CARDIAC GLYCOSIDES OF Cheiranthus allioni. VI.*

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From the seeds of Cheiranthus allioni Hort. we have isolated another two cardiac glycosides. The method of isolation was similar to that described previously [1, 2]. One of the glycosides, which we have called glucoalliside, has the composition $C_{35}H_{54}O_{15}$, corresponding to a bioside. Glucoalliside gives positive Legal, Raymond, Kedde, and Liebermann reactions and a negative Keller–Kiliani reaction. The UV spectrum is characterized by one absorption maximum: λ_{max} (in ethanol) 219 nm (log ε 4.16), due to a butenolide ring. Under the influence of an enzyme preparation from the grape snail, it hydrolyzes forming a monoglycoside and a monosaccharide, which have been obtained in the pure crystalline state and identified as alliside and D-glucose. Alliside is bipindogenin 3-α-L-glucomethylopyranoside [3].

Glucoalliside is not appreciably hydrolyzed by 0.05 N sulfuric acid at 80°C for 30 min. This shows the pyranose form of the monosaccharides present in it [4].

In order to establish the position of attachment of the D-glucose to the L-glucomethylose, the following transformations of glucoalliside were performed.

The glycoside was hydrogenated over a palladium catalyst, and the cardenolide obtained was subjected to periodate oxidation, the consumption of sodium periodate amounting to 3 moles. Hydrolysis of the oxidized product and analysis of the hydrolysate showed the complete absence from it of both D-glucose and L-glucomethylose. These results exclude the possibility of a 1 → 3 glycosidic bond. If the latter were present, the L-glucomethylose would be unaffected and the consumption of sodium periodate would be 2 moles.

Then glucoalliside was exhaustively methylated by Kuhn's method and the product was hydrolyzed. The methylated sugars were separated by adsorption chromatography on alumina and were identified as 2,3,4,6-tetra-O-methyl-D-glucose and 2,3-di-O-methyl-L-glucomethylose. The structure of the second monosaccharide was confirmed by the fact that on treatment with phenylhydrazine it did not form a phenylosazone, but it readily formed a phenylhydrazone. The UV spectrum of the latter is characterized by two absorption maxima, at 239 and 278 nm, which agrees well with literature data for analogous compounds [5]. Phenylosazones show three absorption maxima in the UV spectrum: λ_{max} (in ethanol) 256, 308-314, and 395-399 nm [6].

Thus, on the basis of the results of periodate oxidation and the formation of a phenylhydrazone, it may be unambiguously concluded that in glucoalliside the OH groups at C_2 and C_3 of the L-glucomethylose residue are free. This, in its turn, shows a 1 → 4 bond of the D-glucose and L-glucomethylose in the glycoside.

The formation of 2,3,4,6-tetra-O-methyl-D-glucose shows its pyranose form and also the fact that it occupies the terminal position.

An analysis of molecular rotations in accordance with Klyne's rule [7] shows (Table 1) that the L-glucomethylose is attached by an α-glycosidic bond and the D-glucose by a β-glycosidic bond.

Because of the absence of information in the literature on the specific rotation of the methyl L-glucomethylosides, the comparison was made with methyl α-L-glucoside, the properties of which are known [8].

* For Communication V, see [2].

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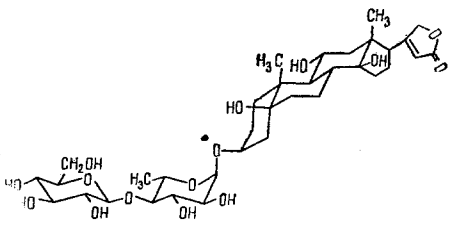
TABLE 1

	M_D, deg	
Glucoalliside, mol. wt. 714.8, $[\alpha]_D$ −47.1 ± 2°	−336.7 ± 14.3	
Alliside, mol. wt. 552.7, $[\alpha]_D$ −47.6 ± 4°	−263.1 ± 22.1	
Bipindogenin, mol. wt. 406.5, $[\alpha]_D$ +29.3 ± 2°	+119.1 ± 8.1	
D-Glucose moiety of glucoalliside	− 73.6 ± 36.4	
L-Glucomethylose moiety of alliside	−382.2 ± 30.2	
Methyl α-D-glucopyranoside, mol. wt. 194.2, $[\alpha]_D$ +158.9° ± ?	+308.6 ± ?	[9]
Methyl β-D-glucopyranoside, mol. wt. 194.2, $[\alpha]_D$ −34.2° ± ?	− 66.4 ± ?	[9]
Methyl α-L-glucopyranoside, mol. wt. 194.2, $[\alpha]_D$ −156.9° ± ?	−304.7 ± ?	[8]

In view of the fact that the difference in the optical activities of the hexosides and the 6-deoxy derivatives corresponding to them are small or completely absent in the majority of cases, it may be assumed that the comparison made gives a correct idea of the configuration of the glycosidic bond of the L-glucomethylose.

