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CARBOHYDRATES IN *DIGITALIS PURPUREA* AT VARIOUS STAGES OF DEVELOPMENT

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Abstract—The concentrations of carbohydrates in *Digitalis purpurea* have been determined at various stages of development. An increase of the carbohydrate content was observed during growth. Glucose was the major carbohydrate component in the leaves and stem, whereas fructose predominated in the inflorescence. The growing tips and other actively growing parts of the plant contained relatively high concentrations of two new compounds.

INTRODUCTION

IT IS well known that the amount and the composition of the cardiac glycosides in *Digitalis* species vary during growth.[1–4] In an extensive study on *Digitalis purpurea* Lemli[5] has pointed out that the digitoxosides are almost absent in the younger parts of the plant, particularly when little or no chlorophyll is present, whereas in the older parts the digitoxosides represent up to 90% of the total glycoside content. The present work was undertaken to discover any correlation between the bound and free carbohydrates in *Digitalis purpurea*, especially with regard to digitoxose or digitoxose precursors.

RESULTS AND DISCUSSION

Whole plants were extracted successively with EtOH 80%, EtOH 70% and H_2O. The solvents were evaporated and the residue was purified on activated charcoal. A preliminary separation of the carbohydrates was performed on a large silica-gel column. The effluent was collected in 7 fractions. Each fraction was concentrated to a small volume and analysed by TLC and GLC. Identification of the carbohydrates was made by comparison of the retention times and R_fs of the parent compounds, of their oximes and of the TMS derivatives, with those of known sugars (Table 1). The following carbohydrates were found: glucose, fructose, two hexitols (probably mannitol and sorbitol), inositol, sucrose and traces of pentoses. No free digitoxose could be detected. Monosaccharides and unknown compounds with similar elution times (GLC) were quantitatively estimated in different parts of the plant. Samples of about 2 g dry weight of respectively the growing tips, the leaves, the roots and the stems were collected from young plants, 2 months after germination, and from second year plants. The growing tips of the second year plants contain almost no chlorophyll since they are protected from light by the leaves of the basal rosette. However, the growing tips of the first year plants are fully exposed to radiation and they are

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almost as green as the leaves. Protection from light was improved by placing a cylinder of porous cardboard around the growing tip in such a way that the light was almost screened off but gaseous exchange remained possible (screened tips). Samples of the inflorescence consisted of flower buds, stamen and filaments, pistils, sepals, petals and stems of the inflorescence.

TABLE 1. CHROMATOGRAPHIC DATA OF CARBOHYDRATES FROM *Digitalis purpurea*

	TLC R_fs relative to 2-deoxy-D-glucose		R_t (relative to mannitol) on GLC of TMS derivatives 3% SE 30		R_t 15% HIEFF 2BP	
	Parent	TMS-derivative	Parent	Oxime	Parent	Oxime
Fructose	0·50	1·90	0·73	1·02	0·46–0·53	1·13
Glucose	0·43	1·78	0·86–1·12	1·12	0·72–1·24	1·53
Mannitol	0·38	2·08	1·00 (12 min)		1·00 (14 min)	
Sorbitol	0·34	2·08	1·03		1·02	
Sucrose	0·12	1·63	3·03			
Compound A	1·78	0·07	0·19	0·42	0·54	0·27
Compound B	1·53	0·11	0·32	0·69	0·38	0·42

TLC of the parent compounds was performed on silica gel G with the solvent EtOAc–secPrOH–H_2O (5:3:2). The TMS derivatives were chromatographed on silanized silica gel with the solvent light petrol.–toluene (3:5). GLC condition (see Experimental).

The estimation of carbohydrates was performed by GLC of their TMS ethers.[6–9] Peak areas of individual compounds were measured with α,D-methylglucopyranoside or α,D-ribose figuring as internal standard. The contents calculated as % of dry weight are presented in Table 2. The total amount of carbohydrates increases gradually during growth: from 0·4% (based on dry weight) in the first leaves of the young plant, to 4·1% in the mature leaves of a second year plant. The same increase is found in the inflorescence: from 0·5% in the flower buds to 9% in the petals and in the pistils. Only traces of carbohydrates could be detected in the roots of young plants. The primary roots of the second year plants contain 0·6% carbohydrates, but it must be emphasized that half of this quantity is represented by an unidentified compound (see Table 2, compound C).

