1990.
5 H. E. HADD AND R. T. BLICKENSTAFF, *Conjugates of the Steroid Hormones*, Academic Press, New York, N. Y., 1969.
6 J. J. SCHNEIDER AND D. K. FUKUSHIMA, *J. Chromatogr.*, 48 (1970) 509.
7 S. BERNSTEIN, J. P. DUSZA, AND J. P. JOSEPH, *Physical Properties of Steroid Conjugates*, Springer-Verlag, New York, N. Y., 1968.
8 S. LIEBERMAN, F. C. CHANG, M. R. BARUSCH, AND C. R. NOLLER, *J. Amer. Chem. Soc.*, 46 (1942) 2581.
9 G. SAUER, M. MATSUI, J. S. LIANG, AND D. K. FUKUSHIMA, *J. Org. Chem.*, 34 (1969) 3525.