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PREPARATION AND DIFFERENTIATION OF ANOMERIC PAIRS OF STEROID TRI-*O*-ACETYL-D-GLUCOPYRANOSIDURONIC METHYL ESTERS AND D-GLUCOPYRANOSIDE TETRAACETATES

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ABSTRACT

Twenty-eight steroid tri-*O*-acetyl-β-D-glucopyranosiduronic methyl esters have been prepared by a standard method, and isomerized to their α-D anomers with titanium tetrachloride. Reduction of seven such pairs with lithium aluminum hydride, followed by re-acetylation, provided the corresponding D-glucopyranoside tetraacetates. The configuration at the anomeric carbon atom assigned in each case was supported by (*a*) the anomerization increment, $M_{D_\alpha} - M_{D_\beta}$, which constituted a regular series within the limits of +704 to +985, and (*b*) the correspondence between observed and calculated M_D values. Members of the anomeric pairs, with the exception of those derived from the isosapogenins, were also differentiated by i.r. spectroscopy, chiefly by a band within the range of 1146–1140 cm^{-1} that is displayed only by the α-D anomers. Methyl (tri-*O*-acetyl-β-D-glucopyranosid)uronates were distinguished from the corresponding β-D-glucopyranoside tetraacetates both by i.r. spectroscopy and mass spectrometry.

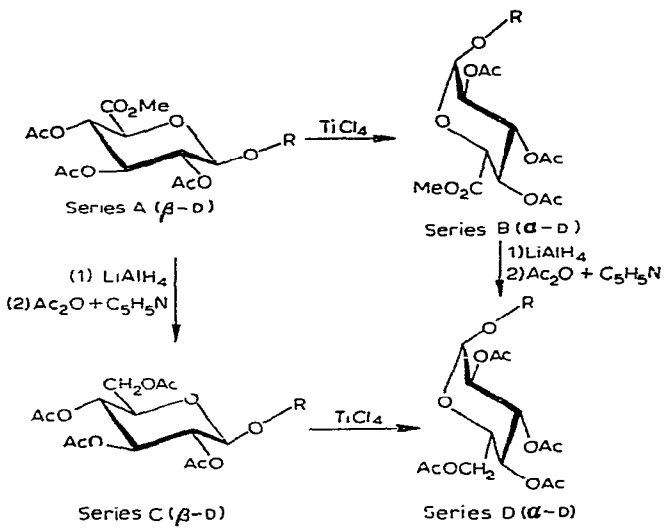
INTRODUCTION

Interest continues in the synthesis and metabolism of steroid β-D-glucopyranosiduronic acids, but it is evident from recent compilations[1] that the preparations of anomeric pairs has received little attention. We have therefore utilized experience gained in a recent synthesis of the methyl (tri-*O*-acetyl-β-D-glucopyranosid)uronate (**1**) of cholesterol[2] to prepare a total of 28 anomeric pairs of steroid D-glucopyranosiduronic acids and D-glucopyranosides, as the tri-*O*-acetyl methyl esters and tetraacetates, respectively. The steroids employed included cholesterol, the four possible stanols derived from it, and various 17-ketosteroids, pregnane derivatives, and isosapogenins. The preparation of these pairs is briefly described, their optical rotatory properties are recorded, and their differentiation by i.r. spectroscopy is discussed.

RESULTS AND DISCUSSION

Preparation of anomeric pairs. — The methyl (tri-*O*-acetyl-β-D-glucopyranosid)-uronates (Series A, Scheme 1) were prepared first, by using those conditions* that

*A benzene solution of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (12 mmoles) and the appropriate steroid (4 mmoles) was shaken for 24 h at room temperature with silver oxide (8 mmoles). For details, see ref. 2.



Scheme 1. Generalized formulas and preparative routes (members of series A and B are listed in Table I, and of series C and D in Table II). R = aglycon moiety.

had provided the cholesterol derivative **1** in a yield of 86%. Applied to the new aglycons, this method gave the β-D anomers of the pure esters in yields ranging from 38 to 77%. For the saturated members, anomerization of each ester to its α-D anomer (Series B) was effected with titanium tetrachloride in benzene or dichloromethane at room temperature. These conditions are notably milder than those employed by Pacsu[3] and Lindberg[4], who anomerized β-D-glucopyranoside tetraacetates of simpler aglycons by treatment with this reagent in refluxing chloroform. Following failure to anomerize **1** with this reagent directly, it was assumed that the procedure would fail with other unsaturated derivatives as well. Accordingly, the derivatives of cholesterol (**1**), pregnenolone (**7**), dehydroisoandrosterone (**12**), and diosgenin (**17**) in Series A were anomerized as their dibromides (assumed to have the 5α,6β configuration, analogous to that of cholesterol dibromide), after which the α-D anomers (**1a**, **7a**, **12a**, and **17a**, respectively, in Series B) were obtained by debromination with zinc in ether–acetic acid. In the saturated series, the β-D-glucopyranoside tetraacetates **23**, **24**, **25**, **26**, and **28** (Series C) were prepared from the corresponding methyl esters of Series A by reduction with lithium aluminum hydride in ether, followed by re-acetylation of the free D-glucopyranosides thus formed. The β-D-glucopyranoside tetraacetates were then anomerized as just described, affording Series D. Conversion of the unsaturated methyl [tri-*O*-acetyl-β(and α)-D-glucopyranosid]uronates (Series A and B, respectively) into the corresponding D-glucopyranoside tetraacetates (Series C and D, respectively) was effected by reduction. This method of preparing D-glucopyranosides was useful in this connection, but has limited utility, as it is restricted to compounds that have reducible groups at C-6 only.

Some interrelationships were established by hydrogenating the unsaturated derivatives **1**, **1a**, **7**, **7a**, **12**, **12a**, **17**, **17a**, **27**, and **27a** in neutral ethanol with palladium-

on-carbon as the catalyst. The products were identical, as judged by their melting points and i.r. spectra, with the saturated 5α derivatives **3**, **3a**, **10**, **10a**, **16**, **16a**, **19**, **19a**, **28**, and **28a**, respectively.

Derivatives **1**, **7**, **10**, **12**, **14**, **15**, **16**, **22**, **22a**, **23**, **23a**, **24**, **24a**, **25**, **26**, and **27** (that included three anomeric pairs) had been prepared or isolated by other workers; the rest are new. In addition to the usefulness of anomeric pairs for intercomparisons, it is necessary only to saponify the acetylated methyl esters of Series A in order to convert them into known, or potential, excretion forms (conjugates) of steroid metabolites. Moreover, the recent demonstration that surviving potato tissue can convert dehydroisoandrosterone into its β-D-glucopyranoside[5] shows that the formation of steroid D-glucopyranosides in plants can be studied by simple, *in vitro* techniques, and justifies further efforts to prepare them by chemical means.

The preparation of the methyl (tri-*O*-acetyl-β-D-glucopyranosid)uronate (**20**) of β-cortolone merits further mention. The 20,21-isopropylidene acetal of β-cortolone can be prepared in high yield and, after the Koenigs–Knorr condensation, hydrolytic removal of the isopropylidene group can be effected in a yield approaching 90%. As this group can be introduced and removed with equal ease in 17,20α,21-glycerols and 20α(and 20β),21-glycols[6], it is clear that this approach to the preparation of the 3-D-glucopyranosiduronates of steroidal side-chain glycols and glycerols has general utility.

Optical rotatory properties of anomeric pairs. — The optical rotations of the anomeric pairs were determined in chloroform, and are given in Tables I and II as molecular rotations (M_D). The value for $M_{D_\alpha} - M_{D_\beta}$, which constitutes the anomerization increment ($\Delta_{anom.}$) for each pair, lies, in this series*, within the narrow range of +704 to +984, with an average value of +823. The configuration at the anomeric center, which is determined by the mechanism of the reaction, is confirmed in each instance by the sign of the increment. The calculated[7] M_D values are also given; their essential agreement with the corresponding observed values supports the proposed assignments.

