CARDENOLIDES OF Gomphocarpus fruticosus AND THE PARTIAL SYNTHESIS OF UZARIGENIN GLYCOSIDES

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In the USSR, Gomphocarpus fruticosus R. Br. (narrow-leaved cotton bush) grows wild along the Black Sea coast of the Caucasus in the region of Poti. At the present time, it is being brought under cultivation in order to ensure the raw material for the production of the preparation gomphotin [1].

The isolation from the leaves of G. fruticosus (of Australian origin) of the cardenolides gemphoside and afroside has been reported previously [2]. We have also studied the glycosidic composition of this plant [3].

The wastes from the production of gomphotin contain a mixture rich in other cardenolides. By chromatographing the combined cardenolides on columns of alumina we obtained, in addition to the glycosides 3 and 5 isolated previously [3], three other substances: 1, 2, and 6. From the unfermented leaves we obtained substance 8. The composition of the cardenolides of the leaves of G. fruticosus is as follows:

Cardenolides	Mp, ° C	Optical rotation, degrees	
Substance 1	261-274	+10	(ethanol)
Uzarigenin	238-247	+15	"
Gomphotin	227-230	+39	(methanol)
Substance 5	241-243	+23	"
Substance 6	222-228	+10	(ethanol)
Desglucouzarin	254-261	–41	"

On oxidation with chromium trioxide in glacial acetic acid, substance 2 formed a 3-oxo derivative which, according to the optical rotatory dispersion spectrum, unlike digitoxigenone [4], belonged to compounds of the trans-A/B series. From its main properties, chromatographic behavior on paper, and the melting point of a mixture, substance 2 was identified as uzarigenin. The main substance of the mother liquors forming the wastes from the production of gomphotin is uzarigenin, its amount being 0.003-0.005% of the weight of the leaves.

On acetylation, substance 8 formed a tetra-O-acetyl derivative. The acid hydrolysis of the glycoside gave uzarigenin as the aglycone. D-Glucose was detected in the acid hydrolysate by paper chromatography. Enzymatic hydrolysis led to results similar to those of acid hydrolysis. Consequently, substance 8 is uzarigenin 3-O-β-D-glucopyranoside and is identical with desglucouzarin. Their identity was confirmed by the partial synthesis of desglucourazin from uzarigenin and acetobromoglucose.

We have also effected a partial synthesis of a new cardiac glycoside of the trans-A/B series – uzarigenin 3-O-α-L-rhamopyranoside. The cardiotonic activities of the uzarigenin glycosides are given below:

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Glycoside	Sugar	Activity, mg/kg weight of the cat
Desglucouzarin [2]	D-Glucose	1.576
Desglucocheiroside [6]	D-Fucose	1.332
Odoroside [7]	D-Diginose	2.102
Uzarigenin-α-L-rhamnopyranoside*	L-Rhamnose	0.315

Some authors [6] have stated that L-rhamnose imparts a higher activity to cardenolides of the cis-A/B series than other sugars. From a comparison of the activity of the uzarigenin rhamnoside that we have obtained with the activities of known uzarigenin monoglycosides, it can be seen that in the trans-A/B series, also, the rhamnosides have a higher activity.

EXPERIMENTAL

Preparation of the Cardenolides. The previously comminuted leaves (10 kg) were fermented in a thermostat at 36-38° C for 3 days and were then extracted in a battery of extractors with 50% ethanol (taking into account the water used for fermentation). The resulting 80 liters of percolate were evaporated until the alcohol had been eliminated completely, and from the aqueous residue the cardenolides were extracted with chloroform, the latter was distilled off, the residue was dissolved in 8 liters of 30% ethanol, and the solution was purified by being stirred with 1.5 kg of alumina. The solid matter was filtered off and washed with 30% ethanol, and the filtrate was evaporated until the ethanol had been eliminated. The aqueous extract was treated with chloroform, and this was distilled off and the residue was crystallized from acetone. This gave 8.5 g of crystalline cardenolides. The benzene-soluble cardenolides remained in the acetonic mother liquor.

The ground cardenolide powder (8.5 g) was dissolved in 5 ml of boiling chloroform, the solution was cooled and filtered from the insoluble part, and the filtrate was passed through a column of alumina (h = 10 cm, d = 7 cm) which was eluted with chloroform (5 liters) and then with chloroform containing 3% of ethanol. After the appearance of cardenolides in the eluate (Raymond's reaction) one-liter fractions were collected and were analyzed for their cardenolide composition by paper chromatography in the chloroform–formamide system. The fractions containing the individual substances were combined and evaporated, and the residues were crystallized. This gave 2.5 g of substance 3 (gomphotin), 1.0 g of substance 5, 0.5 g of substance 6, and 0.25 g of substance 7. The fractions containing mixtures of substances were reseparated on columns of alumina.

The acetonic mother liquor after the separation of the crystalline cardenolides was evaporated, and the residue was dissolved in a mixture of chloroform and benzene (4 : 1) and chromatographed on a column of alumina (h = 20 cm, d = 5 cm). The column was eluted with the same mixture of solvents, and 100-ml fractions were collected after the appearance of cardenolides in the eluate. The qualitative composition of the fractions was analyzed by paper chromatography in the benzene–formamide system. The first six fractions yielded 0.4 g of substance 1, and then the column was eluted with chloroform–benzene (1 : 1) – three fractions – and (4 : 1) – two fractions, from which a mixture of substances 1 and 2 was obtained. Elution of the column with chloroform gave 2.1 g of substance 2.

