ON STEROIDS. CLXI.*

THE SYNTHESIS OF SOME NEW STEROID GLUCOSIDES

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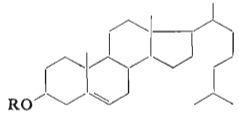
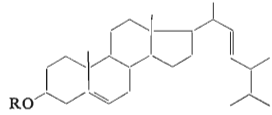
On reaction of hydroxy steroids with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide several anomeric steroid glucosides have been prepared. Configuration of the glycosidic bond has been determined by PMR.

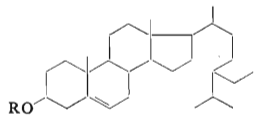
In previous papers[1,2] it was demonstrated that some steroids with a 3β-OH group and 5α-configuration or a double bond in the position 5 may be easily glucosylated



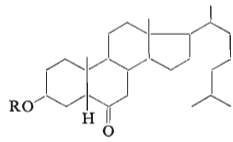
I, R = H
II, R = β-Glc(Ac)$_4$
III, R = β-Glc



IV, R = H
V, R = β-Glc(Ac)$_4$
VI, R = β-Glc



VII, R = H
VIII, R = β-Glc(Ac)$_4$
IX, R = β-Glc



X, R = H
XI, R = α-Glc(Ac)$_4$
XII, R = β-Glc(Ac)$_4$
XIII, R = α-Glc
XIV, R = β-Glc

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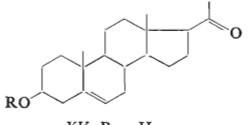
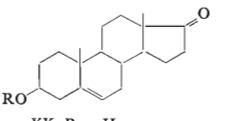
enzymatically with potato tuber tissue. Glucosylation did not take place with steroids carrying a longer side chain in the position 17 even though having the above mentioned structural characteristics. In view of the fact that we expected plant growth activity in some steroidal glucosides we decided to prepare a series of steroid glucosides synthetically and submit them to biological tests. We also wished to correlate the glucoside of dehydroepiandrosterone prepared enzymatically[1] with a synthetically prepared specimen. For glucosylation we chose several plant sterols, sexual hormones and some other steroids.

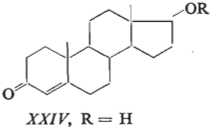
Glucosylation was carried out according to the original Königs-Knorr reaction (see[3]) and a modification of it using cadmium carbonate as catalyst[4]. The reaction always gave a mixture of acetylated α- and β-glucosides which were separated only with difficulty or not at all from the starting components. Even after saponification of the mixtures the anomers of free glucosides could not be separated chromatographically. Therefore we had to saponify first the crude mixture of acetylated glucosides, separate the glucosides from the starting reaction components, and reacetylate the free glucosides. These mixtures of acetylated α- and β- glucosides could be separated chromatographically on preparative silica gel layers. The purified anomers of the acetylated glucosides were then saponified, affording pure anomers of free glucosides. Our experiments have shown that the catalysis with silver oxide[3] gave lower yields than when cadmium carbonate[4] served as the catalyst. The glucosides



XV, R = H
XVI, R = α-Glc(Ac)$_4$
XVII, R = β-Glc(Ac)$_4$
XVIII, R = α-Glc
XIX, R = β-Glc



XX, R = H
XXI, R = α-Glc(Ac)$_4$
XVII, R = β-Glc(Ac)$_4$
XXIII, R = α-Glc
XXIII, R = β-Glc



XXIV, R = H
XXV, R = α-Glc(Ac)$_4$
XXVI, R = β-Glc(Ac)$_4$
XXVII, R = β-Glc

were characterized, if the amount obtained permitted it (for yields see Table I), by the melting point, optical rotation, elementary analysis, and R_F values (Table II). Configurations were determined on the basis of optical rotation values, R_F values

TABLE I

Yields and Ratios of Anomers Formed on Reaction of Some Steroids with 2,3,4,6-Tetra-O-acetyl--α-D-glucopyranosyl Bromide

Compound	*I*	*VII*	*IV*	*XX*	*XV*	*X*	*XXIV*
Method *a*, yield, %	55	53	54	50—55	50—55	51	15
Method *b*, yield, %	23	20	21	—	—	—	—
Ratio α : β[a]	(10—12) : (90—88)					8 : 92	17 : 83

[a] Determined by weighing.