These data were also considered in terms of Hudson's rules of isorotation[8,9]. The second of these states that the A value, which corresponds to half the anomerization increment, is dependent solely on the nature of the aglycon group. As these increments form a regular series (see Tables I and II), it follows that the contributions of the various steroids are similar, irrespective of structural differences. In addition, the magnitude of the A values (which range from +352 to +492) is consistent with the size of the aglycon groups. The first rule states that the B value, which corresponds to half the sum of the observed molecular rotations of a given α,β-pair, is characteristic of the particular carbohydrate, and is independent of the substituent at C-1. As the carbohydrate moiety remains constant within each Table, these data should provide two sets of values, each comprising a series of similar α,β sums; but, when the value

*Anomerization increments based on rotations observed at 365 nm gave a similarly regular series: $M_{365_\alpha} - M_{365_\beta}$ lay within the range of +2028 to +2917; average, +2417.

TABLE I

ANOMERIC PAIRS OF STEROID METHYL (TRI-*O*-ACETYL-D-GLUCOPYRANOSID)URONATES: OPTICAL ROTATORY PROPERTIES[a] AND PRINCIPAL BANDS IN THE 1340–880-cm^{-1} REGION

Compound no.	*Anomeric form*	*Aglycon*	M_D *(obs.)*	$\Delta_{anom.}$	M_D *(calc.)*	*Infrared bands shown at*			
						915–880 cm^{-1}	1150–1130 cm^{-1}	1185–1155 cm^{-1}	1340–1330 cm^{-1}
1	β	Cholesterol	−232	+900	−256	886		1156 (m)	
1a	α		+668		+450	892	1140 (s)	1162 (w)	1330
2	β	Cholesterol dibromide	−518	+984	−341	892		1160 (m)	
2a	α		+466		+365	892	1141 (m)	1160 (m)	1332
3	β	Cholestanol	−35	+796	−12	891		1175 (s)	
3a	α		+761		+694	895	1142 (m)	1172 (w, sh)	1331
4	β	Epicholestanol	−106	+726	−4	890		1161 (m)	
4a	α		+620		+702	895	1145 (m)	1182 (w)	1330
5	β	Coprostanol	−78	+889	0	890		1175 (br, m)	1336 (vw)
5a	α		+811		+706	895	1142 (m)	1172 (vw, sh)	1330
6	β	Epicoprostanol	0	+754	+19	890		1168 (s)	
6a	α		+754		+725	897	1145 (m)	1169 (w)	1335
7	β	Pregnenolone	+44	+854	−21	890		1170 (m)	
7a	α		+898		+685	897	1141 (s)	1168 (m)	1340 (sh)
8	β	Pregnenolone dibromide	−201	+867	−177	896		1160 (m)	
8a	α		+666		+529	896	1142 (m)	1160 (m)	1335 (w, sh)
9	β	Pregnanolone	+273	+736	+246	890		1165 (br, m)	
9a	α		+1009		+952	895	1145 (m)	1170 (vw, sh)	1336 (w, sh)
10	β	Allopregnanolone	+184	+863	+154	892		1168 (m)	
10a	α		+1047		+860	896	1148 (m)	1163(vw)	1330 (sh)

11	β	17-Hydroxypregnanolone	−91	+768	−81	890		1160 (s)		
11a	α		+677		+625	895	1142 (m)	1165 (w, sh)	1330 (vw, sh)	
12	β	Dehydroisoandrosterone	−67	+889	−89	890	1138 (w)	1170 (s)		
12a	α		+822		+617	895	1144 (s)	1165 (w)	1335	
13	β	Dehydroisoandrosterone	−306	+895	−52	895		1159 (m)		
13a	α	dibromide	+589		+654	897	1142 (m)	1160 (m)	1330 (vw)	
14	β	Androsterone	+106	+713	+177	890		1160 (m)		
14a	α		+819		+885	891	1141 (m)	1160 (vw, sh)	1328	
15	β	Etiocholanolone	+194	+704	+216	890		1168 (m)		
15a	α		+898		+922	895	1141 (m)	1165 (w)	1330	
16	β	Isoandrosterone	+146	+849	+317	890		1165 (m)		
16a	α		+995		+1023	895	1146 (m)	1165 (vw, sh)	1328	
17	β	Diosgenin	−629	+943	−599			1169 (s)		
17a	α		+314		+107		1141 (m)	1185–65 (w, sh)	1330–40 (vw)	
18	β	Diosgenin dibromide	−882	+935	−790			1180 (w, sh)		
18a	α		+53		−84		1145 (w, sh)	1185 (w)	1330–40 (vw)	
19	β	Tigogenin	−440	+865	−380		1135 (vw)	1176 (s)		
19a	α		+425		+326		1132 (w, sh)	1172 (m)	1330–40 (vw)	
20	β	β-Cortolone	+27	+765	[b]	890		1168 (w, sh)		
20a	α		+792			891	1141 (m)	1185 (w, sh)	1330	
21	β	β-Cortolone acetonide[c]	+7	+831	+62	891		1160 (s)		
21a	α		+838		+768	891	1140 (w)	1160 (m)	1330	

[a] M_D (obs.) = $[\alpha]_D$ (obs.) × mol.wt./100. $\Delta_{anom.}$ (anomerization increment) = M_{D_α} (obs.) − M_{D_β} (obs.). Bands are characterized as br, broad; m, moderate; sh, shoulder; s, strong; w, weak; and vw, very weak. [b] Calculated M_D values not determined, because of the low solubility of β-cortolone in chloroform. [c] A trivial name for 3α,17α-dihydroxy-20,21-(isopropylidenedioxy)-5β-pregnan-11-one.

TABLE II

ANOMERIC PAIRS OF STEROID D-GLUCOPYRANOSIDE TETRAACETATES: OPTICAL ROTATORY PROPERTIES AND PRINCIPAL BANDS IN THE 1340–880-CM^{-1} REGION

Compound no.	*Anomeric form*	*Aglycon*	M_D *(obs.)*	$\Delta_{anom.}$	M_D *(calc.)*	*Infrared bands shown at* 915–880 cm^{-1}	1150–1130 cm^{-1}	1185–1155 cm^{-1}	1340–1330 cm^{-1}
22	β	Cholesterol	−194	+844	−221	908	1135 (w, sh)	1171 (s)	
22a	α		+652		+319	912	1145 (s)	1169 (m)	1331
23	β	Cholestanol	+22	+740	+23	908	1130 (w, sh)	1172 (s)	
23a	α		+762		+563	895–910	1145 (m)	1170 (s)	1332
24	β	Epicholestanol	−79	+776	+31	909	1135 (w, sh)	1168 (m)	
24a	α		+697		+571	890–905	1141 (s)	1168 (m)	1335
25	β	Coprostanol	−22	+863	+35	908	1136 (w, sh)	1171 (m)	
25a	α		+841		+575	905–913	1140 (m)	1169 (m)	1330
26	β	Epicoprostanol	+79	+711	+54	907	1136 (w, sh)	1169 (m)	
26a	α		+791		+594	910	1142 (m)	1168 (s)	1330
27	β	Diosgenin	−574	+850	−564		1136 (w)	1171 (m)	
27a	α		+276		−23		1141 (m, sh)	1170 (m)	
28	β	Tigogenin	−306	+732	−345		1130 (vw)	1175 (s)	
28a	α		+426		+195		1148 (m, sh)	1172 (s)	

in the Tables were considered in these terms, it was evident that the sums actually form a generally irregular series, which includes several highly anomalous, negative values. When the B values were re-calculated after subtracting the M_D value of the appropriate free steroid from the M_D value of the intact derivative, a somewhat more regular series was obtained. Three distinctly low values were encountered (+160, +76, and +142, from pairs **4**–**4a**, **11**–**11a**, and **13**–**13a**, respectively); the remaining values ranged from +212 to +395, averaging +298. It has been shown[10] that variations in A and B values are due to interactions between the carbohydrate and steroid portions of the molecule, and it is suggested that this factor may account for some of the irregular B values in the present series. In considering the calculated M_D values (see above), it was noted that the agreement, in this series, between calculated and observed M_D values was similar to that recorded by Nitta *et al.*[11] for a number of methyl ester triacetates, but far from the close correspondence observed by Becker[12], who examined several free D-glucopyranosiduronic acids. If divergences between calculated and observed M_D values are also caused by interactions, it may be suggested that such interactions are more marked in acetates. This thesis should readily be testable by re-determining the M_D values of the present series after hydrolysis to the free acids.

