ON STEROIDS. CXXXIII.*

GLUCOSYLATION OF STEROIDS BY POTATO TUBER SLICES; STRUCTURAL REQUIREMENTS

Ž. PROCHÁZKA

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

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The specifity of the enzymatic system of potato tubers glucosylating steroids was investigated. Among more than 60 hydroxy steroids only those containing a sterically unhindered 3β-hydroxy group were glucosylated if they were of the 5α-H or Δ^5-series. Steroids with a long side chain in the position 17 are glucosylated only exceptionally.

In a series of papers (*cf.*[1-4]) it was demonstrated that steroids can be transformed not only by animal tissues or microorganisms, but by vegetable tissue too. We described[5] in the case of 3β-hydroxy-5-androsten-17-one a novel transformation reaction of exogenous steroids typical of plants, *i.e.* glucosylation. Especially the tissue of young potato tubers glucosylated 3β-hydroxy-5-androsten-17-one in a very good yield[5] (up to 60%). We were interested, therefore, to know whether the enzymatic system catalysing the glucosylation is capable of glucosylating other steroid hydroxy derivatives as well, and how specific it is. We therefore submitted more than 60 hydroxy steroids to glucosylation by means of potato tuber slices and observed which were the structural requirements for this enzymatic reaction.

EXPERIMENTAL

Material

The majority of the steroids used was prepared in our laboratories. Some of the samples were of commercial origin or donated by colleagues from abroad. Samples which were not chromatographically pure were recrystallised or, in several instances, chromatographically purified. The melting points are not corrected.

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Potatoes (tubers) were purchased on the marked and their origin and variety was not determined. However, we did not find a single sort which would not glucosylate. Young potatoes were most active. On ageing their activity dropped but it never completely disappeared.

Solvents, adsorbents, detection reagents *etc.* were described earlier[5].

17β-Hydroxysteroid dehydrogenase was prepared by Dr I. Bartošek, Research Institute for Pharmacy and Biochemistry, Prague, from guinea-pig liver by a method described earlier[6].

Methods

A solution of the steroid (3 mg) in methanol (0·3 ml) was added under stirring to a suspension of freshly grated potato tubers (10 g) in water (75 ml). The mixture was shaken on a reciprocal shaker at 20—25°C for 15—20 hours, usually overnight. It was then filtered and the filtrate extracted twice with 75 ml of chloroform. The extract was dried over sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in 0·3 ml of methanol and an aliquot part (40 μl) was chromatographed on a thin layer of silica gel bound with calcium sulfate, in chloroform–ethanol (9 : 1) or chloroform–methanol (9 : 1). If it was necessary to increase the R_F values a larger proportion of the polar solvent was employed or the chromatography was repeated (multiple chromatography). Detection was carried out either with a chloroform solution of antimony trichloride and heating, or by spraying with conc. sulfuric acid and heating in an oven at 120°C or by direct flame. The colours appearing or fluorescence were observed. As a control, an extract from potatoes was simultaneously chromatographed by the above described procedure (without the addition of the steroid), or an extract after the transformation of 3β-hydroxy-5-androste-17-one, prepared in the same manner. Those tests were considered to be positive in which a distinct spot appeared on the chromatogram, having an R_F value close to the glucoside of 3β-hydroxy-5-androsten-17-one and giving a coloration of fluorescence similar to the starting steroid.