Glucose is the major carbohydrate component in most parts of the plant except in the inflorescence, where fructose predominates. Pentoses are only present in trace amounts, mainly in the growing tips. Inositol, found throughout the plant, shows only minor variations. The absolute concentration fluctuates around 0·2% with a peak level (1·5%) in the pistils. The highest relative concentrations of inositol were found in the growing tips of the first year plants and in the newly developed leaves (30%), whereas the young stem contained only 6%. The hexitols from the young plants are more equally distributed between leaves and stem, with a slight predominance in the latter. In the second year plants, the hexitols are chiefly stored in the leaves. In addition to these carbohydrates, several other compounds were found in varying amounts. Most of them did not react with the common reagents for carbohydrates. However, two among them, compounds A and B, deserve some attention since they accumulate in plant organs with low digitoxoside levels, and inversely, they

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TABLE 2. THE DISTRIBUTION OF FREE CARBOHYDRATES AS A PERCENTAGE OF DRY MATTER IN *Digitalis purpurea*

Compound	*Young plant, 2 months after germination* 1st+2nd leaf	3rd leaf	4th leaf	5th+6th leaf	Stem	Root
Fructose	0·04	0·10	0·10	0·20	0·21	0·04
Glucose	0·10	0·11	0·21	0·45	0·45	Traces
Mannitol	0·03	0·10	0·05	0·10	0·30	Traces
Sorbitol	Traces	0·10	0·05	0·05	0·12	Traces
Inositol	0·13	0·20	0·10	0·20	0·10	Traces
Compound A	Traces	Traces			0·04	
Compound B					Traces	
Compound C	0·10	0·04	0·05	0·10	0·30	Traces
Total	0·40	0·65	0·56	1·10	1·52	—

Compound	*Second year plant* Mature leaf	Leaf-stalk	Prim. root	Second root
Fructose	0·60	0·25	0·03	
Glucose	1·50	0·65	0·14	0·02
Mannitol	0·15	0·15	0·04	0·01
Sorbitol	1·50	0·07	0·06	0·04
Inositol	0·2		0·03	
Compound A			0·02	
Compound B				
Compound C	0·20		0·30	0·10
Total	4·15	1·12	0·62	0·17

Compound	*Inflorescence* Stem of inflorescence	Bract leaves	Sepals	Petals	Flower buds	Stamen and filament	Pistil and ovaries
Fructose	0·50	0·04	0·64	3·2	0·11	0·70	2·80
Glucose	0·37	0·11	0·77	3·0	0·10	0·45	2·40
Mannitol	Traces	0·02	0·30	0·9	0·02	0·20	0·90
Sorbitol	0·20	0·01	0·35	0·4	0·01	0·10	0·70
Inositol	0·05	0·10	0·20	0·2	0·11	0·07	1·50
Compound A	0·01	Traces	0·02	0·1	0·08	0·02	0·40
Compound B					Trace		0·10
Compound C		0·04		0·8	0·14	0·50	
Total	1·13	0·32	2·28	8·6	0·57	2·0	8·8

Compound	*Cotyledons and growing tips* Cotyledons	Growing tips 1st year plant	Growing tips 2nd year plant	Screened growing tips
Fructose		Traces	Traces	Traces
Glucose	0·2	0·16	2·1	1·1
Mannitol	0·6	0·06	0·9	0·6
Sorbitol	0·3	0·06	0·6	0·7
Inositol		0·13	0·2	
Compound A	0·1	0·03	0·6	1·0
Compound B	Traces	Traces	0·2	0·8
Compound C			0·7	0·2
Total	1·2	0·44	5·3	4·4

The levels of unknown compounds (A, B, C) are calculated as glucose. Leaves are numbered from the top downwards.

disappear when the digitoxoside content increases. It is not impossible that these compounds represent some intermediate steps in the biosynthetic pathway of digitoxose or in some other way are involved in digitoxose biogenesis. Considerable amounts of compounds A and B were found in the actively growing parts of the plant, particularly when little or no chlorophyll was present: 0·4% in the pistils, 0·08% in the flower buds and 0·03% in the

growing tips of the first year plant. These levels increased to 0·8 and 1·8% in the growing tips of the second year plants and in the screened tips, which both contained only traces of chlorophyll. Compounds A and B were not found in the mature leaves and stems.

