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THIN-LAYER CHROMATOGRAPHY OF ANOMERIC STEROID TRI-O-ACETYL-D-GLUCOPYRANOSIDURONIC METHYL ESTERS, D-GLUCOPYRANOSIDE TETRAACETATES, AND 2'-ACETAMIDO-2'-DEOXY-TRI-O-ACETYL-D-GLUCOPYRANOSIDES

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SUMMARY

Sixteen anomeric pairs of steroid tri-O-acetyl-D-glucopyranosiduronic methyl esters, seven pairs of D-glucopyranoside tetraacetates, and four pairs of 2'-acetamido-2'-deoxy-tri-O-acetyl-D-glucopyranosides, together with the free steroids from which they were derived, were chromatographed on thin layers of silica gel. In all cases except one, the α member was more mobile than its anomer.

INTRODUCTION

The considerable information available on the chromatographic characteristics of steroid D-glucopyranosiduronic acids, D-glucopyranosides, and their derivatives[1] is limited almost entirely to members of the β series since very few of their anomers (α forms) have been synthesized. Recently, however, a number of anomeric pairs were prepared, including tri-O-acetyl-α (and β)-D-glucopyranosiduronic methyl esters, α (and β)-D-glucopyranoside tetraacetates, and 2'-acetamido-2'-deoxy-tri-O-acetyl-α (and β)-D-glucopyranosides[2,3]. The assignment of configuration at the anomeric carbon atom for each pair was made from a consideration of optical rotatory, infrared spectral and, in some instances, NMR spectral data. It seemed of interest to extend these points of comparison to a consideration of the relative mobilities of members of each pair in suitable chromatographic systems. These derivatives are ideally suited for thin-layer chromatography on silica gel and, accordingly, this report details the results of such a study.

MATERIALS AND METHODS

Two new anomeric pairs (18,18a and 19,19a) were prepared by methods described in an earlier publication[2]. These derivatives were characterized as follows:

Methyl (*17α-hydroxy-20β,21-isopropylidenedioxy-5β-pregnan-3α-yl 2',3',4'-tri-O-acetyl-β-D-glucopyranosid)uronate* (**18**). Prepared from 20β,21-isopropylidenedioxy-5β-pregnane-3α,17α-diol[4]. Crystallization from acetone–*n*-hexane gave needles: $[\alpha]_D$ — 4° (chloroform); ν_{max}^{KBr} 3600–3500 (17α-OH), 1770–1745 and 1260–1205 (acetate), 1161, 890, and 860 cm^{-1} (20,21-acetonide).

Anal. Calc. for $C_{37}H_{56}O_{13}$: C, 62.69; H, 7.96. Found: C, 62.60; H, 8.20.

Methyl (*17α-hydroxy-20β,21-isopropylidenedioxy-5β-pregnan-3α-yl 2',3',4'-tri-O-acetyl-α-D-glucopyranosid)uronate* (**18a**) *from* **18**. Anomerization of **18**, followed by re-acetonation, gave needles from acetone–methanol: m.p. 223–224°; $[\alpha]_D$ +116° (chloroform); ν_{max}^{KBr} 3580–3500 (17α-OH), 1770–1745 and 1260–1205 (acetate), 1165, 1141 (weak), 895, and 860 cm^{-1} (20,21-acetonide). The anomerization increment, $M_{D\alpha}$ (**18a**)—$M_{D\beta}$ (**18**), = + 850 (see Table I in ref. 2).

Anal. Calc. for $C_{37}H_{56}O_{13}$: C, 62.69; H, 7.96. Found: C. 62.80; H, 7.94.

Methyl (*17α,20β,21-trihydroxy-5β-pregnan-3α-yl 2',3',4'-tri-O-acetyl-β-D-glucopyranosid)uronate* (**19**) *from* **18**. Hydrolysis of **18** with aqueous acetic acid gave needles from aqueous methanol: $[\alpha]_D$ —18° (chloroform); ν_{max}^{KBr} 3620–3250 (OH), 1770–1740 and 1270–1205 (acetate), 1165, and 890 cm^{-1}.

