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SHORT COMMUNICATION

BIOSYNTHESIS OF DIGITOXOSE AND GLUCOSE IN THE PURPUREA GLYCOSIDES OF *DIGITALIS PURPUREA**

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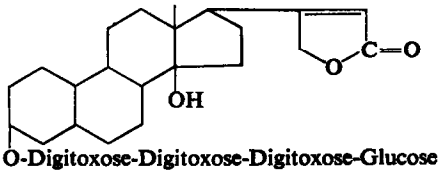
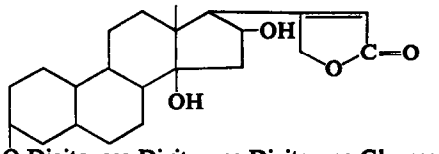
Abstract—A study of the biosynthesis of digitoxose and D-glucose in the purpurea glycosides A and B of *Digitalis purpurea* leaves by feeding ^{14}C-labeled D-glucose, D-fructose, D-mannose and D-galactose demonstrated that D-glucose was the most effective precursor of digitoxose in these glycosides. Degradation of the labeled digitoxose and D-glucose indicated that D-glucose was directly transformed to this 2,6-dideoxy sugar and to the bound D-glucose of the glycosides without essential rearrangement to its carbon skeleton.

INTRODUCTION

IN HIGHER plants dideoxy sugars are mainly found in cardiac glycosides. Digitoxose (2,6-dideoxy-D-ribohexose) is one of the earliest dideoxy sugars found, and is present in the purpurea glycosides A and B (Fig. 1) of *Digitalis purpurea*.[1] Since the only information available about the biosynthesis of deoxy sugars in higher plants[2,3] is that pertaining to 6-deoxy-sugars, it was of interest to investigate the formation of 2,6-dideoxy sugars.



O-Digitoxose-Digitoxose-Digitoxose-Glucose
(Purpurea glycoside A)



O-Digitoxose-Digitoxose-Digitoxose-Glucose
(Purpurea glycoside B)

FIG. 1.

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[1] A. STOLL and W. KREIS, *Helv. Chim. Acta* **18**, 120 (1935).
[2] J. E. WATKIN and A. C. NEISH, *Phytochem.* **1**, 52 (1961).
[3] G. A. BARBER, *Arch. Biochem. Biophys.* **103**, 276 (1963).

In order to obtain information about the most likely sugar precursor of digitoxose, digitalis leaves were fed with different radioactive sugars, and after a time the glycosides were isolated, hydrolyzed, and the radioactivity in the resulting aglucons, digitoxose and D-glucose was determined. The carbon skeleton of both sugars, obtained after hydrolysis, was degraded in order to learn whether any rearrangement of the carbon chain had taken place.

RESULTS

D-Glucose-^{14}C, D-fructose-^{14}C, D-mannose-^{14}C and D-galactose-^{14}C were fed to *Digitalis purpurea* leaves and allowed to metabolize for 24 and 48 hr. The purpurea glycosides were then extracted, hydrolyzed, and the extent of the radioactivity in the products examined. The results are given in Table 1.

TABLE 1. INCORPORATION OF DIFFERENT ^{14}C-LABELED SUGARS INTO DIGITOXOSE, D-GLUCOSE AND AGLUCON OF PURPUREA GLYCOSIDES EXTRACTED FROM *Digitalis purpurea* LEAVES