Infrared spectra of anomeric pairs. — Considerable information is available on the i.r. spectra of the peracetates of the common hexoses and of simpler glycosides and glycosiduronic acids (see reviews[13], and papers by Isbell and associates[14,15] and Nitta *et al.*[16]), but interest in the spectra of steroid D-glucopyranosiduronic acids and D-glucopyranosides has developed only within the past few years. Spectra of the compounds prepared in this study were obtained for potassium bromide dispersions, and were closely compared in the region of 1350–850 cm^{-1}, the range considered most useful for distinguishing between members of anomeric pairs. These data are compiled in Tables I and II for bands observed within the following regions: 915–880, 1150–1130, 1185–1155, and 1340–1330 cm^{-1}.

All of the methyl (tri-*O*-acetyl-D-glucopyranosid)uronates exhibit a sharp band, having medium to strong intensity, in the range 897–890 cm^{-1}. This band has no value for differentiating anomeric pairs, but it distinguishes the methyl ester triacetates from the D-glucopyranoside tetraacetates, since the latter display a similar band lying *above* 900 cm^{-1} (a sharp band in the range of 912–908 cm^{-1} for **22**, **22a**, **23**, **24**, **26**, and **26a***, and broader bands, as indicated, for **23a**, **24a**, and **25a**). This differentiation cannot be applied to derivatives of diosgenin and tigogenin, which show a strong, obscuring band at 900 cm^{-1} that is characteristic of isosapogenins.

All of the derivatives examined display a band (which sometimes appears as a shoulder or is flanked by a second, weaker band) in the range 1185–1156 cm^{-1}, varying in intensity from compound to compound, from very weak to strong. This

*The β-D-glucopyranoside tetraacetates of pregnenolone, pregnanolone, allopregnanolone, 17-hydroxypregnanolone, dehydroisoandrosterone, androsterone, etiocholanolone, isoandrosterone, and β-cortolone, which we have recently prepared, but which are not formally described in this paper, show sharp bands in the range 906–909 cm^{-1}.

band is usually weaker for the α-D member of an anomeric pair, but members cannot be differentiated by this means alone. Such differentiation depends on the observation that only α-D anomers, with the inevitable exceptions provided by the isosapogenins, also display a sharp band, of moderate to strong intensity, in the range 1146–1140 cm^{-1}. One methyl ester triacetate (**12**) and all of the β-D-glucopyranoside tetraacetates show a second, usually very weak, band in the range 1138–1130 cm^{-1}; its low intensity and displacement serve to distinguish it from the band at 1146–1140 cm^{-1}. It would appear that the "α" band, in the range 1146–1140 cm^{-1}, permits the assured differentiation of anomeric pairs of steroid tri-*O*-acetyl-D-glucopyranosiduronic methyl esters and D-glucopyranoside tetraacetates.

In Tables I and II are also listed bands generally observed in the range 1335–1330 cm^{-1}; these range in intensity from moderate to weak (but well defined) bands, to weak, broad bands. With one exception (**5**), they were noted only for the α-D anomers; they were either absent or very obscure for the isosapogenins. Where clearly present, these bands serve to supplement data from the region of 1150–1130 cm^{-1}, but their usefulness is limited by their low intensity.

A general comparison of these bands with those recorded for simple methyl (tri-*O*-acetyl-D-glucopyranosid)uronates and D-glucopyranoside tetraacetates is unfortunately not possible, as most of the latter spectra were determined for chloroform solutions, rather than for potassium bromide dispersions; significantly different spectra may be obtained with the two media, as shown, for example, in Fig. 3 of the paper of Nitta *et al.*[16]. The spectral characteristics of one anomeric pair, in potassium chloride dispersion, may be mentioned, namely the methyl α(and β)-D-glucopyranoside tetraacetates[15]. Bands for the α anomer include 1180 (w), and *1145* cm^{-1} (s), whereas those for the β anomer are at *1175* (s), 1140 (m), and 1110 cm^{-1} (m). The italicized values clearly link these results with those in Tables I and II. A few i.r. spectra of steroidal β-D-glucopyranosiduronic acids and esters have been reported; these include the spectra of eleven methyl ester triacetates in the amorphous and crystalline states[17], four free acids recorded in the same way[12], and five methyl ester triacetates in a crystalline form[11]. From these data and our observations, it is evident that the most useful spectra, particularly as regards the 1185–1130-cm^{-1} region, are derived from acetylated forms in a dispersed, crystalline state.

Mass-spectral studies. — The fragmentation patterns of seven pairs of Series A and C derivatives were determined, in order to extend earlier observations[2] bearing on their differentiation. The results are summmarized in Table III, in which ion intensities are expressed as percentages of the base ion, *m/e* 43 (CH_3CO^+).

Molecular (M^+) ions were observed for all compounds except one (*e*), and were unexpectedly prominent for the *g*,*g'* pair, indicating the considerable stability of these rather complex compounds. As the free 17,20β,21-glycerol derived from *g* also provided a large M^+ ion, it follows that the noted stability is not conferred by the isopropylidene group alone.

The pyronium ions *m/e* 317 and *m/e* 331 (see Scheme 2) are derived from Series A and C, respectively. As Table III illustrates, these ions provide a reliable means for

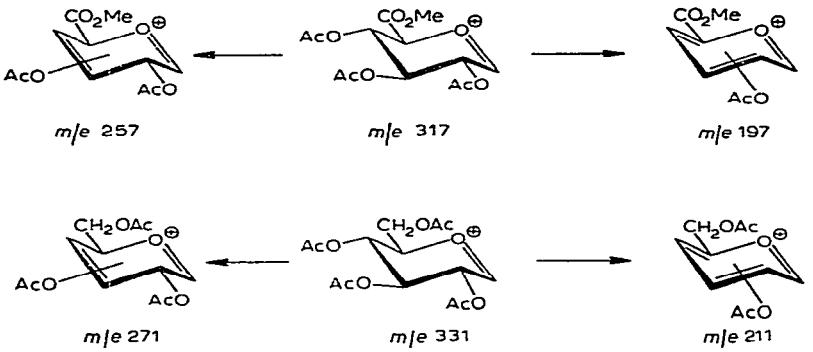
TABLE III

RELATIVE INTENSITIES OF IONS IN METHYL (TRI-*O*-ACETYL-β-D-GLUCOPYRANOSID)URONATE (SERIES A) AND β-D-GLUCOPYRANOSIDE TETRAACETATE (SERIES C) DERIVATIVES

Series	*Compound no.*	*Aglycon*	M^+	m/e					
				331	*317*	*271*	*257*	*211*	*197*
A	a (1)[a]	Cholesterol	0.03[b]		2		6		6
	b (3)	Cholestanol	0.03		3		15		3
	c (7)	Pregnenolone	0.05		3		4		5
	d (10)	Allopregnanolone	0.01		2		6		2
	e (12)	Dehydroisoandrosterone			4	40	7		6
	f (16)	Isoandrosterone	0.04		1	3	29	1	2
	g (21)	β-Cortolone acetonide	0.25	6	63	3	30	2	16
C	a′ (22)	Cholesterol	0.02	6		2		2	
	b′ (23)	Cholestanol	0.08	2		2	9		
	c′	Pregnenolone	0.02	10		2	1	3	1
	d′	Allopregnanolone	0.02	3		1	9	1	
	e′	Dehydroisoandrosterone	0.02	11		51		4	2
	f′	Isoandrosterone	0.05	2		2	4		
	g′	β-Cortolone acetonide	0.87	19		6		2	

[a]Lettered designations are for the purpose of this Table and discussion only; arabic numbers in parentheses are those generally assigned. Compounds *c′* through *g′* were prepared especially for this study, and are referred to in the i.r. section only. [b]Intensities are expressed as percent of that observed for the *m/e* 43 base ion.

distinguishing between the two classes. The apparently exceptional *m/e* 331 ion furnished by *g* is believed not to be a pyronium ion, since the required *m/e* 317 ion was also present. The free 17,20β,21-glycerol obtained from *g* provided a wholly analogous pattern, and it is therefore suggested that the *m/e* 331 ion observed for *g* was derived from a steroid residue lacking an isopropylidene group, possibly corresponding to $C_{21}H_{31}O_3$ (331.46). The presence of the isopropylidene group in *g* and *g'*, and its absence in the 17,20β,21-glycerol, was clearly shown by the characteristic *m/e* 101 ion in the spectra of the first two.