Thus, glucoalliside can be characterized as 5β,11α,14β-trihydroxycard-20(22)-enolide 3β-O-[O-β-D-glucopyranosyl-(1 → 4)-α-L-glucomethylopyranoside]:



The conformation of the L-glucomethylose can be judged from the results of investigations performed for 3-O-methyl-L-glucomethylose (L-thevetose) [16].

The second glycoside isolated from Cheiranthus allioni was identified by its properties and hydrolysis products as cheiroside A. The latter is uzarigenin 3-β-D-fucosyl-β-D-glucoside [10]. In view of the fact that the position in which the glucose is attached in this glycoside has not been determined previously, we performed the transformations analogous to those for glucoalliside. In the periodate oxidation of cheiroside A, both the D-glucose and the D-fucose were destroyed. After methylation followed by hydrolysis, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3-di-O-methyl-D-fucose were obtained, which shows a 1 → 4 bond between the D-glucose and the D-fucose in the glycoside.

EXPERIMENTAL

For analysis, the substances were dried in a vacuum of 0.01 mm Hg at 100°C over phosphorus pentoxide for 2 h. The elementary analyses corresponded to the calculated figures.

Glucoalliside. The glycoside crystallized from isopropanol; mp 203-206°C; $[\alpha]_D^{19}$ − 47.1 ± 2° (c 1.00; methanol). In conc. H_2SO_4, it forms the following colorations changing with time: 0 min, orange; 5 min, brown; 80 min, red; 140 min, violet.

Found %: mol. wt. 708.1 (spectrophotometrically). $C_{35}H_{54}O_{16}$. Calculated %: mol. wt. 714.8.

Enzymatic Hydrolysis of Glucoalliside. A solution of 0.25 g of glucoalliside and 0.3 g of a dry enzyme preparation from the grape snail in 25 ml of water was left in a thermostat at 40°C for 30 h. Then 100 ml of hot ethanol was added and the enzyme precipitate was separated off by filtration. The filtrate was concentrated under vacuum to a volume of about 15 ml and was then treated with a mixture of ethanol and chloroform (1:2; 4 × 25 ml). The ethanolic−chloroformic extract was washed with 10 ml of water and evaporated to dryness. The residue, which consisted of the monoglycoside, was crystallized from acetone−benzene. The aqueous solution was concentrated to a syrupy consistency. The monosaccharides were

crystallized from ethanol–ether. The substance obtained had mp 145-146°C; $[\alpha]_D^{20}$ +54.5 ± 5° (c 0.67; aqueous solution after 2 h). On paper chromatography, the substance had the same R_f values as D-glucose. The melting point of a mixture with authentic D-glucose was 145-146°C.

The melting point of the monoglycoside was 180-183°C; $[\alpha]_D^{19}$ – 47.6 ± 4° (c 0.71; methanol). These results, and also those obtained by paper chromatography and the mixed melting point test, correspond to alliside [3].

The monoglycoside (80 mg) was hydrolyzed with hydrochloric acid in acetone by the Mannich–Siewert method [11] for nine days. After the usual working up, a crystalline aglycone (30 mg) and an amorphous monosaccharide were obtained. The latter was identified by paper chromatography as L-glucomethylose. The aglycone had mp 231-235/294-300°C (ethanol), $[\alpha]_D^{21}$ +29.3 ± 2° (c 0.87; methanol). A mixture with a sample of bipindogenin gave no depression of the melting point.

Hydrogenation of Glucoalliside. Palladium–carbon (100 mg; 5% of palladium) catalyst in 5 ml of absolute ethanol was first hydrogenated for 1 h. Then 35 mg of glucoalliside dissolved in 7 ml of ethanol was added, and hydrogenation was continued until the reaction for cardenolides was negative. The solution was filtered and evaporated, and the substance was crystallized from acetone; mp 192-199°C.

Periodate Oxidation of Dihydroalliside. With the addition of three drops of acetic acid to improve the solubility of the sodium metaperiodate, 20 mg of the substance and 500 mg of sodium metaperiodate were dissolved in 250 ml of water. After every hour, a 10-ml sample was taken and this, after the addition of 0.15 g of potassium iodide, was kept in the dark for 15 min. The liberated iodine was titrated with 0.01 N sodium thiosulfate solution. In parallel on each occasion, an equal volume of a blank sample was titrated. After fairly constant results had been achieved (after 6-8 h), showing the end of the oxidation reaction, the analyses were stopped. The difference between two parallel experiments was used to calculate the amount of iodine liberated. Then from the amount of iodine, after suitable recalculation, the number of moles of sodium periodate consumed in the oxidation of the glycoside was calculated.