Preparative Glucosylation of 3β,17β-Dihydroxy-5-androstene

3β,17β-Dihydroxy-5-androstene (950 mg) dissolved in 95 ml of methanol was added to a suspension of 2·85 kg of coarsely grated potatoes in 9·5 l of tap water, and the mixture was shaken on a reciprocal shaker at 20°C for 18 hours. It was then filtered through a tissue and the filtrate was extracted with 9 l and 5 l of chloroform and then with 9 l and 5 l of ethyl acetate. After the concentration of the ethyl acetate extract 53·2 mg of a substance crystallised out which according to thin-layer chromatography, *i.e.* according to its R_F value, was predominantly a transformation product corresponding to monoglucoside. The mother liquors were combined with the chloroform extract, evaporated, and chromatographed on alumina (act. III—IV, neutral), first with ethyl acetate and then with methanol (see[5]). The ethyl acetate eluate gave approximately 350 mg of the starting substance and the methanolic eluate 58 mg of a non-crystalline fraction in which a substance was present having the R_F value identical with the above described glucoside. Therefore, both materials were combined and crystallised first from aqueous ethanol and then from a large excess of ethanol. The yield was 41 mg of a compound, m.p. 285—290°C. For the monoglucoside* $C_{25}H_{40}O_7$ (452·6) calculated: 66·34% C, 8·91% H; found: 65·77% C, 8·99% H.

* The evidence that it was a β-glucoside was brought about by enzymatic hydrolysis of the glucoside by means of β-glucosidase (emulsin) and by chromatographic proof of the starting steroid[5].

Preparative Glucosylation of 3β-Hydroxy-5-pregnen-20-one

3β-Hydroxy-5-pregnen-20-one (900 mg) was dissolved in 100 ml of methanol and added to a suspension of 3·07 kg of grated potatoes in 9 l of tap water. Paraffin oil (20 ml) was added to the incubation mixture to prevent foaming and this was then stirred with a current of air for 15 hours. It was then filtered through gauze and the filtrate was extracted four times with 5 l of chloroform. The extract was washed with 1 l of sodium hydrogen carbonate and 1 l of water and dried over sodium sulfate. The filtrate was concentrated to dryness and the residue was dissolved in a mixture of 25 ml of 90% methanol and 25 ml of light petroleum. This mixture was submitted to countercurrent distribution in three funnels by the double-withdrawal method.

After three shifts the methanolic layers were combined and evaporated to dryness (466 mg). The residue was dissolved in 100 ml of ethyl acetate, this was washed with water and dried. After filtration the solution was filtered through a column of 10 g of alumina (act. III—IV, neutral) and the column was washed with 50 ml of ethyl acetate. The eluate was evaporated and the residue (380 mg) gave after crystallisation 160 mg of the starting compound. The alumina column was then eluted with 200 ml of methanol. The eluate was evaporated to dryness and the residue weighed 105 mg. On crystallisation from methanol 48 mg of the product were obtained, m.p. 270—280°C, which after an additional crystallisation from methanol gave 38 mg of a sample melting at 270—277°C. For $C_{27}H_{42}O_7$ (478·6) calculated: 67·75% C, 8·85% H; found: 67·72% C, 8·97% H.

Reduction of the Glucoside of 3β-Hydroxy-5-androsten-17-one with Sodium Boro-hydride

The glucoside of 3β-hydroxy-5-androsten-17-one[5] (11·6 mg) was dissolved in 1 ml of ethanol and mixed with a solution of 41·5 mg of sodium borohydride in ethanol (1 ml). The mixture was shortly heated to boiling point (until the dissolution was complete) and then allowed to stand at room temperature for 6 hours. It was evaporated and the residue was mixed with 1 ml of acetone and evaporated again to dryness. The residue was dissolved in a mixture of 0·5 ml of ethanol, 0·2 ml of acetic acid, and 0·5 ml of water and it was diluted with 5 ml of water. A substance crystallised out which was filtered off and washed with 3 ml of water and dried. Yield 11·0 mg, m.p. 280—289°C. After crystallisation from ethanol 8·2 mg of product were obtained, m.p. 280—285°C, which melted undepressed on admixture of a product obtained on glucosylation of 3β,17β-dihydroxy-5-androstene (see above). Their R_F values were also identical. For $C_{25}H_{40}O_7$ (452·5) calculated: 66·34% C, 8·91% H; found: 66·36% C, 9·05% H.