Scheme 2. Ion fragments useful in distinguishing between steroid tri-*O*-acetyl-β-D-glucopyranosiduronic methyl esters and β-D-glucopyranoside tetraacetates.

It is clear from Table III that prominent among the further reactions of the *m/e* 317 ion, is the loss of both one and two molecules of acetic acid, yielding the *m/e* 257 and *m/e* 197 ions, respectively. The further fragmentation of the *m/e* 331 ion appears to follow a similar course, furnishing, in this case, the *m/e* 271 ion and, less regularly, the well known[18,19] *m/e* 211 ion*. Our earlier suggestion[2] that the *m/e* 331 ion undergoes an initial expulsion of the $CH_3COOCH_2^+$ fragment followed by the loss of one molecule of acetic acid, successively yielding the *m/e* 257 and *m/e* 197 ions, is not supported by these data. It seems probable that the large *m/e* 271 ion derived from *e* and *e'* represents a steroid component, possibly 3,5-androstadiene-17-one (270.4).

It has been suggested[18] that, under favorable circumstances, it may be possible to distinguish anomers on the basis of their mass spectra. We have attempted to test this view with the present pairs, but differences, if present, were too small to be detected by using the direct-introduction technique.

EXPERIMENTAL

General. — Melting points were obtained 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.

*The presence of the M-60, M-120, and M-180 ions of various intensities in the spectra of all derivatives indicates the successive loss of three molecules of acetic acid.

spectra were recorded with a Beckman IR-8 spectrometer; they were in accord with the assigned structures, but, as most of them displayed few bands in the 4000–1330-cm^{-1} region, except those regularly occurring at 1745–1760 and 1260–1205 (acetate), 1445–1435, and 1375–1365 cm^{-1}, they are referred to only where they provide additional information. Mass spectra were determined by Dr. Robert Schaffer, of the Morgan–Schaffer Corporation, Montreal, Canada, with a Hitachi–Perkin–Elmer RMU-6D spectrometer. T.l.c. was performed on silica gel (Camag DF-5) supported on glass plates. Columns were prepared with silica gel (Davison, grade 923); the mobile phases consisted of appropriate mixtures of ethyl acetate and 2,2,4-trimethylpentane. Yields reported are based on products that had been twice recrystallized and dried over anhydrous calcium chloride *in vacuo*. Specimens for elementary analysis (by August Peisker-Ritter, Brugg, Switzerland, and Alfred Bernhardt, Elbach über Engelskirchen, West Germany) were dried to constant weight over phosphoric anhydride under high vacuum at an appropriate temperature.

Methyl (5α,6β-dibromocholestan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**2**) *from* **1**. — To a solution of methyl (cholest-5-en-3β-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate[2] (**1**, 500 mg) in ether (25 ml) was added, in one portion, a solution of bromine (75 μl) and anhydrous sodium acetate (20 mg) in acetic acid (2.5 ml). After the mixture had been stirred for about 3 min at room temperature, the product separated spontaneously in crystalline form. The flask was chilled, and the crystals were dispersed by the addition of methanol, collected by filtration, and washed with methanol. Recrystallization from ether–methanol afforded 532 mg (87%) of the pure dibromide, m.p. 173–174° (dec.); $[\alpha]_D$ −60°, $[\alpha]_{365}$ −197°.

Anal. Calc. for $C_{40}H_{62}Br_2O_{10}$: C, 55.68; H, 7.24; Br, 18.52. Found: C, 55.50; H, 7.21; Br, 18.65.

Methyl (5α,6β-dibromocholestan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**2a**) *from* **2**. — To a solution of **2** (466 mg) in benzene (5 ml) was added 0.1 ml of titanium tetrachloride (Fisher, purified grade). As the solution darkened rapidly, it was diluted with ethyl acetate after 30 min, washed with neutral brine, dried, and evaporated. Crystallization from ethyl acetate–methanol furnished 311 mg (67%) of needles, m.p. 185–186° (dec.); $[\alpha]_D$ +54°, $[\alpha]_{365}$ +141°.

Anal. Calc. for $C_{40}H_{62}Br_2O_{10}$: C, 55.68; H, 7.24; Br, 18.52. Found: C, 56.01; H, 7.20; Br, 18.42.

Methyl (cholest-5-en-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**1a**) *from* **2a**. — To a solution of **2a** (573 mg) in ether (30 ml) and acetic acid (10 ml) was added zinc powder (1.5 g), and the suspension was stirred for 10 min at room temperature. The supernatant liquor was diluted with ethyl acetate, washed successively with water, cold 2M sodium hydroxide, and neutral brine, dried, and evaporated. Crystallization from ethyl acetate–ethanol provided 420 mg (90%) of needles, m.p. 191–192°; $[\alpha]_D$ +95°, $[\alpha]_{365}$ +274°.

Anal. Calc. for $C_{40}H_{62}O_{10}$: C, 68.35; H, 8.89; OCH_3, 4.41. Found: C, 68.31; H, 8.93; OCH_3, 4.46.

Methyl (5α-cholestan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**3**). —

Compound **3** was prepared from 5α-cholestan-3β-ol (cholestanol) as described for the preparation of **1** from cholesterol[2]. Crystallization from ethyl acetate–methanol gave needles in a yield of 67%, m.p. 180–181°; $[\alpha]_D$ −5°, $[\alpha]_{365}$ −15°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.18; H, 9.14; OCH_3, 4.42.

Methyl (5α-cholestan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**3a**) *from* **3**. — To a solution of **3** (100 mg) in benzene (2 ml) was added 0.05 ml of titanium tetrachloride. After the yellow complex had been dispersed with glass beads, the suspension was kept for 12 h at room temperature. The product was isolated as in the preparation of **2a** from **2**, to give 78 mg (78%) of needles from acetone–methanol, m.p. 175–175.5°; $[\alpha]_D$ +108°, $[\alpha]_{365}$ +315°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.14; H, 9.30; OCH_3, 4.70.

Methyl (5α-cholestan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**4**). — Compound **4** was prepared from 5α-cholestan-3α-ol (epicholestanol) as for the synthesis of **1**. Crystallization of the product from ethyl acetate–methanol gave long needles; yield 46%, m.p. 191–192°, $[\alpha]_D$ −15°, $[\alpha]_{365}$ −45°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.20; H, 9.11; OCH_3, 4.38.

Methyl (5α-cholestan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**4a**) *from* **4**. — Treatment of **4** (100 mg) with titanium tetrachloride, as in the preparation of **3a** from **3**, gave 74 mg (74%) of needles from acetone–methanol, m.p. 147–147.5°; $[\alpha]_D$ +88°, $[\alpha]_{365}$ +258°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.36; H, 9.21; OCH_3, 4.42.

Methyl (5β-cholestan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**5**). — Compound **5** was prepared from 5β-cholestan-3β-ol (coprostanol) as in the synthesis of **1**. Crystallization from acetone furnished needles (38%), m.p. 182–183°, $[\alpha]_D$ −11°, $[\alpha]_{365}$ −29°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.19; H, 9.15; OCH_3, 4.39.

Methyl (5β-cholestan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**5a**) *from* **5**. — Treatment of **5** (100 mg) with titanium tetrachloride, as in the preparation of **3a** from **3**, afforded 50 mg (50%) of needles from hexane, m.p. 159–160°; $[\alpha]_D$ +115°, $[\alpha]_{365}$ +334°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.20; H, 9.19; OCH_3, 4.59.

Methyl (5β-cholestan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**6**). — Compound **6** was obtained from 5β-cholestan-3α-ol (epicoprostanol) as in the preparation of **1**. Crystallization from ethyl acetate–methanol provided needles (56%), m.p. 176–177°; $[\alpha]_D$ 0°, $[\alpha]_{365}$ 0°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.12; H, 9.05; OCH_3, 4.48.

