NEW SEMISYNTHETIC CARDIAC GLYCOSIDES AND THEIR BIOLOGICAL ACTIVITY

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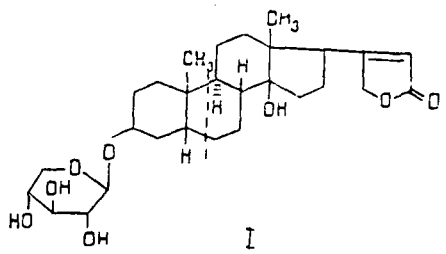
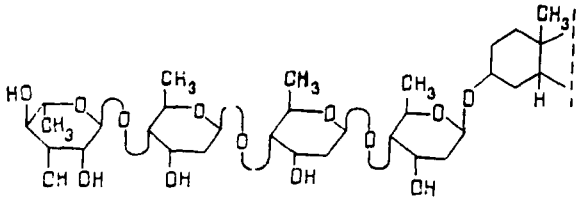
Digitoxigenin, digitoxin, D-xylose and L-rhamnose have been used as the starting materials for the synthesis of the new cardiac glycosides: digitoxigenin 3-O-β-D-xylopyranoside and digitoxin 4'''-O-α-L-rhamnopyranoside. It has been established that the compounds obtained possess comparatively high biological activities.

In order to study the relationship between chemical structure and biological activity, we have synthesized two new cardiac glycosides. One of them is the monoglycoside digitoxigenin 3-O-β-D-xylopyranoside (I) and the other the tetraglycoside digitoxin 4'''-O-α-L-rhamnopyranoside (II).



I



II

In approaching the performance of this investigation, we set ourselves the task of determining, in the first place, how the biological activity changes if in Purpurea glycoside A the D-glucose residue is replaced by a L-rhamnose residue. The driving motive for this was the circumstance that L-rhamnose in monoglycosides usually imparts a higher biological activity than the majority of sugars of the D-series [1]. There was no information on the effect of the presence of a L-rhamnose residue in those cases where it is the terminal residue in an oligoside chain. No natural glycosides with such a structure have been found. In the second place, the addition to the three D-digitose units in the known glycoside digitoxin of a, more polar, D-xylose unit should, so we expected, also lead to a change in biological activity, and this in the direction of an increase.

The glycosides were synthesized by the Koenigs–Khnorr method in V. T. Chernobai's modification [2]. A mixture of mercuric oxide and silver carbonate was used as the HBr acceptor. In order to bring the formation of by-products in the synthesis of glycoside (II) to a minimum, the calculated amounts of 1-bromo-2,3,4-tri-O-acetyl-L-rhamnose and of digitoxin were used. The synthesis took place stereospecifically: the D-xylose added to the aglycon by a

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TABLE 1

Glycoside	Biological activity, mg/kg body weight of the cat	Biological activity in experiments on pigeons	
		mg/kg	PAU
Digitoxin xyloside (I)	0,213*	0,277±0,013	3627
Digitoxin	0,325–0,420	0,48	2091
Digitoxin rhamnoside (II)	0,238*	0,310±0,009	3230
Purpurea glycoside A	0,337	0,44	1916

*Calculated figures obtained on the basis of the results of analysis in experiment on pigeons.

β-glycosidic bond, and the L-rhamnose added to digitoxin by an α-glycosidic bond. It was possible to judge this from the values of the increments of the molecular rotation of the D-xylose in glycoside (I). ($\Delta C = -7.4 \pm 17.6°$) and of L-rhamnose in glycoside (II) ($\Delta C = -310.8 \pm 33.5°$) and also from their PMR spectra. The PMR spectra of glycoside (I) showed the presence of a signal at 4.76 ppm in the form of a doublet (J = 6.0 Hz) belonging to an axial proton at an anomeric carbon atom.

To prove the position of addition of the L-rhamnose in glycoside (I), we used the kinetic method of conformational analysis [3]. After the addition of the L-rhamnose derivative to digitoxin, the inermediate product obtained [triacetyl-(II)] was subjected to controlled further acetylation. Analysis of the course of the reaction by the method described showed that the intermediate product lacked an equatorial hydroxyl that was present in the initial digitoxin at C-4'''. It followed unambiguously from this that the position of addition of the L-rhamnose residue was the 4''' position.

Investigation of biological activity by Hatcher's method (Table 1) showed that the digitoxigenin xyloside (I) that had been synthesized was 1.7 times more active than digitoxin, while the digitoxin rhamnoside (II) was 1.4 times more active than the Purpurea glycoside. These results, in the main, confirmed our expectations. Nevertheless, the striking finding was that digitoxin L-rhamnoside (II) and digitoxin D-glucoside (Purpurea glycoside A) differed so greatly in biological activity in favor of the rhamnoside. In spite of the remoteness of the L-rhamnose unit from the aglycon, its influence on biological activity was considerably greater than the influence of a D-glucose residue present at the same distance.