Enzymatic Dehydrogenation of the Monoglucoside of 3β,17β-Dihydroxy-5-androstene

A solution of the monoglucoside of 3β,17β-dihydroxy-5-androstene (3 mg) in tert-butanol (1·5 ml) and water (1·5 ml) was poured into a mixture of 6·6 ml of a 0·1M Tris-HCl buffer of pH 9·2, 3 ml of a solution of NAD (35 mg NAD), and 3 ml of an enzymatic preparation of 17β-hydroxysteroid dehydrogenase which contained 250 mg of proteins. Water (14·4 ml) was further added to the mixture which was then incubated for 1 hour at 37·5°C and then extracted with four 30 ml portions of chloroform. The extract was dried over anhydrous sodium sulfate, filtered and evaporated. The residue was extracted twice with 0·3 ml portions of light petroleum, dissolved in a mixture of 0·5 ml of methanol and 0·1 ml of water. A part of the solution (10 µl) and the standards (10 µl) of the starting compound and of the glucoside of 3β-hydroxy-5-androsten-17-one (1 mg of compound in 0·2 ml of methanol and 0·1 ml of water) were chromatographed on a thin layer of silica gel bound with calcium sulfate in a chloroform–ethanol mixture (85 : 15). Detection was carried out with antimony trichloride in chloroform. According to the chromatogram the dehydrogenation of the monoglucoside took place with a high yield.

TABLE I

The Results of the Glucosylation of Hydroxy Steroids by the Enzymatic System from Potato Tubers

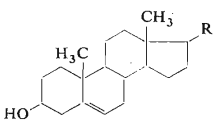
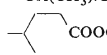
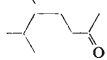
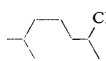
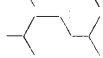
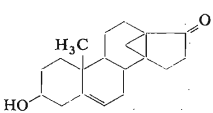
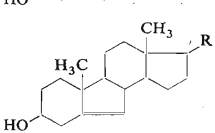
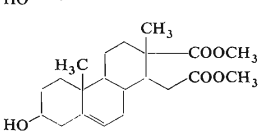
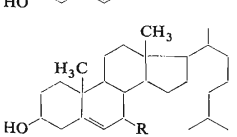
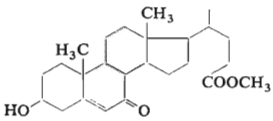
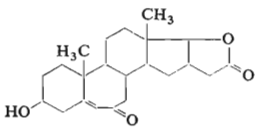
Formula	R	Cpd. No	Glucosylation
	=O	1	+++++
	—CO.CH_3	2	+++
	—OH	3	++
	—$COOCH_3$	4	+++
	—$CH(CH_3).COOH$	5	—
	—$CH(CH_3).COOCH_3$	6	—(?)
		7	+
		8	—
		9	—
		10	—
	— H	11	++
		12	++++
	=O	13	+++
	—CO.CH_3	14	++
		15	+++
	⁓OH	16	+(tr.)
	=O	17	—

TABLE I
(*Continued*)

Formula	R	Cdp. No	Glucosylation
		18	—
		19	—

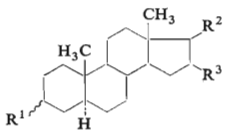
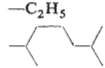
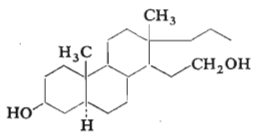
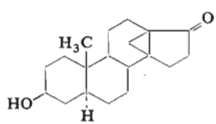
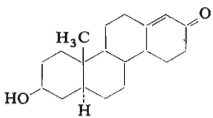
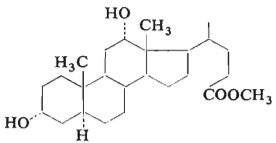
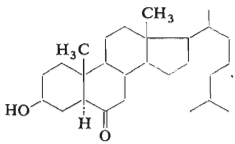
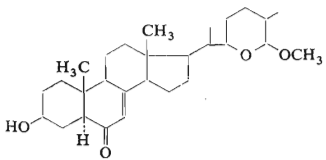
Formula	R^1	R^2	R^3	No	Glucosylation
	—OH	=O	—H	20	++++
	····OH	=O	—H	21	—
	—OH	—H	=O	22	++++
	····OH	—H	=O	23	—
	—OH	—H	—H	24	++
	····OH	—H	—H	25	—
	—OH	$—C_2H_5$	—OH	26	+++
	—OH	$—C_2H_5$	=O	27	++++
	—OH		—H	28	—
				29	++
				30	++

