GITOXIGENIN GLYCOSIDES

I. F. Makarevich, S. G. Kislichenko,
D. G. Kolesnikov, L. Ya. Topchii,
and M. A. Angarskaya

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The synthesis of cardiac glycosides with the sugar component in different positions in the aglycone is interesting from the point of view of the influence of this factor on the biological action of the glycosides. The introduction of the sugar component at C-16 is particularly interesting. As early as 1944 there was a paper [1] from Japanese authors who obtained from Digitalis purpurea L. a glycoside digicorin with a biological activity 100-1000 times greater than that of digitoxin. In digicorin, according to these workers, the aglycone is gitoxigenin and the sugar component is D-digicuronic acid, attached by a glycosidic bond at C-16. In this connection, attention is attracted to three points: the unusual position in which the sugar component is attached, the extremely high biological activity, and also the fact that until now the compound has never been isolated again in spite of the intensive study of the foxglove carried out in various countries.

Gitoxigenin has already been used for the semisynthesis of glycosides. Thus, Voigtlander et al. [2], by glycosylating gitoxigenin with triacetyl-L-rhamnosyl bromide, obtained gitoxigenin 3-α-L-rhamnoside and, in addition, $\Delta^{14,16}$-dianhydrogitoxigenin 3-α-L-rhamnoside. There is no information whatever on the preparation of glycosides with a different position of the sugar component in the paper cited.

Previously, in the synthesis of digoxigenin glycosides, we noted [3] that in glycosylation 2,3,4-tri-O-acetyl-1-bromo-L-rhamnose is more subject to the influence of steric hindrance than 2,3,4,6-tetra-O-acetyl-1-bromo-D-glucose or the analogous derivative of D-xylose. Consequently, in the experiments to be described we used D-glucose.

The synthesis was performed under mild conditions by the Koenigs-Knorr method in Chernobai's modification [4] by glycosylating gitoxigenin with acetylbromoglucose. The protective acetyl groups were eliminated by saponification with potassium bicarbonate. It was established by paper chromatography that the synthetic product consisted of not less than five substances, which were shown up by trichloroacetic acid in the form of spots with a blue fluorescence in UV light. The cardenolides obtained were separated by partition column chromatography in the solvent systems toluene–butan-1-ol (3:1 and 1:2)/water. Four cardenolides were isolated in the individual state, and these were denoted by the symbols I, II, III, and IV in order of increasing polarity. Substance (I) was shown to be identical with the initial gitoxigenin.

Cardenolide (II) was obtained with a yield of 49% with respect to the weight of the gitoxigenin taken for the synthesis. Its properties were similar to those of natural gitorin [9], which is the 3-O-β-D-glucoside. The structure of the glycoside (II), corresponding to gitorin, was also confirmed by the following facts. The results of elementary analysis agreed well with those calculated for the structure $C_{29}H_{44}O_{10}$, i.e., they show that it is a monoglycoside. On enzymatic hydrolysis, D-glucose and gitoxigenin were formed. Analysis of the molecular rotations in accordance with Klyne's rule showed the β configuration of the glycosidic bond. The prolonged standing of the glycoside adsorbed by alumina (activity grade I) gave $\Delta^{14,16}$-dianhydrogitoxigenin glucoside. An analysis of the rate of acetylation (by acetic anhydride in pyridine) of glycoside (II) in accordance with the method described previously [5] showed the absence of axial OH groups (in the D-glucose residue all the secondary OH groups are equatorial; and the alcoholic group at C-16 of the aglycone acetylates at a rate approximately equal to that of the acetylation of equatorial OH groups). This last fact unambiguously shows that in the glycoside (II) the D-glucose is attached at C-3 and not at C-16.

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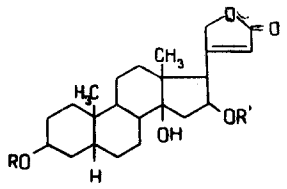
Glycosides III and IV were isolated in amounts of only 15 and 22 mg, respectively. Both glycosides gave a positive Legal reaction and, in contrast to the preceding three substances, a virtually negative Raymond reaction. On enzymatic hydrolysis they formed gitoxigenin and D-glucose (both identified by paper chromatography). The UV spectrum of each substance is characterized by a single absorption maximum, at 216 nm for substance (III) and 217 nm (log ε 4.11) for substance (IV).



I. R = R' = H
II. R = β-D G , R' = H
III R = H, R' = β-D-G
IV. R = R' = β-D-G

The elementary analysis of the glycoside (IV) corresponded to a bioside with the composition $C_{35}H_{54}O_{15}$. The high polarity of the substance also agrees with the assumption that it is a bioside.