Methyl (5β-cholestan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**6a**) *from* **6**. — Anomerization of **6** (100 mg), as in the preparation of **3a** from **3**, gave 64 mg (64%) of needles from methanol, m.p. 136.5–137.5°; $[\alpha]_D$ +107°, $[\alpha]_{365}$ +311°.

Anal. Calc. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; OCH_3, 4.40. Found: C, 68.07; H, 9.05; OCH_3, 4.38.

Methyl (20-oxopregn-5-en-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**7**). — Compound **7** was prepared from 3β-hydroxypregn-5-en-20-one (pregnenolone) as in the synthesis of **1**. The product was obtained as needles from methanol (54%), m.p. 184–185°; $[\alpha]_D$ +7°, $[\alpha]_{365}$ +150°; lit.[20] m.p. 183–184°, $[\alpha]_D$ +5° (chloroform).

Methyl (5α,6β-dibromo-20-oxopregnan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**8**) *from* **7**. — To a solution of **7** (633 mg; 1 mmole) in chloroform was added, in one portion, a solution of bromine (61 μl; 1.2 mmoles) in 0.15 ml of acetic acid. As soon as decolorization was complete (~3 min), the solution was successively washed with cold, dilute sodium hydroxide and water, dried, and evaporated. Crystallization of the product from dichloromethane–methanol provided 525 mg (66%) of needles, m.p. 171–172° (dec.); $[\alpha]_D$ −38°, $[\alpha]_{365}$ −18°.

Anal. Calc. for $C_{34}H_{48}Br_2O_{11}$: C, 51.52; H, 6.10; Br, 20.17. Found: C, 51.40; H, 6.08; Br, 20.31.

Methyl (5α, 6β-dibromo-20-oxopregnan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**8a**) *from* **8**. — To a solution of **8** (400 mg) in dichloromethane (20 ml) was added titanium tetrachloride (0.20 ml). After 2 h at room temperature, the solution was successively washed with dilute, cold sodium hydroxide and water, dried, and evaporated. Crystallization of the product from dichloromethane–methanol provided 305 mg (75%) of needles, m.p. 196.5–197.5° (dec.); $[\alpha]_D$ +84°, $[\alpha]_{365}$ +346°.

Anal. Calc. for $C_{34}H_{48}Br_2O_{11}$: C, 51.52; H, 6.10; Br, 20.17. Found: C, 51.44; H, 6.00; Br, 19.98.

Methyl (20-oxopregn-5-en-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**7a**) *from* **8a**. — To a stirred solution of **8a** (150 mg) in ether (10 ml) and acetic acid (3 ml) was added powdered zinc (500 mg) during 10 min. Isolation of the product as in the preparation of **1a** from **2a**, followed by crystallization from dichloromethane–methanol, gave 120 mg (80%) of needles, m.p. 197–198°; $[\alpha]_D$ +142°, $[\alpha]_{365}$ +564°.

Anal. Calc. for $C_{34}H_{48}O_{11}$: C, 64.54; H, 7.65; OCH_3, 4.90. Found: C, 64.31; H, 7.70; OCH_3, 4.79.

Methyl (20-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**9**). — Compound **9** was prepared from 3α-hydroxy-5β-pregnan-20-one (pregnanolone) as in the synthesis of **1**. The product crystallized as needles from ethyl acetate–methanol; yield 44%, m.p. 197.5–198.5°; $[\alpha]_D$ +43°, $[\alpha]_{365}$ +270°.

Anal. Calc. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.94; OCH_3, 4.98. Found: C, 64.64; H, 8.02; OCH_3, 5.15.

Methyl (20-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**9a**) *from* **9**. — To a solution of **9** (100 mg) in benzene (3 ml) was added 0.05 ml of titanium tetrachloride. After 5 h at room temperature, the product was isolated;

it furnished, from ethyl acetate–methanol, 80 mg (80%) of plates, m.p. 222–223°; $[\alpha]_D$ +159°, $[\alpha]_{365}$ +610°.

Anal. Calc. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.94; OCH_3, 4.98. Found: C, 64.21; H. 7.74; OCH_3, 4.93.

Methyl (20-oxo-5α-pregnan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**10**). — Compound **10** was obtained from 3β-hydroxy-5α-pregnan-20-one (allopregnanolone) as in the synthesis of **1**. Crystallization of the product from ethyl acetate–methanol gave needles; yield 51%, m.p. 203–204°; $[\alpha]_D$ +29°, $[\alpha]_{365}$ +244°; lit.[21] m.p. 201–202°.

Methyl (20-oxo-5α-pregnan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**10a**) *from* **10**. — Anomerization of **10** (100 mg), as in the preparation of **9a** from **9**, gave 68 mg (68%) of needles from ethyl acetate–methanol, m.p. 177–178°; $[\alpha]_D$ +165°, $[\alpha]_{365}$ +629°.

Anal. Calc. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.94; OCH_3, 4.98. Found: C, 64.31; H, 7.80; OCH_3, 4.94.

Methyl (17α-hydroxy-20-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**11**). — Compound **11** was prepared from 3α,17α-dihydroxy-5β-pregnan-20-one (17-hydroxypregnanolone), as in the synthesis of **1**. The product crystallized from ethyl acetate as needles, yield 47%, m.p. 213–213.5°; $[\alpha]_D$ −14°, $[\alpha]_{365}$ −11°.

Anal. Calc. for $C_{34}H_{50}O_{12}$: C, 62.75; H, 7.75; OCH_3, 4.77. Found: C, 62.72; H, 7.69; OCH_3, 5.01.

Methyl (17α-hydroxy-20-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**11a**) *from* **11**. — Treatment of **11** (100 mg) with titanium tetrachloride, as in the preparation of **9a** from **9**, gave 83 mg (83%) of needles from ethyl acetate–methanol, m.p. 211–212°; $[\alpha]_D$ +104°, $[\alpha]_{365}$ +313°.

Anal. Calc. for $C_{34}H_{50}O_{12}$: C, 62.75; H, 7.75; OCH_3, 4.77. Found: C, 62.66; H, 7.50; OCH_3, 4.91.

Methyl (17-oxoandrost-5-en-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**12**). — Compound **12** was prepared from 3β-hydroxyandrost-5-en-17-one (dehydroisoandrosterone) as in the synthesis of **1**. Crystallization from methanol furnished needles (77%), m.p. 194–194.5°; $[\alpha]_D$ −11°, $[\alpha]_{365}$ −102°; lit.[20] m.p. 195–195.5°, $[\alpha]_D$ −11° (chloroform).

Methyl (5α,6β-dibromo-17-oxoandrostan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**13**) *from* **12**. — Bromination of compound **12** was effected as in the preparation of **8** from **7**. Crystallization from dichloromethane–methanol afforded needles (74%), m.p. 153–154° (dec.); $[\alpha]_D$ −40°, $[\alpha]_{365}$ −19°.

Anal. Calc. for $C_{32}H_{44}Br_2O_{11}$: C, 50.27; H, 5.80; Br, 20.90. Found: C, 50.14; H, 5.84; Br, 21.06.

Methyl (5α,6β-dibromo-17-oxoandrostan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**13a**) *from* **13**. — Treatment of compound **13** with titanium tetrachloride was conducted as in the preparation of **8a** from **8**, except that a reaction time of

3 h was used. Crystallization from dichloromethane–methanol gave needles (90%), m.p. 180.5–181.5° (dec.); $[\alpha]_D$ +77°, $[\alpha]_{365}$ +323°.

Anal. Calc. for $C_{32}H_{44}Br_2O_{11}$: C, 50.27; H, 5.80; Br, 20.90. Found: C, 49.96; H, 5.72; Br, 21.85.

*Methyl (17-oxoandrost-5-en-3β-yl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosid)uronate* (**12a**) *from* **13a**. — Debromination of compound **13a** was performed as in the preparation of **7a** from **8a**. The product crystallized from aqueous methanol as needles (88%), m.p. 156.5–157°; $[\alpha]_D$ +136°, $[\alpha]_{365}$ +539°.

Anal. Calc. for $C_{32}H_{44}O_{11}$: C, 63.56; H, 7.33; OCH_3, 5.13. Found: C, 63.67; H, 7.30; OCH_3, 5.04.