Anal. Calc. for $C_{34}H_{52}O_{13}$: C, 61.06; H, 7.84. Found: C, 60.96; H, 8.00.

Methyl (*17α,20β,21-trihydroxy-5β-pregnan-3α-yl 2',3',4'-tri-O-acetyl-α-D-glucopyranosid)uronate* (**19a**) *from* **18a**. Hydrolysis of **18a** as above gave needles from aqueous acetone: $[\alpha]_D$ +97° (chloroform); ν_{max}^{KBr} 3620–3250 (OH), 1770–1735 and 1260–1205 (acetate), 1165 (weak), 1145, and 892 cm^{-1}. The anomerization increment, $M_{D\alpha}$ (**19a**) — $M_{D\beta}$ (**19**), = + 769.

Anal. Calc. for $C_{34}H_{52}O_{13}$: C, 61.06; H, 7.84. Found: C, 61.05; H, 7.84.

Chromatography was carried out at 25 ± 2° by the ascending method on Silica Gel IB-F (Baker) sheets, applying two concentrations of each anomer together with the appropriate aglycon as a compound of reference. Systems (Table I) consisted of ethyl acetate–isooctane mixtures so proportioned as to provide R_F values of the derivatives in the range of 0.1 to 0.3. Following chromatography, the sheets were air-dried, sprayed with a 20 % solution of *p*-toluenesulfonic acid in ethanol, and heated at around 120° for from 5 to 15 min. This converted the steroids and their derivatives to colored products, best visualized under a UV lamp emitting maximally at around 360 nm.

TABLE I

COMPOSITION OF CHROMATOGRAPHIC SYSTEMS

System No.	Composition
1–8	Ethyl acetate, 6, 8, 10, 12, 14, 18, 20, and 22 ml, diluted in each case to 25 ml with isooctane (2,2,4-trimethylpentane)
9	Toluene–isooctane–methanol (abs.)–water (3:2:4:1)
10	Isopropyl ether–*n*-heptane–methanol (abs.)–water (14:6:13:7)

TABLE II

R_F VALUES OF ANOMERIC STEROID TRI-O-ACETYL-D-GLUCOPYRANOSIDURONIC METHYL ESTERS AND AGLYCONS

Compound No.	Anomeric form	Aglycon[a]	System No.	R_F Derivative	Aglycon
1	α	Cholesterol	1	0.21	0.29
1a	β			0.19	
2	α	Cholestanol	1	0.23	0.27
2a	β			0.18	
3	α	Epicholestanol	1	0.28	0.37
3a	β			0.20	
4	α	Coprostanol	1	0.23	0.35
4a	β			0.18	
5	α	Epicoprostanol	1	0.26	0.33
5a	β			0.19	
6	α	Diosgenin	2	0.25	0.27
6a	β			0.22	
7	α	Tigogenin	2	0.26	0.26
7a	β			0.23	
8	α	Dehydroisoandrosterone	3	0.18	0.19
8a	β			0.16	
9	α	Etiocholanolone	3	0.19	0.16
9a	β			0.18	
10	α	Androsterone	3	0.20	0.21
10a	β			0.18	
11	α	Isoandrosterone	3	0.19	0.18
11a	β			0.16	
12	α	Pregnenolone	3	0.21	0.23
12a	β			0.20	
13	α	Pregnanolone	3	0.24	0.19
13a	β			0.21	
14	α	Allopregnanolone	3	0.23	0.21
14a	β			0.21	
15	α	17-Hydroxypregnanolone	4	0.22	0.13
15a	β			0.21	
16	α	β-Cortolone acetonide	5	0.27	0.10
16a	β			0.28	