TABLE I
(*Continued*)

Formula	Cpd. No	Glucosylation
	31	++
	32	–
	33	++
	34	++

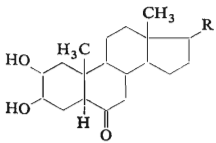
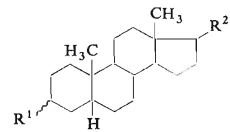
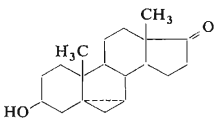
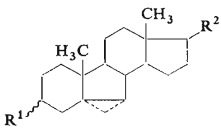
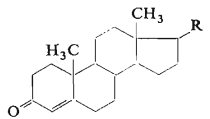
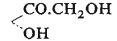
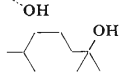
Formula	R	Cpd. No	Glucosylation
	—H	35	–
	—C_8H_{17}	36	–

TABLE I
(*Continued*)

Formula	R^1	R^2	Cpd. No	Glucosylation
	—OH	=O	37	—
	····OH	=O	38	—
	—OH	—OH	39	—
			40	—
	—OH	=O	41	++++
	—OH	$-CO.CH_3$	42	+++
	····OH	=O	43	—

Formula	R	Cpd. No	Glucosylation
	—OH (testosterone)	44	—
	$-CO.CH_2OH$	45	—
		46	—
		47	—

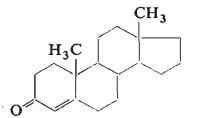
Formula	Substituents	Cpd. No	Glucosylation
	11α-hydroxy, 17-keto	48	—
	7β-hydroxy, 17-keto	49	—
	6β-hydroxy	50	—

TABLE I
(*Continued*)

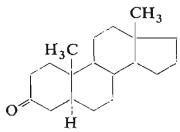
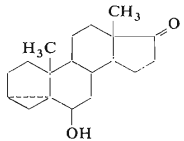
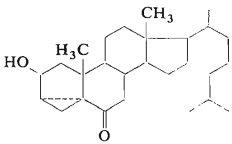
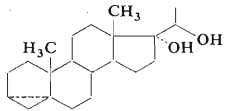
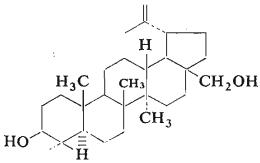
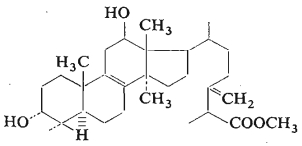
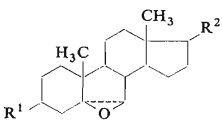
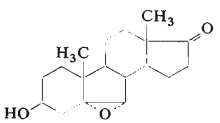
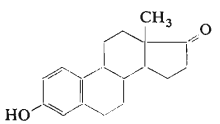
Formula	Substituents	Cpd. No	Glucosylation
	16β-hydroxy 11α-hydroxy, 6-keto	51 52	—
		53	—
		54	—
		55	—
		56	—
		57	—

TABLE I
(*Continued*)