*Methyl (17-oxo-5α-androstan-3α-yl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosid)uronate* (**14**). — Compound **14** was prepared from 3α-hydroxy-5α-androstan-17-one (androsterone) as in the synthesis of **1**. Crystallization of the product from ethyl acetate–hexane afforded needles in a yield of 42%, m.p. 181–182°; $[\alpha]_D$ +17°, $[\alpha]_{365}$ +196°; lit.[22] m.p. 175.5–176°, $[\alpha]_D$ +12° (chloroform).

*Methyl (17-oxo-5α-androstan-3α-yl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosid)uronate* (**14a**) *from* **14**. — Treatment of compound **14** (100 mg) with titanium tetrachloride was conducted as in the preparation of **9a** from **9**. The product was crystallized (with difficulty) from aqueous methanol (yield, 70%); m.p. indefinite*; $[\alpha]_D$ +135°, $[\alpha]_{365}$ +541°.

Anal. Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OCH_3, 5.11. Found: C, 63.20; H, 7.39; OCH_3, 5.09.

*Methyl (17-oxo-5β-androstan-3α-yl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosid)uronate* (**15**). — Compound **15** was prepared from 3α-hydroxy-5β-androstan-17-one (etiocholanolone) as in the synthesis of **1**. The product crystallized from ethyl acetate–hexane as needles (63%), m.p. 181–182°; $[\alpha]_D$ +32°, $[\alpha]_{365}$ +235°; lit.[22] m.p. 176.5–178°, $[\alpha]_D$ +29° (chloroform).

*Methyl (17-oxo-5β-androstan-3α-yl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosid)uronate* (**15a**) *from* **15**. — Anomerization of compound **15** was performed as in the preparation

*The melting points of **14a**, **15a**, **16a**, **20**, and **21** were indefinite, and are therefore not recorded. Extended drying over a suitable desiccant *in vacuo* at 60–100° was without effect. In order to determine whether these derivatives were contaminated with unreacted free steroid, samples of each were treated with acetic anhydride and pyridine, recovered in the usual way, and examined by t.l.c. This procedure would readily reveal contaminating free steroid, as the mobilities of the methyl ester triacetates and the acetates of the steroidal aglycons differ greatly. In all cases, this test gave negative results. (Contaminating free steroid can be detected by mass spectrometry, provided that the steroid is appreciably more volatile than the corresponding methyl ester triacetate; see ref. 2 in this regard).

Another possibility was that **14a**, **15a**, **16a**, and **20a** might contain some unreacted anomer; its presence or absence was difficult to establish with certainty by t.l.c., as the R_F differences between members of anomeric pairs are frequently too small to be useful. As an alternative test, fresh samples of each β-D precursor, namely **14**, **15**, **16**, and **20**, were treated with titanium tetrachloride for a period of time twice that originally employed. The crystallizing characteristics and melting-point behavior of the products were the same as those originally observed for **14a**, **15a**, **16a**, and **20a**.

These results, together with the satisfactory elemental analyses and the optical rotatory behavior, which was as expected, indicated that the compounds were substantially pure.

of **9a** from **9**. Crystallization from acetone–hexane gave needles (82%); $[\alpha]_D$ +148°, $[\alpha]_{365}$ +569°.

Anal. Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OCH_3, 5.11. Found: C, 63.47; H, 7.43; OCH_3, 5.10.

Methyl (17-oxo-5α-androstan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**16**). — Compound **16** was prepared from 3β-hydroxy-5α-androstan-17-one (iso-androsterone) as in the synthesis of **1**. The product crystallized from ethyl acetate–hexane as needles (63%), m.p. 147–148°; $[\alpha]_D$ +24°, $[\alpha]_{365}$ +217°; lit. m.p. 163–164°, $[\alpha]_D$ +19° (chloroform[23]; m.p. 168–170°, $[\alpha]_D$ +28° (chloroform)[20].

Methyl (17-oxo-5α-androstan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**16a**) *from* **16**. — Treatment of compound **16** with titanium tetrachloride, as in the preparation of **9a** from **9**, gave crystals from aqueous methanol (with difficulty) in a yield of 70%; $[\alpha]_D$ +164°, $[\alpha]_{365}$ +626°.

Anal. Calc. for $C_{32}H_{46}O_{11}$: C, 63.35; H, 7.64; OCH_3, 5.11. Found: C, 63.23; H, 7.44; OCH_3, 5.09.

Methyl ($25_{\alpha F}$-spirost-5-en-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**17**). — Compound **17** was prepared from $25_{\alpha F}$-spirost-5-en-3β-ol (diosgenin) as in the synthesis of **1**. The product crystallized from ethyl acetate–methanol as long needles (60%), m.p. 207–208°; $[\alpha]_D$ −86°, $[\alpha]_{365}$ −246°.

Anal. Calc. for $C_{40}H_{58}O_{12}$: C, 65.73; H, 8.00; OCH_3, 4.25. Found: C, 65.78; H, 7.96; OCH_3, 4.14.

Methyl (5α,6β-dibromo-$25_{\alpha F}$-spirostan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**18**) *from* **17**. — Bromination of compound **17** was effected as in the preparation of **13** from **12**. Crystallization of the product from ethyl acetate–methanol (with avoidance of heat) gave needles (66%), m.p. 166–167° (dec.); $[\alpha]_D$ −99°, $[\alpha]_{365}$ −308°.

Anal. Calc. for $C_{40}H_{58}Br_2O_{12}$: C, 53.94; H, 6.56; Br, 17.94. Found: C, 53.69; H, 6.51; Br, 18.24.

Methyl (5α,6β-dibromo-$25_{\alpha F}$-spirostan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**18a**) *from* **18**. — Treatment of compound **18** with titanium tetrachloride was conducted as in the anomerization of **13**, except that the reaction time was extended to 4 h. Crystallization from dichloromethane–methanol afforded needles (68%), m.p. 217–218° (*dec.*); $[\alpha]_D$ +6°, $[\alpha]_{365}$ +6°.

Anal. Calc. for $C_{40}H_{58}Br_2O_{12}$: C, 53.94; H, 6.56; Br, 17.94. Found: C, 53.98; H, 6.72; Br, 17.34.

Methyl ($25_{\alpha F}$-spirost-5-en-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**17a**) *from* **18a**. — Debromination of compound **18a** was performed as in the preparation of **12a** from **13a**. The product crystallized from dichloromethane–methanol as needles (82%), m.p. 218–219°; $[\alpha]_D$ +43°, $[\alpha]_{365}$ +131°.

Anal. Calc. for $C_{40}H_{58}O_{12}$: C, 65.73; H, 8.00; OCH_3, 4.25. Found: C, 65.70; H, 8.04; OCH_3, 4.25.

Methyl ($25_{\alpha F}$-5α-spirostan-3β-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**19**). — Compound **19** was prepared from $25_{\alpha F}$-5α-spirostan-3β-ol (tigogenin) as in

the synthesis of **1**. Crystallization from methanol gave needles (43%), m.p. 234–235°; $[\alpha]_D$ −60°, $[\alpha]_{365}$ −171°.

Anal. Calc. for $C_{40}H_{60}O_{12}$: C, 65.55; H, 8.23; OCH_3, 4.23. Found: C, 65.68; H, 8.31; OCH_3, 4.33.

Methyl (25$_{\alpha F}$-5α-spirostan-3β-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**19a**) *from* **19**. — Anomerization of compound **19**, as in the preparation of **9a** from **9**, gave needles from ethyl acetate (70%), m.p. 246.5–247.5°; $[\alpha]_D$ +58°, $[\alpha]_{365}$ +172°.

Anal. Calc. for $C_{40}H_{60}O_{12}$: C, 65.55; H, 8.23; OCH_3, 4.23. Found: C, 65.57; H, 8.02; OCH_3, 4.16.

3α,17α-Dihydroxy-20β,21-(isopropylidenedioxy)-5β-pregnan-11-one. — To a concentrated solution of 3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one[24] (5 g) in warm methanol was added acetone (2 liters). The solution was cooled to room temperature, and *p*-toluenesulfonic acid (1.25 g) was added. After 20 min at room temperature, M sodium hydroxide (7.5 ml) was added, and the solution was concentrated almost to dryness *in vacuo*. The residue was dissolved in wet ethyl acetate, and the solution was washed with neutral brine, dried, and evaporated. Crystallization from acetone gave 5.05 g (90%) of prisms, m.p. 186–187°, $[\alpha]_D$ +40°.