TABLE II

Analytical and Physical Data of the Prepared Steroidal Glucosides and their Derivatives

Compound	Formula (m.w.)	Calculated/Found % C	% H	m.p., °C[a] $[\alpha]_D^{20}$	R_F[b]
II	$C_{41}H_{64}O_{10}$	68·88	9·00	159—160	0·80[b]
	(716·9)	68·94	8·96	−23·6	
III	$C_{33}H_{56}O_6$	72·22	10·28	290 (dec.)	
	(548·8)	71·83	10·12	—	0·31[c]
V	$C_{43}H_{68}O_{10}$	69·42	9·08	137—138	
	(744·0)	69·60	8·98	−32·8°	0·80[b]
VI	$C_{35}H_{60}O$	73·00	10·33	290 (dec.)	
	(575·8)	72·78	10·16	—	0·31[c]
VIII	$C_{43}H_{70}O_{10}$	69·23	9·32	166—167	
	(746·0)	68·90	8·83	−24·6°	0·80[b]
IX	$C_{35}H_{62}O_6$	72·75	10·64	295 (dec.)	
	(577·8)	72·63	10·49	—	0·31[c]
XI	$C_{41}H_{64}O_{11}$	67·18	8·80	187—188	
	(733·0)	67·57	9·08	+41·2°	0·63[b]
XII	$C_{41}H_{64}O_{11}$	67·18	8·80	183—184	
	(733·0)	67·19	8·82	−12·8°	0·53[b]
XIII	$C_{33}H_{56}O_6$	70·17	10·01	216—220 and	
	(564·8)	70·30	9·82	258—263	0·29[c]

TABLE II
(*Continued*)

Compound	Formula (m.w.)	Calculated/Found % C	Calculated/Found % H	m.p., °C[a] $[\alpha]_D^{20}$	R_F[b]
XIV	$C_{33}H_{56}O_6$	70·17	10·01	206—211	
	(564·8)	70·04	9·89	—	0·30[c]
XVI	$C_{35}H_{50}O_{11}$	64·99	7·79	94—96	
	(646·8)	64·54	7·87	—	0·61[b]
XVII	$C_{35}H_{50}O_{11}$	64·99	7·99	190—192	
	(646·8)	65·18	7·81	+4·0°	0·52[b]
XVIII	$C_{27}H_{42}O_6$	67·75	8·85	273—276	
	(478·6)	68·03	8·66	—	0·28[c]
XIX[d]	$C_{27}H_{42}O_6$	67·75	8·85	270—278	
	(478·6)	67·72	8·95	—	0·28[c]
XXI	$C_{33}H_{46}O_{11}$	63·06	7·49	115—118	
	(618·7)	64·25	8·41	—	0·57[b]
XXII	$C_{33}H_{46}O_{11}$	64·06	7·49	183—184	
	(618·7)	64·19	7·35	−2·9°	0·46[b]
XXIII[e]	$C_{25}H_{38}O_6$	66·64	8·50	215—218	
	(450·5)	66·34	8·53	−16·9°	0·27[c]
XXV	$C_{33}H_{46}O_{11}$	64·06	7·49	75—79	
	(618·7)	64·08	7·52	—	0·25[b]
XXVI	$C_{33}H_{46}O_{11}$	64·06	7·49	166—167	
	(618·7)	63·92	7·65	−43·4°	0·16[b]
XXVII	$C_{25}H_{38}O_6$	66·64	8·50	122—125	
	(450·5)	66·35	8·51	—	0·26[c]

[a] Crystallised from methanol. [b] These values were measured after double development in a mixture of tetrachloromethane and ether 3 : 2. [c] Chloroform–methanol 9 : 1. [d] The substance is identical with a sample prepared enzymatically[2]. [e] The substance is identical with a sample prepared enzymatically[1].

and PMR spectra (Table III). As in this reaction the β-anomer was formed predominantly we endeavoured to obtain the α-anomer in a larger amount by isomerisation of the β-anomer with titanium(IV) chloride[5]. However, in this reaction Δ^5-steroids gave an inseparable mixture and the isolation of both anomers from the mixture after isomerisation was feasible only with 3β-hydroxycholestan-6-one.