EXPERIMENTAL

The elementary analysis of the glycosides was performed with the use of an automatic C–H–N–S analyzer. The results of the analysis agreed with the calculated figures.

Digitoxigenin 3-O-β-D-xyloside (I). $C_{28}H_{42}O_8$. With heating 2.0 g of digitoxigenin was dissolved in 0.12 liter of dichloroethane, and 4.0 g of silver carbonate and 10.0 g of mercury oxide were added. The mixture was boiled for 3 min, after which a solution of 8 g of 1-bromo-2,3,4-tri-O-acetyl-D-xylose dissolved in 30 ml of dichloroethane was added with continuous stirring over 5 min. Boiling with stirring was continued for another 30 min. During the reaction, another 40 ml of dichloroethane was added, and the precipitate was washed with chloroform [sic]. The filtrate was purified with a 2 N solution of sodium carbonate (50 ml × 3), washed with water to neutrality (30 ml × 3), and evaporated. The residue was dissolved in 200 ml of methanol, and 20 ml of methanol saturated with ammonium was added.

The saponification process, which lasted 17 h, was monitored by paper chromatography. The salt that had deposited was filtered off, and the filtrate was evaporated. The resudue was dissolved in chloroform–ethanol (2:1; 200 ml). The ethanolic chloroform solution was washed with water (30 ml × 2), and dried with anhydrous sodium sulfate; it was purified with a ninefold amount of Al_2O_3 activated at 120°C, and was evaporated. The glycoside was crystallized three times from benzene. The individual glycoside was obtained with mp 216-218°C, $[\alpha]_D^{20}$+ 12.2 ± 2° (c 1.0; ethanol).

Digitoxin–4'''-O-α-L-Rhamnoside (II). $C_{47}H_{74}O_{17}$. In a similar way to that described above for the glycosylation of digitoxigenin, 3.0 g of digitoxin was glycosylated with 1-bromo-2,3,4-tri-O-acetyl-L-rhamnose. After the protective acetyl groups had been eliminated, the technical product was chromatographed on 150 g of silica gel that had been activated at 120°C for 2 h. Elution was performed with mixtures of ethyl acetate and methanol of increasing

polarity. The product was crystallized from the two-phase methanol—ethyl acetate—water—petroleum ether (2:2:3:3) system. This gave the individual glycoside with mp 169-170/255-261°C, $[\alpha]_D^{20}$ −20.1 ± 2° (c 1.0; ethanol).

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TRITERPENE GLYCOSIDES OF Hedera taurica

VI. STRUCTURES OF HEDEROSIDES G, H_1, H_2, AND I FROM THE BERRIES OF CRIMEAN IVY

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We have isolated from Crimean ivy berries in addition previously known triterpene glycosides — 3-O-α-L-arabinopyranosyl-28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glycopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin, 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glycopyranosyl]hederagenin, the new triterpene glycosides hederoside H_2-3-O-[O-β-D-glycopyranosyl-(1 → 2)-β-D-glycopyranosyl-(1 → 2)-β-D-glucopyranosyl]-28-O-[O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]oleanolic acid- and hederoside I-3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-28-O-[O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin. Details of their ^{13}C NMR spectra are given.

We have previously studied the weakly polar triterpene glycosides of the berries of crimean ivy Hedera taurica Carr., family Araliaceae [1-3]. In the present paper we describe the isolation and determination of the structures of glycosides of medium polarity which have been called, in order of increasing polarity, hederosides G, H_1, H_2, and I.

For the isolation of these glycosides, the berries were subjected to boiling with the aim of denaturing the enzymes, and were comminuted and extracted with aqueous ethanol. The extract was evaporated, and the residue was diluted with water and was extracted successively with chloroform to eliminate weakly polar substances and with butanol, into which the triterpeneglycosides of medium polarity passed together with phenolic compounds. The preliminary separation of the glycosides and their freeing from phenolic compounds was carried out by reversed-phase chromatography on silica gel with grafted-on heptyl groups [4]. Under these conditions, free sugars, phenolic compounds, and glicosides I, H, and G, were eluted successively. The latter were additionally purified by chromatography on silica gel.

TLC analysis of the acetates of the glycosides showed that glycosides G and I were individual compounds, while the acetate of glycoside H contained two components — H_1 and H_2. Additional purification of hederosides G and I and the separation of hederosides H_1 and H_2 were achieved by the chromatography of their acetates on silical gel, followed by deacetylation.

The complete acid hydrolysis of the hederosides permittted the identification as their aglycons of hederagenin for glycosides G, H_1, and I. and oleanolic acid for glycoside H_2. The carbohydrate composition of hederosides G and H_1 was represented by arabinose, rhamnose, and glucose, and that of hederoside H_2 and I by glucose.

The alkaline hydrolysis of the hederosides gave progenins the structures of which it was possible to establish by comparing their chromatographic mobilities with glycosides of

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