[a] Formal names of aglycons referred to trivially in this and other tables are: cholesterol, cholest-5-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; epicholestanol, 5α-cholestan-3α-ol; coprostanol, 5β-cholestan-3β-ol; epicoprostanol, 5β-cholestan-3α-ol; diosgenin, (22S,25S)-spirost-5-en-3β-ol; tigogenin, (22S,25S)-5α-spirostan-3β-ol; dehydroisoandrosterone, 3β-hydroxyandrost-5-en-17-one; etiocholanolone, 3α-hydroxy-5β-androstan-17-one; androsterone, 3α-hydroxy-5α-androstan-17-one; isoandrosterone, 3β-hydroxy-5α-androstan-17-one; pregnenolone, 3β-hydroxypregn-5-en-20-one; pregnanolone, 3α-hydroxy-5β-pregnan-20-one; allopregnanolone, 3β-hydroxy-5α-pregnan-20-one; 17-hydroxypregnanolone, 3α,17α-dihydroxy-5β-pregnan-20-one; β-cortolone acetonide, 3α,17α-dihydroxy-20β,21-isopropylidenedioxy-5β-pregnan-11-one; testosterone, 17β-hydroxyandrost-4-en-3-one; androstanediol, 5β-androstane-3α,17β-diol. The point of attachment of the carbohydrate and steroid involves the C-3 hydroxyl group of the latter in all cases except pair 27,27a (testosterone).

RESULTS AND DISCUSSION

Table II gives R_F values for sixteen anomeric pairs of tri-O-acetyl-D-glucopyran-osiduronic methyl esters and the aglycons from which they were obtained. Although these values (as well as those in subsequent tables) were obtained from individual chromatograms, it was noted that R_F differences between α,β pairs, however small, could be demonstrated repeatedly. These differences are considered sufficiently large to demonstrate (perhaps with some overrunning in certain cases) both components of a given α,β mixture, but too small to permit separation on silica gel columns.

With the exception of the **16,16a** pair, the α member is always more mobile than its anomer. This general relationship may be due, at least in part, to fixed differences in their conformations. The β member has an essentially linear shape, which appears to limit carbohydrate–steroid interaction* and, possibly, to promote binding (particularly of the carbohydrate moiety) to the adsorbent. In contrast, the α member has an angular form in which the carbohydrate, irrespective of its freedom about the C-1' (carbohydrate):C-3 (steroid) axis, tends to approach the steroid portion. It is probable that such a conformation serves to encourage carbohydrate–steroid interaction, and thus increase the mobility of the molecule.

TABLE III

PAPER CHROMATOGRAPHY OF ANOMERIC TRI-O-ACETYL-D-GLUCOPYRANOSIDURONIC METHYL ESTERS OF STEROIDAL $17\alpha,20\beta,21$-GLYCEROLS

Compound No.	Anomeric form	Aglycon	System No.	R_F	System No.	R_F
17	α	$3\alpha,17\alpha,20\beta,21$-Tetrahydroxy-$5\beta$-pregnan-11-one	9	0.18	10	0.25
17a	β			0.12		0.12
19	α	5β-Pregnane-$3\alpha,17\alpha,20\beta,21$-tetrol	9	0.49	10	0.66
19a	β			0.35		0.49

The anomalous result provided by the **16,16a** pair led to attempts to explain it. The $17\alpha,20\beta,21$-glycerols (**17,17a**), obtained from **16** and **16a** by hydrolysis with aqueous acetic acid[2], appeared also to give the anomalous result, but judgment was uncertain since they tended to streak. Thin-layer chromatography of the 11-deoxo derivatives corresponding to **16** and **16a** (**18** and **18a**, in which the aglycon is $20\beta,21$-isopropylidenedioxy-5β-pregnane-$3\alpha,17\alpha$-diol) gave irregular results, meaning that the relative mobilities of the anomeric members varied unpredictably from trial to trial. Hydrolysis of the 11-deoxoacetonides and chromatography of the two pairs of $17\alpha,20\beta,21$-glycerols (**17,17a** and **19,19a**) by the partitioning method on paper gave the results presented in Table III. It is seen that under these circumstances, both