EXPERIMENTAL

The melting points are not corrected. Optical rotations were measured in chloroform (acetates) or chloroform–methanol (1 : 1) (free glucosides). Samples for analysis were dried *in vacuo* (oil pump) at 50°C for 30 minutes. The PMR spectra were measured on Varian 100 Mc in deuteriochloroform using tetramethylsilane as internal standard. Silica gel for column

TABLE III

PMR Data of Acetylated Anomers of Some Prepared Steroidal Glucosides (chemical shifts in δ, coupling constants in Hz)

Compound	C_1,—H (J)	C_2,—H ($J_{1',2'}$, $J_{2',3'}$)	C_3,—H (J)
XI	5·21 d	4·77 dd	5·47 t
	(3·5)	(3·5, 10·0)	(10·0)
XII	4·61 d	4·97 m	5·13 t
	(7·5)	—	(8·0)
XVI	5·24 d	4·73 dd	5·49 t
	(3·5)	(3·5, 10·0)	(9·0)
XVII	4·61 d	4·97 m	5·19 t
	(8·0)	—	(8·0)
XXI	5·22 d	4·80 dd	5·48 t
	(3·5)	(3·5, 10·0)	(9·5)
XXII	4·59 d	4·97 m	5·18 t
	(8·0)	—	(8·0)
XXVI	4·52 d	4·99 m	5·12 t
	(8·0)	—	(8·0)

chromatography (according to Pitra, 60—120 μ) was supplied by the Service Laboratories of our Institute (Lysolaje), for thin-layer chromatography silica gel G (Woelm) was used.

Preparation of Acetylated Steroid Glucosides

a) A mixture of steroid alcohol (1 mmol), 344 mg (2 mmol) of $CdCO_3$, and 20 ml of toluene was freed of traces of humidity by azeotropic distillation off of 2 ml of solvent. A solution of 795 mg (2 mmol) of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in 20 ml toluene was added dropwise to the mixture over one hour under simultaneous distillation off of the solvent. Additional toluene (20 ml) was added dropwise to the mixture under the same conditions. The cold reaction mixture was filtered through a layer of diatomaceous earth and evaporated to dryness.

b) To a solution of steroidal alcohol (0·2 g; dried by azeotropic distillation with benzene) in 10 ml of ether freshly prepared silver oxide (0·2 g; precipitated at 80°C and dried over P_2O_5) was added in several portions and the mixture shaken for 48 h. After filtration and washing of the precipitate with ether the filtrate was washed with water, dried over sodium sulfate, and evaporated.

Saponification of Acetylated Glucosides

a) A solution of a mixture of acetate in 85 ml of methanol was diluted with 15 ml of water and then refluxed in the presence of 1 g of potassium carbonate for 1 h. Methanol was distilled off under reduced pressure and the residue extracted three times with chloroform. The Combined extracts were dried and evaporated to dryness. *b*) The above mentioned mixture was stirred with 100 ml of 1% methanolic KOH for 1 h and worked up as under *a*).

The residue was separated chromatographically on a 50 fold amount of silica gel first with chloroform (which eluted impurities) and then chloroform–methanol 9 : 1 that eluted the mixture of α and β glucosides. The weight of the residue of this mixture, expressed as yield, is given in Table I.

Acetylation of the Mixture of α and β Glucosides

The mixture of epimeric glucosides from the above reaction (100 mg) was acetylated with acetic anhydride (3 ml) in pyridine (5 ml) at room temperature for 18 h. The reaction mixture was evaporated, the residue dissolved in benzene and again evaporated. The residue was dissolved in ether, the solution was washed twice with 2% hydrochloric acid, 5% sodium hydrogen carbonate and water, dried and evaporated to dryness. The residue was submitted to preparative chromatography on two silica gel plates (20 × 20 cm), using double development with a mixture of tetrachloromethane and ether (3 : 2) as eluent. The faster moving substance is the α-anomer, the slower one is the β-anomer. The separated zones were detected with morin (0·2% solution in methanol) and the substances were eluted with chloroform and evaporated. The ratio of the weights of the residues is given in Table I.

Isomerization: Tetraacetate of β-glucoside of 3β-hydroxycholestan-6-one (200 mg) in 6 ml of chloroform was refluxed with 0·1 ml $TiCl_4$ for 1 h. The reaction mixture was diluted with chloroform, washed with a solution of sodium hydrogen carbonate and water, dried over sodium sulfate, filtered and evaporated to dryness. The mixture of both anomers was chromatographed on four preparative silica gel G plates as in the preceding experiment. The weight of the eluate of the α-anomer was 127 mg, of the β-anomer 11 mg.

Saponification of Pure Anomers of Acetylated Glucosides

a) A solution of 100 mg of acetate in 8·5 ml of methanol was diluted with 1·5 ml of water and then boiled in the presence of 100 mg of potassium carbonate for 1 h. Methanol was distilled off under reduced pressure and the residue extracted three times with chloroform. The combined extract was dried and evaporated. The residue was crystallised from methanol.

b) 100 mg of acetate were stirred with 30 ml of a 1·5% methanolic KOH solution and the mixture worked up as under *a*).

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