In both the glycosides (III and IV), the D-glucose is present in the pyranose form and is attached by a β-glycosidic bond, as is shown by the resistance of the substance to hydrolysis with 0.05 M sulfuric acid [6] and by the values of the molecular rotations in accordance with Klyne's rule [7]:

Substance	$[M]_D$, deg	
Gitoxigenin 3,16-diglucoside (IV), mol. wt. 714.8, $[\alpha]_D$ +2.6 ± 4°	+ 18.6 ± 28	
Gitoxigenin 3-glucoside (II), mol. wt. 552.7, $[\alpha]_D$ +8.0 ± 2°	+ 44.2 ± 11	
Gitoxigenin 16-glucoside (III), mol. wt. 552.7, $[\alpha]_D$ +11.8 ± 5°	+ 65.2 ± 28	
Gitoxigenin, mol. wt. 390.5, $[\alpha]_D$ +34.5 ± 2°	+134.7 ± 8	
Rotation of the D-glucose moiety in substance (II)	− 90.5 ± 19	
Rotation of the D-glucose moiety in substance (III)	− 69.5 ± 36	
Rotation of the D-glucose moiety at C-16 in substance (IV)	− 25.6 ± 39	
Methyl α-D-glucopyranoside, mol. wt. 194.2, $[\alpha]_D$ +158.9° ± ?	+308.6 ± ?	[8]
Methyl β-D-glucopyranoside, mol. wt. 194.2, $[\alpha]_D$ −34.2° ± ?	− 66.4 ± ?	[8]

Judging from the polarity of substance (III), it may be assumed that this is a monoglycoside. An analysis of the rate of its acetylation clearly shows the presence of an axial OH group in this substance. In this case, an axial alcoholic group can be present only at C-3 of the aglycone. From this it follows that the sugar component in glycoside (III) is present at C-16.

Thus, the results obtained permit the assumption that cardenolides (III) and (IV) are, respectively, gitoxigenin 16β-O-β-D-glucopyranoside and gitoxigenin 3β,16β-di-O-β-D-glucopyranoside. The fact that these cardenolides do not give the Raymond reaction (reaction for butenolides) is obviously due to the influence of the sugar component at C-16, which considerably shields the lactone ring and, apparently, forms intramolecular hydrogen bonds with it.

The very small yield with which the synthesis of gitoxigenin 16-glycosides takes place can also be explained by steric factors: the approach to the OH group at C-16 of such a voluminous substituent as tetraacetyl-D-glucosyl bromide is made difficult by the presence of the closely adjacent methyl group at C-13 OH group at C-14, and butenolide ring at C-17.

A determination of the biological activity of the glycosides synthesized, carried out on cats by Hatcher's method, showed that gitoxigenin 3-O-β-D-glucoside possesses a comparatively high cardiotonic activity (0.25 mg/kg); working solution 1 : 50,000. Gitoxigenin 16-O-β-D-glucoside and gitoxigenin 3,16-di-O-β-D-glucoside do not exert the specific action on the heart which is characteristic of "ordinary" cardenolides. When they were administered in a dose of 2 mg/kg the animals remained alive; in this test the working solutions were 1 : 25,000.

Consequently, the results of our investigations differ sharply from those given by the Japanese authors [1], who indicated a high biological activity of a gitoxigenin 16-glycoside.

EXPERIMENTAL

The substances were analyzed after being dried for two hours in vacuum (0.01 mm Hg) at 80°C over phosphorus pentoxide. The melting points were determined on a Kofler block.

Synthesis. With heating, 5.8 g of anhydrous gitoxigenin was dissolved in 1.5 liter of dichloroethane, and 25 g of silver oxide and 1.2 g of calcium oxide were added. With continuous stirring, a dichloroethane solution of 18.5 g of acetylglucosyl bromide was added uniformly over 7 min to the boiling reaction mixture. The mixture was boiled for another 5 min and the resulting solution was filtered through a layer of Kieselguhr under vacuum. The filtrate was treated twice with water (100 ml each time) and evaporated. The residue was dissolved in 1 liter of methanol and the solution was treated with 540 ml of 2.2% potassium bicarbonate solution and left at room temperature (20-25°C) for seven days. Then it was concentrated in vacuum to a volume of about 1 liter and the cardenolides were exhaustively (according to the Legal reaction) extracted with a mixture of chloroform and ethanol (2:1). The extracts were dried with anhydrous sodium sulfate and evaporated. The residue was dissolved in 300 ml of ethanol, and 250 ml of water was added. The solution was slowly concentrated to a volume of about 300 ml. The crystals of gitoxigenin that deposited (2.1 g) were separated off and the filtrate, after saturation with sodium chloride, was treated with a mixture of ethanol and chloroform (1:2; 6 × 350 ml). The alcoholic-chloroformic extract was washed with 20 ml of water and evaporated.