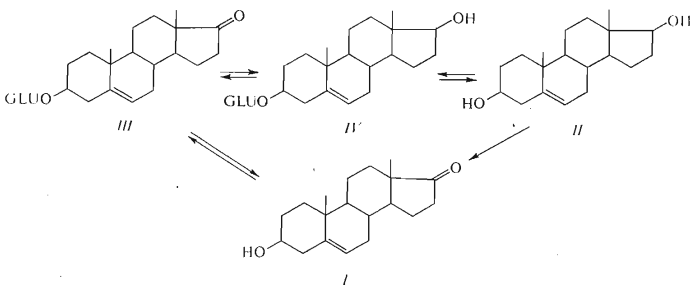
Formula	R^1	R^2	Cpd. No	Glucosylation
	—OH	$—C_8H_{17}$	58	—
	····OH	$—C_8H_{17}$	59	—
	—OH	=O	60	—
			61	+
			62	—

[a] The number of crosses indicates the approximate intensity of the spots of the glucosides on chromatograms.

RESULTS

The first steroids which we submitted to the glucosylation test were those close to 3β-hydroxy-5-androsten-17-one (*I*), *i.e.* 3β,17β-dihydroxy-5-androstene (*II*) and 3β-hydroxy-5-pregnen-20-one. Both were glucosylated, but distinctly slower than 3β-hydroxy-5-androsten-17-one. If the latter was glucosylated in an almost 60% yield, the first two substances were glucosylated to a maximum of 20 to 30% (judging from the chromatograms and the preparative experiments). In the case of the glucoside of 3β,17β-dihydroxy-5-androstene (*IV*) it was necessary, however, to prove on which hydroxy group glucosylation took place, because the R_F value and the analysis indicated that it was a monoglucoside. We therefore prepared an authentic 3β-monoglucoside of 3β,17β-dihydroxy-5-androstene (*IV*) by reduction of 3β-glucoside of 3β-hydroxy-5-androsten-17-one[5] (*III*) with sodium borohydride. The obtained product was compared with a product of glucosylation of 3β,17β-dihydroxy-5-androstene(*II*) and they were found identical (m. p., R_F values, analyses). A further

proof was brought about by specific oxidation of the free 17β-hydroxy group of the glucoside *IV* by 17β-hydroxysteroid dehydrogenase from guinea-pig liver. According to chromatographic analysis (R_F, detection) the oxidation took place under the formation of 3β-glucoside of 3β-hydroxy-5-androsten-17-one (*III*) which shows clearly that in compound *II* only the 3β-hydroxy-group was glucosylated, but not the 17β-hydroxyl.



SCHEME 1

The specificity of the 17β-hydroxysteroid dehydrogenase was tested on 3β,17β-dihydroxy-5-androstene (*II*) as a model substance. This diol was oxidised to 3β-hydroxy-5-androsten-17-one (*I*).

In view of the fact that the suspension of grated potato tubers glucosylated 3β,17β-dihydroxy-5-androstene only at the position 3β, but not at the position 17β we started to study the specificity of the enzymatic system glucosylating steroids more thoroughly The results are presented in Table I.

From the experiments it follows that the enzymatic system is very specific. It glucosylates only Δ^5 and 5α-steroids at the 3β-hydroxy group with the exception of substances mentioned below. 3β-Hydroxy steroids of the 5β-series and 3α-hydroxy steroids of the 5α-series were not glucosylated. Interesting results were obtained with 3-hydroxy-5,7-cyclosteroids and 5,6-oxides of B-norsteroid. In those cases where the methylene bridge (compound No 40) or the oxido group (No 58–60) were above the plane of the five-membered ring (B), glucosylation did not take place. In the opposite case, *i.e.* where the bridge atom was below the plane of the five-membered ring, glucosylation did take place if the hydroxy group in the position 3 had the β-configuration (compounds No 41, 42, 61). The 3-hydroxy group on the aromatic ring A (No 62) was not glucosylated. The hydroxy groups in other positions of the steroid molecule could not be glucosylated either.

Among the steroids of the Δ^5 or the 5α-series only those were not glucosylated at the position 3β which contained in the vicinal position either a hydroxy group (No 35, 36) or 2 methyl groups (No 56). Steroids with a longer side chain in the position 17 were glucosylated only exceptionally (No 33, 34).

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