Anal. Calc. for $C_{24}H_{38}O_5$: C, 70.90; H, 9.43. Found: C, 70.68; H, 9.15.

Methyl [17α-hydroxy-11-oxo-20β,21-(isopropylidenedioxy)-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate (**21**). — Compound **21** was prepared from 3α,17α-dihydroxy-20β,21-(isopropylidenedioxy)-5β-pregnan-11-one as in the synthesis of **1**. The product crystallized from acetone–hexane as fine needles (69%); $[\alpha]_D$ +1°, $[\alpha]_{365}$ +48°; ν_{max}^{KBr} 3680–3550 (17-OH), 1705 (11-;carbonyl), 1160, 936, and 860 cm^{-1} (20,21-acetonide)[6].

Anal. Calc. for $C_{37}H_{54}O_{14}$: C, 61.48; H, 7.53; OCH_3, 4.29. Found: C, 61.19; H, 7.70; OCH_3, 4.40.

Methyl (17α,20β,21-trihydroxy-11-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**20**) *from* **21**. — A solution of compound **21** (1.5 g) in acetic acid (525 ml) and water (225 ml) was kept for 16 h at room temperature. After removal of the solvents *in vacuo*, the product was isolated in the usual way and gave, from acetone–hexane, 1.24 g (87%) of needles; $[\alpha]_D$ +4°, $[\alpha]_{365}$ +40°; ν_{max}^{KBr} 3700–3200 (OH) and 1701 cm^{-1} (11-carbonyl).

Anal. Calc. for $C_{34}H_{50}O_{14}$: C, 59.81; H, 7.38; OCH_3, 4.55. Found: C, 59.97; H, 7.60; OCH_3, 4.53.

Methyl (17α,20β,21-trihydroxy-11-oxo-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate (**20a**) *from* **21***. — To a solution of compound **21** (200 mg) in benzene (10 ml) was added 0.1 ml of titanium tetrachloride. After 6 h at room temperature, the suspension was diluted with ethyl acetate, and the neutral product was isolated in the usual way. Crystallization from methanol gave 152 mg (80%)

*The acetonide **21** is the preferred starting material for the preparation of **20a**, as it is more soluble than **20** in benzene. The isopropylidene group is probably removed by hydrolysis during processing of the acidic reaction mixture.

of fine needles; $[\alpha]_D$ +116°, $[\alpha]_{365}$ +364°; ν_{max}^{KBr} 3700–3150 (OH) and 1701 cm^{-1} (11-carbonyl).

Anal. Calc. for $C_{34}H_{50}O_{14}$: C, 59.81; H, 7.38; OCH_3, 4.55. Found: C, 59.68; H, 7.16; OCH_3, 4.50.

Methyl [17α-hydroxy-11-oxo-20,21-(isopropylidenedioxy)-5β-pregnan-3α-yl 2,3,4-tri-O-acetyl-α-D-glucopyranosid]uronate (**21a**) *from* **20a**. — Acetonation of compound **20a**, as in the previous example, furnished needles from ethyl acetate–methanol (85%), m.p. 224.5–225.5°; $[\alpha]_D$ +116°, $[\alpha]_{365}$ +364°; ν_{max}^{KBr} 3680–3350 (17-OH), 1701 (11-carbonyl), 1160, 935, and 859 cm^{-1} (20,21-acetonide).

Anal. Calc. for $C_{37}H_{54}O_{14}$: C, 61.48; H, 7.53; OCH_3, 4.29. Found: C, 61.40; H, 7.65; OCH_3, 4.10.

Cholest-5-en-3β-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**22**) *from* **1**. — To a solution of compound **1** (200 mg) in dry ether (20 ml), lithium aluminum hydride (100 mg) was added in one portion. After the suspension had been refluxed for 3 h, the excess of hydride was decomposed by the sequential addition of ethyl acetate and water. The mixture was diluted with ethyl acetate, and washed successively with 2M hydrochloric acid, dilute sodium hydroxide, water, and neutral brine, dried with anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was treated with acetic anhydride and pyridine for 12 h at room temperature, and the resulting acetate was isolated in the usual way. Crystallization from ethyl acetate–methanol gave 130 mg of needles (64%), m.p. 161.5–162°; $[\alpha]_D$ −27°, $[\alpha]_{365}$ −90°; lit. m.p. 159–160°, $[\alpha]_D$ −24° (chloroform)[25]; m.p. 159–160°, $[\alpha]_D$ −23° (chloroform)[26]; m.p. 157–159°, $[\alpha]_D$ −25° (chloroform)[27].

Cholest-5-en-3β-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**22a**) *from* **1a**. — Reduction of compound **1a** (100 mg), as in the preparation of **22** from **1**, afforded 43 mg (42%) of needles from ethyl acetate–methanol, m.p. 195.5–196.5°; $[\alpha]_D$ +91°, $[\alpha]_{365}$ +257°; lit. m.p. 195°, $[\alpha]_D$ +88° (chloroform)[28]; m.p. 195–197°, $[\alpha]_D$ +88° (chloroform)[27].

5α-Cholestan-3β-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**23**) *from* **3**. — Reduction of **3** (300 mg), as in the preparation of **22** from **1**, gave only 52 mg (17%)* of needles from ethanol, m.p. 174.5–175.5°; $[\alpha]_D$ +3°, $[\alpha]_{365}$ +5°; lit. m.p. 175°, $[\alpha]_D$ +5° (chloroform)[29]; m.p. 174–175° (ref. 30); m.p. 164–165°, $[\alpha]_D$ +6.3° (chloroform)[31].

5α-Cholestan-3β-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**23a**) *from* **23**. — Treatment of **23** (100 mg) in benzene (2 ml) with 0.05 ml of titanium tetrachloride

*When it was noted that the amount of mother liquor was very small, it became clear that much of the product, which has a very limited solubility in ether–ethyl acetate, had been lost during the processing. A modified procedure was therefore devised: after the addition of ethyl acetate and water, the remaining ether was evaporated with a stream of nitrogen. The residue was suspended in aqueous methanol, the pH adjusted to ~7, the solution re-evaporated, and the residue well dried (still in the original flask) *in vacuo* over an effective desiccant. The dry residue was treated with acetic anhydride and pyridine, and the acetate was recovered in the usual way. When a fresh sample of **3** was reduced as indicated, and subsequently processed by the modified technique, the yield of **23** was 85%. This method was also used for recovering the reduction products from **17**, **17a**, and **19**.

for 9 h at room temperature, followed by the usual processing, furnished 55 mg (55%) of needles from ethyl acetate–methanol, m.p. 186–187°; $[\alpha]_D$ +106°, $[\alpha]_{365}$ +303°; lit. m.p. 184°, $[\alpha]_D$ +114° (chloroform)[29]; m.p. 182°, $[\alpha]_D$ +108° (chloroform)[26].

5α-Cholestan-3α-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**24**) *from* **4**. — Compound **24** was obtained by the reduction of **4**, as in the preparation of **22** from **1**. The product crystallized from acetone–hexane as needles (79%), m.p. 175–176°; $[\alpha]_D$ −11°, $[\alpha]_{365}$ −35°; lit. m.p. 174°, $[\alpha]_D$ −3° (chloroform)[29]; m.p. 170–172° (ref. 30).

5α-Cholestan-3α-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**24a**) *from* **24**. — Treatment of compound **24** with titanium tetrachloride was conducted as in the preparation of **23a** from **23**. Crystallization of the product from ethyl acetate–methanol gave needles (50%), m.p. 137–139°; $[\alpha]_D$ +97°, $[\alpha]_{365}$ +278°; lit. m.p. 130°, $[\alpha]_D$ +93° (chloroform)[29].

5β-Cholestan-3β-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**25**) *from* **5**. — Compound **25** was obtained by the reduction of **5**, as in the preparation of **22** from **1**. Crystallization from ethanol–acetone gave needles (59%), m.p. 202.5–203.5°; $[\alpha]_D$ −3°, $[\alpha]_{365}$ −16°; lit.[30] m.p. 198–200°.