EXPERIMENTAL

Plants were grown in a greenhouse. Immediately after collection, they were divided into distinct morphological units and freeze-dried. The dry samples were ground in a mortar and stored in a vacuum-desiccator until required for analysis.

Extraction and purification. About 200 g of freeze-dried and finely ground plant material was successively stirred for 2 hr at room temp. with 2 l. 80% EtOH, 2 l. 70% EtOH and 1 l. H_2O. The combined extracts were concentrated at 45° under vac. to a small vol. and purified on a column of activated charcoal (70 g Darco G 60), from which the carbohydrates were eluted with 15% EtOH. The syrupy residue obtained upon evaporation, was applied to a large silica-gel (0·02–0·5 mm) column (200 g). The column was developed with EtOAc–secBuOH–secPrOH–H_2O (5:5:5:2). The effluent was monitored by TLC and collected in 7 fractions: fractions 1 and 2, 200 ml; fractions 3 and 4, 300 ml; fractions 5, 6 and 7, 2 l.

Identification by TLC and GLC. Aliquots of the different fractions, together with a standard mixture of known sugars, were chromatographed on 0·25 mm silica-gel layers with the solvent EtOAc–secPrOH–H_2O (25:15:10). Subsequently, the individual carbohydrates were isolated by preparative TLC on 1 mm silica-gel layers. The carbohydrates were converted to the corresponding TMS ethers.[10] These TMS ethers were chromatographed on 0·1 mm layers of silanized silica gel (Merck) using the solvent light petrol.–toluene (3:5). Spots were localized by spraying with a 0·5% solution of $KMnO_4$ in 1 N aq. NaOH. Two instruments were used for analysis by GLC. The Pye Argon chromatograph 12001 with straight Pyrex glass columns (1·2 m × 4 mm o.d.) packed with 15% HI-EFF 2BP, was operated isothermally at 159° (flow rate 32 ml/min). The Pye series 104/64 chromatograph with a dual FID, was employed with coiled glass columns (1·5 m × 4 mm o.d.) containing 3% SE 30 on 80–100 mesh Gaschrom Q. The flow rate of Argon carrier gas was 40 ml/min. The column oven was programmed from 150 to 220° at 3°/min. Detector oven and injection head were at 250 and 170° respectively. Retention times, corrected for dead vol. according to the method of Peterson and Hirsch,[11] were measured relative to the TMS ether of D-mannitol.

Estimation of carbohydrates. Samples of 2 g dry weight from the different plant parts were extracted as above. After removal of the solvent, the residue was further dried over H_2SO_4 for at least 24 hr. The residue was then taken up in a few ml of pyridine and treated with an excess of silylation reagents (TMCS and HMDS). The TMS ethers were purified on a small column of activated charcoal (5 g), from which they were eluted with 20 ml light petrol. After evaporation of the solvent, the TMS ethers were redissolved in 1 ml light petrol. containing 0·5 mg of an appropriate internal standard (TMS ether of α,D-methylglucopyranoside or α,D-ribose). Aliquots of this solution were injected on the SE 30 column (same GLC conditions as above) and peak areas were measured with an electro-mechanical integrator (Sefram).[12] The weight (W_x) of carbohydrates was calculated with the following equation: $W_x = A_1 \times W \times (A_2 \times k)^{-1}$, where A_1 and A_2 are the peak areas of the estimated sugar and of the internal standard, respectively; W_1 is the weight of the internal standard and k is the relative response factor.[13]

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