Preparation of Desglucouzarin. This substance was found only in the extract obtained from unfermented leaves. Comminuted G. fruticosus leaves (5 kg) were extracted with a tenfold amount of ethanol, and this was distilled off and the resinous residue was dissolved in 3 liters of distilled water. The precipitate that deposited on standing was separated off, and the filtrate was treated with chloroform. A voluminous yellow crystalline precipitate separated out at the boundary of the immiscible phases. On investigation, the substance proved to be of flavonoid nature and was identified as rutin ($C_{27}H_{30}O_{16}$, mp 187-189° C, $[\alpha]_D^{19}$ – 24° in ethanol). After the purification of the aqueous fraction with chloroform, the cardenolides were extracted with a mixture of chloroform and ethanol (9 : 1) (9 × 1 liter). The extracts, which mainly contained substance 8, were evaporated, and the residue was dissolved in 200 ml of chloroform containing 5% of ethanol and the solution was chromatographed on a column of alumina (h = 40 cm, d = 3 cm). The

*Activity determined by P. I. Bezruk and Zh. I. Lyubetskaya.

column was eluted with chloroform and then with chloroform containing ethanol in gradually increasing concentration up to 7%. The fractions containing substance 8 yielded 1.37 g of crystals with mp 254-261° C $[\alpha]_D^{18}$ – 41.6° in ethanol.

Oxidation of Uzarigenin to Uzarigenone. A solution of 0.32 g of uzarigenin in 30 ml of glacial acetic acid was treated with 8.5 ml of a 1% solution of chromium trioxide in glacial acetic acid. After 4-5 h, 6 ml of methanol were added and the mixture was left until the following day. Then it was poured into 100 ml of ice water and the solution was treated with chloroform (5 × 70 ml), and the chloroform extracts were combined, washed with water, and evaporated. The residue was crystallized from acetone to give acicular crystals (0.25 g), mp 269-273° C $[\alpha]_D^{20}$ + 33° (in chloroform).

Enzymatic Hydrolysis of Substance 8. The substance (80 mg) was hydrolyzed with the enzyme of the grape snail [9] for 3 days. After the usual working up of the reaction mixture, 33 mg of the aglycone uzarigenin was obtained, and paper chromatography of the concentrated aqueous phase showed the presence of D-glucose.

Synthesis of Uzarigenin 3-O-β-D-Glucopyranoside. A reaction flask was charged with 250 mg of uzarigenin, 1 g of silver acetate, 0.5 g of calcium oxide, 0.7 g of activated carbon, and 100 ml of dry dichloroethane. Then the flask was immersed in an oil bath previously heated to 140° C. Five minutes after the dichloroethane began to boil, the addition of a solution of 1 g of acetobromoglucose in 100 ml of dichloroethane in 10-ml portions was begun with the continuous stirring of the boiling mixture. The synthesis was complete after 2 h; the mixture was cooled to room temperature, the solid matter was filtered off, and the filtrate was evaporated. The residue was dissolved in 20 ml of methanol and then 2 ml of methanol previously saturated with ammonia at 0° C were added and the mixture was left for 18 h. The solvent was distilled off and the residue was dissolved in chloroform containing 3% of ethanol and this solution was chromatographed on a column of alumina (h = 6 cm, d = 3 cm). The column was eluted with the same mixture of solvent until the uzarigenin had passed into the eluate completely (0.5 liter), after which the ethanol concentration was increased to 10%, when the desglucouzarin rapidly passed into the eluate (0.5 liter). This eluate was evaporated and the residue was crystallized from aqueous ethanol giving 160 mg of crystals with mp 254-261° C. The properties of the substance obtained and the products of its enzymatic hydrolysis were identical with those of substance 8, and a mixed melting point gave no depression.

Synthesis of Uzarigenin 3-O-α-L-Rhamnopyranoside. A flask was charged with 0.3 g of uzarigenin, 1.5 g of silver acetate, 0.9 g of calcium oxide, 0.9 g of activated carbon, and 100 ml of dry dichloroethane. The further operations were as described for desglucouzarin. A total of 1.1 g of acetobromorhamnose was added. The residue was deacetylated and was then crystallized from aqueous ethanol. This gave 0.23 g of a substance with mp 227-235° C, $[\alpha]_D^{20}$ – 46° (in ethanol). After the Mannich hydrolysis of the uzarigenin rhamnoside synthesized, the hydrolysate was found by paper chromatography to contain the aglycone uzarigenin and L-rhamnose.

SUMMARY

1. Six cardenolides have been isolated from the fermented leaves of Gomphocarpus fruticosus R. Br.: cardenolide 1, uzarigenin, gomphotin, and cardenolides 5, 6, and 7, and from the unfermented leaves, desglucouzarin.

2. From uzarigenin by partial synthesis desglucouzarin and a new glycoside – uzarigenin 3-O-α-L-rhamnopyranoside – have been obtained.

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