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* The nature of such carbohydrate–aglycon interactions remains unknown, but it was earlier suggested[5] that they account for the failure of many anomeric pairs (especially those bearing large aglycon groups) to conform to Hudson's "rules of isorotation". It is assumed, for the purposes of this discussion, that the carbohydrate portion is in close contact with the adsorbent, and that such interactions influence mobility chiefly by inducing conformational changes within this portion. It can be argued that any of several abnormal conformers of the carbohydrate moiety may be less strongly fixed to the surface than the normal (chair) arrangement.

pairs of anomers behave normally: the α anomer is more mobile in each case, and the members are well separated in both instances. Comparable results were obtained in three other, similar, systems. Although this comparison is not wholly valid (since acetonides are compared with the corresponding $17\alpha,20\beta,21$-glycerols), it suggests the importance of surface effects.

Table IV provides R_F values for seven anomeric pairs of D-glucopyranoside tetraacetates; all were derived from the first seven pairs in Table II. These results are wholly regular in that the α anomer is the more mobile member of each pair.

The results from four anomeric pairs of 2'-acetamido-2'-deoxy-tri-O-acetyl-D-glucopyranosides (Table V) also form a regular series. In each case the α anomer is more mobile, and in one instance (pair 30,30a), a notable separation was obtained.

TABLE IV

R_F VALUES OF ANOMERIC STEROID D-GLUCOPYRANOSIDE TETRAACETATES AND AGLYCONS

Compound No.	Anomeric form	Aglycon	System No.	R_F	
				Derivative	Aglycon
20	α	Cholesterol	1	0.22	0.28
20a	β			0.18	
21	α	Cholestanol	1	0.23	0.26
21a	β			0.17	
22	α	Epicholestanol	1	0.27	0.37
22a	β			0.18	
23	α	Coprostanol	1	0.23	0.35
23a	β			0.18	
24	α	Epicoprostanol	1	0.25	0.33
24a	β			0.18	
25	α	Diosgenin	2	0.26	0.28
25a	β			0.21	
26	α	Tigogenin	2	0.26	0.26
26a	β			0.21	

TABLE V

R_F VALUES OF ANOMERIC STEROID 2'-ACETAMIDO-2'-DEOXY-TRI-O-ACETYL-D-GLYCOPYRANOSIDES AND AGLYCONS

Compound No.	Anomeric form	Aglycon	System No.	R_F	
				Derivative	Aglycon
27	α	Testosterone	7	0.15	0.36
27a	β			0.10	
28	α	Androsterone	6	0.20	0.44
28a	β			0.13	
29	α	Isoandrosterone	6	0.19	0.39
29a	β			0.12	
30	α	Androstanediol	8	0.25	0.35
30a	β			0.11	

The data in Tables II, IV, and V also show that the aglycon is in most cases slightly more mobile than either of its derivatives. When this relationship is examined in terms of ΔR_{Mr} values[6], which express the contribution to mobility of the carbohydrate portion, a series of small, generally positive, values is obtained. Some pairs, namely 9,9a, 13,13a, 15,15a and 16,16a, provide small to moderate negative values. If this range in values exceeds that due to experimental error, it may be suggested that the anomalous (negative) values are due to unusual interactions or conformational changes. From the practical point of view, it is evident that many of the aglycon–derivative mixtures listed in Tables II and IV could not be separated by this chromatographic procedure alone*.

In contrast, the 2'-acetamido-2'-deoxy-tri-O-acetyl-D-glucopyranosides (Table V) are well separated from the free aglycons. This is due to the inherently greater polarity of the carbohydrate portion as is evident from the composition of the systems. It is also to be noted that D-glucopyranosiduronic acid and D-glucopyranoside derivatives bearing the same aglycon have very similar mobilities, and therefore cannot be distinguished by the present method. However, the 2'-acetamido-2'-deoxy-tri-O-acetyl-D-glucopyranosides readily can be discerned in a mixture containing all three classes.

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* Such mixtures readily can be resolved by acetylation followed by re-chromatography.