	Expt. 1*	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
Radioactive sugar fed (^{14}C)	D-Glucose	Glucose	Glucose	Fructose	Galactose	Mannose
Metabolic period (hr)	24	48	24	24	24	24
Dry weight of plant (g)	3·8	2·4	2·6	1·5	1·1	1·4
Total radioactivity fed (μc)	12·8	12·8	12·8	1·45	22·4	1·6
Dose of ^{14}C sugar fed†	$3{\cdot}7\times10^{-2}$	$5{\cdot}8\times10^{-2}$	$5{\cdot}4\times10^{-2}$	$2{\cdot}2\times10^{-2}$	$1{\cdot}6\times10^{-1}$	$5{\cdot}1\times10^{-1}$
Incorporation into digitoxose‡	$5{\cdot}7\times10^{-4}$	$8{\cdot}5\times10^{-4}$	$7{\cdot}6\times10^{-4}$	$2{\cdot}4\times10^{-4}$	$1{\cdot}3\times10^{-4}$	$1{\cdot}7\times10^{-4}$
Incorporation into glucose‡	$4{\cdot}5\times10^{-3}$	$6{\cdot}2\times10^{-3}$	$6{\cdot}1\times10^{-3}$	$3{\cdot}3\times10^{-4}$	$1{\cdot}1\times10^{-3}$	$7{\cdot}6\times10^{-4}$
Incorporation into aglucon‡	$2{\cdot}9\times10^{-5}$	$2{\cdot}6\times10^{-4}$	$1{\cdot}2\times10^{-4}$	$5{\cdot}0\times10^{-5}$	$3{\cdot}3\times10^{-5}$	—

* In Expt. 1 old leaves were used; in Expt. 2–6 leaves 5–6 weeks old were used.
† Expressed as μmoles of sugar per g of dry weight.
‡ Expressed as μmoles of the ^{14}C-labeled sugar incorporated per dose fed to the plant.

D-Glucose was shown to be the most effective precursor of digitoxose. D-Fructose was about 70 per cent less effective, whereas D-mannose and D-galactose were incorporated in still smaller amounts. The transformation of D-glucose to digitoxose was approximately 30 per cent higher in younger plants (5–6 weeks old) than in older plants. The length of the metabolic period from 24 to 48 hr did not seem to have considerable influence upon the rate of incorporation of D-glucose into digitoxose or bound D-glucose, whereas incorporation of the radioactivity into the aglucon after 48 hr of incubation was considerably higher than after 24 hr.

Since D-glucose was found to be the most effective precursor of digitoxose, ^{14}C-labeled D-glucose in only one position (C-1 or C-6) was fed to the 5–6 weeks old leaves in order to learn if there is any redistribution of radioactivity in the carbon skeletons during the process of incorporation. The D-glucose and digitoxose obtained after hydrolysis of the purpurea glycosides were chemically degraded, and the amount of radioactivity in the carbon atoms 1–6 was determined. The results given in Table 2 indicate that both sugars of the glycosides were formed from the carbon skeleton of the exogenous D-glucose without being completely split and rearranged.

TABLE 2. PERCENTAGE OF RADIOACTIVITY IN THE DIFFERENT CARBON ATOMS OF D-GLUCOSE AND DIGITOXOSE OBTAINED FROM PURPUREA GLYCOSIDES BY HYDROLYSIS AFTER FEEDING ^{14}C-LABELLED D-GLUCOSE

Position of C atoms	Glucose-1-^{14}C		Glucose-6-^{14}C			
			Experiment 1		Experiment 2	
	D-Glucose	Digitoxose	D-Glucose	Digitoxose	D-Glucose	Digitoxose
C-1	74	73	14	14	16	12
C-2	9		5		7	
C-3						
C-4		*		1		2
C-5		3		6		10
C-6	17	24	81	79	77	76

* Sample was lost.

DISCUSSION

D-Glucose was shown to be a more effective precursor of digitoxose in purpurea glycosides of *Digitalis purpurea* than other hexose sugars. This might indicate that fructose, glucose and mannose are first transformed to D-glucose and then to digitoxose. The radioactivity in the bound D-glucose was essentially higher than in the digitoxose. This fact suggests that gitoxin and digoxin (glycosides lacking D-glucose) are formed in an earlier step and that D-glucose is bound to the glycosides during the final reaction. The radioactivity in the aglucon was also found to be higher from D-glucose than when the other sugars were fed to the plant. Furthermore it was demonstrated that the rate of incorporation in younger plants was more effective than in older plants. This shows that the glycosides are mainly formed in the early stages of growth.

Watkin and Neish[2] showed that the labeling pattern of the D-glucose fed to buckwheat had been redistributed to some extent in the bound D-glucose and L-rhamnose. The results of our experiments similarly show that the main percentage of the D-glucose fed is transformed directly to the dideoxy sugar and the bound D-glucose. Only a small percentage of the hexose sugar undergoes rearrangement, probably via the Embden–Meyerhof phosphate pathway.[4] This would explain the radioactivity found in carbon atom C-6 after feeding glucose-1-^{14}C and the radioactivity in C-1 after feeding glucose-6-^{14}C, respectively.