The resulting mixture of cardenolides was separated by partition chromatography in the solvent systems toluene–butan-1-ol (3:1 and 1:2)/water. The support for the stationary phase was cellulose (1.5 kg) impregnated with water. The ratio of the combined substances to be separated to cellulose (calculated as air-dry) was 1:300.

Gitoxigenin 3-O-β-D-Glucoside (II). The glycoside was obtained in an amount of 2.85 g, with mp 241-243°C (ethanol); $[\alpha]_D^{19}$ +8.0 ± 2° (c 1.00; methanol). In conc. H_2SO_4 it formed the following colorations changing with time: 0 min, orange; 60 min, red-orange; 320 min, brown; 500 min, green; 25 h, blue with a precipitate.

The glycoside (60 mg) was dissolved in 1.0 ml of dimethylformamide, and 60 mg of an enzyme preparation from the grape snail dissolved in 20 ml of water was added. The solution was left in the thermostat for 40 h. Then 120 ml of hot ethanol was added. The precipitate of enzymes was filtered off. The filtrate was concentrated to a volume of about 3 ml. Crystals of the aglycone deposited, and these were separated off and recrystallized from ethanol. The aglycone melted at 222-225°C and, according to paper chromatography and the colorations with conc. H_2SO_4, it was identical with gitoxigenin. The mother liquor contained a monosaccharide which was identified by paper chromatography as D-glucose.

The glycoside (0.2 g) was dissolved with heating in 40 ml of dioxane. The solution was mixed with 100 g of alumina (activity grade I) and kept at 35°C for 15 days. Then the adsorbent was deactivated with water and the cardenolides were extracted with a mixture of ethanol and chloroform (1:2). The extract was evaporated, and the residue was chromatographed on 10 g of alumina (activity grade III). The cardenolide eluted first [with a mixture of chloroform and ethanol (93:7)] was crystallized from acetone–benzene. It melted at 189-196°C, and its UV spectrum had two absorption maxima: λ_{max} (in ethanol) 224 and 332 nm (log ε 4.08 and 4.27). The enzymatic hydrolysis of the anhydroglycoside gave D-glucose, identified by paper chromatography, and $\Delta^{14,16}$-dianhydrogitoxigenin with mp 211-215°C, $[\alpha]_D^{20}$ +531.3 ± 10° (c 0.73; chloroform). The UV spectrum of the anhydroaglycone also showed two absorption maxima: λ_{max} (in ethanol) 224 and 333 nm (log ε 4.08 and 4.24). Both the anhydroglycoside and the anhydroaglycone had a blue fluorescence in UV light.

Gitoxigenin 3,16-di-O-β-D-Glucoside (IV). The substance crystallizes from isopropanol, mp 238-246°C; $[\alpha]_D^{18}$ +2.6 ± 4° (c 0.42; methanol). In conc. sulfuric acid it forms the following colorations: 0 min, yellow; 5 min, yellow-orange; 30 min, orange-brown; 96 min, red; 17 h, gray-green. In the toluene–butan-1-ol (1:2)/water system, R_f 0.28. Enzymatic hydrolysis carried out on the micro scale and analysis of the hydrolysis products by paper chromatography showed the presence of gitoxigenin and D-glucose.

Gitoxigenin 16-O-β-D-Glucoside (III). This cardenolide was obtained in an amorphous, but chromatographically homogeneous, state; $[\alpha]_D^{19}$ +11.8 ± 5° (c 0.34; methanol). In conc. H_2SO_4 it gave the following colorations: 0 min, yellow; 5 min, yellow-orange; 30 min, orange-brown; 240 min, brown; 270 min, red; 360 min, violet; 20 h, blue. On enzymatic hydrolysis, gitoxigenin and D-glucose were formed.

SUMMARY

Three cardiac glycosides have been synthesized from gitoxigenin and D-glucose: gitoxigenin 3β-O-β-D-glucopyranoside, gitoxigenin 16β-O-β-D-glucopyranoside, and gitoxigenin 3β,16β-di-O-β-D-glucoside.

The first glycoside, obtained with a yield of 49% on the weight of the initial aglycone, possesses a fairly high biological activity (0.25 mg/kg), while the other two, formed in a yield of about 0.3%, do not possess the action on the heart that is specific for the cardenolides.

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