5β-Cholestan-3β-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**25a**) *from* **25**. — Anomerization of compound **25**, as in the preparation of **23a** from **23**, gave needles from ethyl acetate–methanol (62%), m.p. 120–121°; $[\alpha]_D$ +117°, $[\alpha]_{365}$ +332°.

Anal. Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.66; H, 9.20.

5β-Cholestan-3α-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**26**) *from* **6**. — Reduction of compound **6**, as in the preparation of **22** from **1**, gave a filtrable gel from acetone–methanol (72%), m.p. 173–173.5°; $[\alpha]_D$ +11°, $[\alpha]_{365}$ +23°; lit.[30] m.p. 140–141° (?).

Anal. Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.45; H, 9.03.

5β-Cholestan-3α-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**26a**) *from* **26**. — Treatment of compound **26** with titanium tetrachloride, as in the preparation of **23a** from **23**, gave needles (71%) from ethyl acetate–methanol, m.p. 144.5–145.5°; $[\alpha]_D$ +110°, $[\alpha]_{365}$ +314°.

Anal. Calc. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.37; H, 9.05.

$25_{\alpha F}$-Spirost-5-en-3β-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**27**) *from* **17**. — Reduction of compound **17**, as in the preparation of **23** from **3**, gave needles from ethyl acetate–methanol (72%), m.p. 208–209°; $[\alpha]_D$ −77°, $[\alpha]_{365}$ −228°; lit. m.p. 200–202°, $[\alpha]_D$ −75° (chloroform)[32]; m.p. 204–205°, $[\alpha]_D$ −71° (*p*-dioxane)[33].

Anal. Calc. for $C_{41}H_{60}O_{12}$: C, 66.10; H, 8.12. Found: C, 66.24; H, 7.88.

$25_{\alpha F}$-Spirost-5-en-3β-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**27a**) *from* **17a**. — Reduction of compound **17a**, as in the preparation of **23** from **3**, gave a filtrable gel from ethyl acetate–methanol (62%), m.p. 161.5–162.5°; $[\alpha]_D$ +37°, $[\alpha]_{365}$ +107°.

Anal. Calc. for $C_{41}H_{60}O_{12}$: C, 66.10; H, 8.12. Found: C, 66.10, H, 7.98.

$25_{\alpha F}$-5α-Spirostan-3β-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**28**) *from* **19**.

— Reduction of compound **19**, as in the preparation of **23** from **3**, gave plates from ethyl acetate–methanol (72%), m.p. 207–208°; $[\alpha]_D$ −41°, $[\alpha]_{365}$ −149°.

Anal. Calc. for $C_{41}H_{62}O_{12}$: C, 65.93; H, 8.37. Found: C, 66.06; H, 8.13.

$25_{\alpha F}$-5α-Spirostan-3β-yl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**28a**) *from* **28**. — Treatment of compound **28** with titanium tetrachloride, as in the preparation of **23a** from **23**, gave needles from ethyl acetate–methanol (71%), m.p. 210–211°; $[\alpha]_D$ +57°, $[\alpha]_{365}$ +164°.

Anal. Calc. for $C_{41}H_{62}O_{12}$: C, 65.93; H, 8.37. Found: C, 65.86; H, 8.22.

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REFERENCES

1 S. BERNSTEIN, E. W. CANTRALL, J. P. DUSZA, AND J. P. JOSEPH, *Steroid Conjugates*, American Chemical Society, Washington, D. C., 1966; S. BERNSTEIN, J. P. DUSZA, AND J. P. JOSEPH, *Physical Properties of Steroid Conjugates*, Springer-Verlag, New York, N. Y., 1968; H. E. HADD AND R. T. BLICKENSTAFF, *Conjugates of the Steroid Hormones*, Academic Press, New York, N. Y., 1969.
2 J. J. SCHNEIDER AND N. S. BHACCA, *J. Org. Chem.*, 34 (1969) 1990.
3 E. PACSU, *J. Amer. Chem. Soc.*, 52 (1930) 2563, 2568, 2571.
4 B. LINDBERG, *Acta Chem. Scand.*, 2 (1948) 426.
5 Z. PROCHAZKA, *Collect. Czech. Chem. Commun.*, 33 (1968) 4039.
6 M. L. LEWBART AND J. J. SCHNEIDER, *J. Org. Chem.* (in press).
7 W. KLYNE, *Biochem. J.*, 47 (1950) xli.
8 C. S. HUDSON, *J. Amer. Chem. Soc.*, 31 (1909) 66.
9 E. L. ELIEL, N. L. ALLINGER, S. J. ANGYAL, AND G. A. MORRISON, *Conformational Analysis*, Interscience, New York, N. Y., 1967, pp. 383–384.
10 W. KORYTNYK, *J. Chem. Soc.*, (1959) 650.
11 Y. NITTA, M. SHINDO, AND K. TAKAMURA, *Chem. Pharm. Bull.* (Tokyo), 12 (1964) 450.
12 J. F. BECKER, *Biochim. Biophys. Acta*, 100 (1965) 574.
13 H. SPEDDING, *Advan. Carbohyd. Chem.*, 19 (1964) 23; R. S. TIPSON, *Nat. Bur. Stand. Monograph 110* (1968).
14 H. S. ISBELL, F. A. SMITH, E. C. CREITZ, H. L. FRUSH, J. D. MOYER, AND J. E. STEWART, *J. Res. Nat. Bur. Stand.*, 59 (1957) 41.
15 R. S. TIPSON AND H. S. ISBELL, *J. Res. Nat. Bur. Stand.*, 64A (1960) 405.
16 Y. NITTA, Y. NAKAJIMA, M. KURANARI, A. MOMOSE, AND J. IDE, *Yakagaku Zasshi*, 81 (1961) 1160.
17 E. SMAKULA, J. H. LEFTIN, AND H. H. WOTIZ, *J. Amer. Chem. Soc.*, 81 (1959) 1708.
18 K. BIEMANN, D. C. DE JONGH, AND H. K. SCHNOES, *J. Amer. Chem. Soc.*, 85 (1963) 1763.
19 H. BUDZIKIEWICZ, C. DJERASSI, AND D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry, Vol. II, Steroids, Terpenoids, Sugars, and Miscellaneous Classes*, Holden-Day, San Francisco, 1964, pp. 206, 207.
20 H. H. WOTIZ, E. SMAKULA, N. N. LICHTIN, AND J. H. LEFTIN, *J. Amer. Chem. Soc.*, 81 (1959) 1704.
21 R. A. LUCAS, D. F. DICKEL, R. L. DZIEMAIN, M. J. CEGLOWSKI, B. L. HENSEL, AND H. B. MACPHILLAMY, *J. Amer. Chem. Soc.*, 82 (1960) 5688.
22 F. FOGGITT AND A. E. KELLIE, *Biochem. J.*, 91 (1964) 209.
23 H. PELZER, *Z. Physiol. Chem.*, 314 (1959) 234.

24 J. J. SCHNEIDER AND N. S. BHACCA, *J. Biol. Chem.*, 241 (1966) 5313.
25 A. H. SALWAY, *J. Chem. Soc.*, 103 (1913) 1022.
26 H. LETTRE AND A. HAGEDORN, *Z. Physiol. Chem.*, 242 (1936) 210.
27 A. YA. KHORLIN, A. F. BOCHKOV, L. V. BAKINOVSKIĬ, AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 143 (1962) 1119.
28 E. HARDEGGER AND J. DEPASCUAL, *Helv. Chim. Acta*, 31 (1948) 281.
29 R. P. LINSTEAD, *J. Amer. Chem. Soc.*, 62 (1940) 1766.
30 K. MIESCHER, C. MEYSTRE, AND J. HEER, *Helv. Chim. Acta*, 24 (1941) 988.
31 P. A. PLATTNER AND A. UFFER, *Helv. Chim. Acta*, 28 (1945) 1049.
32 W. Y. HUANG, Y. C. CHEN, AND J. H. CHU, *Hua Hsueh Hsueh Pao*, 22 (1956) 409.
33 S. LIEBERMAN, F. C. CHANG, M. R. BARUSCH, AND C. R. NOLLER, *J. Amer. Chem. Soc.*, 64 (1942) 2581.