EXPERIMENTAL

Radioactive sugars were purchased from New England Nuclear Corp. The radioactive paper strips were dried and counted in toluene with Liquifluor (New England Nuclear Corp.) in a Tri-Carb Liquid Scintillation Spectrometer and with a gas flow Geiger-Müller counter.

The *Digitalis purpurea* plants were grown in a greenhouse at 20–22° under constant illumination. The plant material in experiment 1, Table 1, was obtained from outdoor-grown plants. Labeled sugars were administered as follows: the petioles of the leaves were cut under water with a razor blade and the fresh cut end placed in the solution of the radioactive sugars contained in plastic planchets. The solutions (20–50 μl) were absorbed in 15–20 min. The last traces of substrate were taken up by the plant by addition of a few drops of water to the planchet. The leaves were then transferred to glass tubes containing water and allowed to metabolize for 24 and 48 hr, respectively. The isolation of the purpurea glycosides was carried out by the method of Stoll and Kreis.[1] The hydrolysis of the glycosides was performed as described by Rees.[5]

[4] J. EDEDMAN, V. GINSBURG and W. Z. HASSID, *J. Biol. Chem.* **213**, 843 (1955).
[5] R. REES, *Helv. Chim. Acta* **44**, 1607 (1961).

The resulting sugars were separated by paper chromatography using the solvent ethylacetate–pyridine–water (2:1:2). The radioactive sugars were eluted on to plastic planchets, dried in a desiccator over KOH, and the radioactivity was determined. The aglucons were isolated and purified from the two glycosides (Fig. 1)[5] and dissolved in a small amount of methanol, transferred to a plastic planchet, dried in a desiccator, and their radioactivity determined. The degradation of labeled D-glucose was carried out by the method of Andrews, Hough and Picken,[6] whereby the radioactivity of the carbon atoms C-1, C-2 to C-5, and C-6 can be determined. The resulting $^{14}CO_2$ was collected in hydroxide of Hyamine (Packard), dissolved in toluene counting fluid and the radioactivity was determined.

The degradation of radioactive digitoxose was carried out by adapting the method described for rhamnose by Andrews, Hough and Picken.[7] The radioactive digitoxose was mixed with 100 mg carrier and oxidized with periodate, resulting in the formation of malondialdehyde (C-1 to C-3), formic acid (C-4), and acetaldehyde (C-5 and C-6).[8] Permanganate oxidation of the latter gave acetic acid, which was degraded by the Schmidt reaction.[7] The separation of formic acid and malondialdehyde was carried out by adding saturated sodium bisulfite solution (40 per cent) in large excess, whereby the aldehyde forms an insoluble addition compound which was separated by centrifuging. The supernatant solution containing formic acid (C-4) was acidified with 1 N H_2SO_4 (pH 4) and steam distilled until the distillate no longer showed an acid reaction. The oxidation to $^{14}CO_2$ was performed as previously described.[6] The aldehyde–bisulfite compound was washed with sodium bisulfite solution, ethanol and ether, dissolved in a small quantity of water, and 0·1 N HCl was added to regenerate the free aldehyde, which was extracted with ether, evaporated to dryness, redissolved in toluene counting fluid and the radioactivity was determined.

Note. When this work was concluded, a publication by von Euw and Reichstein[9] appeared pertaining to biosynthesis of digitoxose in *Digitalis lanata*. Their results are in accord with our data concerning the incorporation of D-glucose-6-^{14}C and the distribution of radioactivity in the carbon skeleton of digitoxose.

[6] P. ANDREWS, L. HOUGH and J. M. PICKEN, *Biochem. J.* **94**, 75 (1965).
[7] P. ANDREWS, L. HOUGH and J. M. PICKEN, *Biochem. J.* **97**, 27 (1965).
[8] V. S. WARAVDEKAR and L. D. SASLAW, *J. Biol. Chem.* **234**, 1945 (1959).
[9] J. VON EUW and T. REICHSTEIN, *Helv. Chim. Acta* **49**, 1477 (1966).