In order to determine the residual sugars, 100 ml of the reaction mixture was acidified with acetic acid to pH 5 and treated with ethanol–chloroform (1:2) (3 × 150 ml). The extract was washed with 20 ml of water and evaporated. The oxidized substance was hydrolyzed by Kiliani's method [12]. The hydrolysate was evaporated in vacuum at 30°C. When the residue was chromatographed on paper, treatment with aniline phthalate reagent revealed no sugars.

Methylation of Glucoalliside. A solution of 240 mg of glucoalliside in 8 ml of dimethylformamide was treated with 4 ml of methyl iodide and 10 g of silver oxide. The mixture was heated with continuous stirring for 6 h. During this time two further amounts of 2 ml of methyl iodide and 4 g of silver oxide each were added. The solution was diluted with chloroform, filtered, and evaporated. The residue was remethylated similarly. The methylated substance was hydrolyzed by Kiliani's method [12]. The monosaccharides were extracted from the hydrolysate with chloroform–ethanol (2:1). The ethanolic–chloroformic extract was treated with 2 N sodium carbonate solution and with water and was evaporated. The residue was chromatographed on 3 g of alumina (activity grade III). One of the methylated monosaccharides (1) was eluted with chloroform and a second (2) with a mixture of chloroform and ethanol (3:1).

The monosaccharide (I) was crystallized from petroleum and diethyl ethers. The crystals obtained had mp 90-95°C; $[\alpha]_D^{18}$ +107.9 ± 5° (c 0.56; methanol) initially and +83.1 ± 5° after the establishment of equilibrium. These figures correspond to the properties of 2,3,4,6-tetra-O-methyl-D-glucose [13]. Paper chromatography also showed the identity of these substances.

For the monosaccharide (2), mp 64-71°C (acetone–ether); $[\alpha]_D^{19}$ – 49.3 ± 10° (c 0.32; methanol) after the establishment of equilibrium. In the butanol–acetic acid (4:1)/water system, the R_f value with respect to 2,3,4,6-tetra-O-methyl-D-glucose was 0.89.

A solution of 15 mg of the monosaccharide (2), 30 mg of phenylhydrazine hydrochloride, and 45 mg of sodium acetate in 1.5 ml of water was heated at 100°C for 1 h. Then it was extracted with chloroform and the chloroform extract was evaporated. The phenylhydrazone obtained was purified by preparative chromatography on paper in the benzene/formamide system, in which it had R_f 0.25. UV spectrum of the substance: λ_{max} (in absolute ethanol) 239 and 278 nm.

Cheiroside A. The melting point of the glycoside was 295-297°C (from methanol); $[\alpha]_D^{25} - 23.7 \pm 2°$ (c 1.00; pyridine). In conc. H_2SO_4, it formed a coloration changing with time: 0 min, orange; 5 min, yellow-orange; 50 min, blue; 190 min, violet. Literature data for cheiroside A: mp 293°C; $[\alpha]_D - 23.8°$ (pyridine) [10].

After the enzymatic hydrolysis (for method, see above) of this glycoside, an aglycone and a mixture of two monosaccharides were obtained. The latter were identified by paper chromatography as D-glucose and D-fucose. The aglycone had mp 236-245°C (methanol); $[\alpha]_D^{20} +12.6 \pm 2°$ (c 0.92; chloroform). In conc. H_2SO_4 it gave a coloration: 0 min, orange; 55 min, green; 70 min, blue-green. The monoacetate of the aglycone was obtained, mp 257-263°C. An analysis of the rate of acetylation as described previously [14] showed that the OH group undergoing acetylation was equatorial. These properties of the aglycone agree well with the properties reported for uzarigenin [15].

The periodate oxidation of cheiroside A, its methylation, hydrolysis, and the separation of the methylated sugars, and also their identification, were performed as for the case of glucoalliside. It was found that both the D-glucose and the D-fucose residues in the glycoside were oxidized. The consumption of sodium periodate was three moles. One of the methylated monosaccharides was identified as 2,3,4,6-tetra-O-methyl-D-glucose. The second monosaccharide, consisting of 2,3-di-O-methyl-D-fucose was obtained in the amorphous state; $[\alpha]_D^{18} +49.6 \pm 15°$ (c 0.40; ethanolic solution after 10 h); R_f with respect to 2,3,4,6-tetra-O-methyl-D-glucose 0.83 in the butanol–acetic acid (4:1)/water system. The phenyl hydrazone of this monosaccharide had R_f 0.21 in the benzene/formamide system. UV spectrum: λ_{max} (in absolute ethanol) 239 and 277 nm ($\log \varepsilon \approx 3.7$ and 4.1).

SUMMARY

Another two cardiac glycosides have been isolated from the seeds of Cheiranthus allioni Hort. It has also been established that in cheiroside A the D-glucose is attached at C_4 of the D-fucose. The second glycoside, which we have called glucoalliside, is new, and has the structure of bipindogenin 3-O-[O-β-D-glucopyranosyl-(1 → 4)-α-L-glucomethylopyranoside].

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