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CARDENOLIDE GLYCOSIDE PRODUCTION IN *DIGITALIS LANATA*

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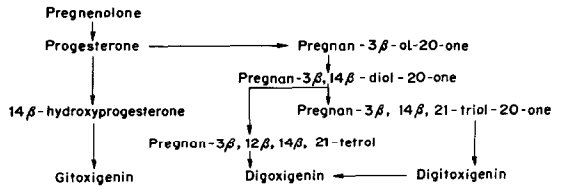
Abstract—Leaves of *Digitalis lanata* metabolize progesterone more rapidly than pregnenolone when applied to the surface of the leaves. Their transformation into digitoxigenin, gitoxigenin, digoxigenin, digitoxin, gitoxin, digoxin and the corresponding cardenolides was demonstrated.

INTRODUCTION

The biosynthesis of cardenolides by *Digitalis lanata* has been investigated by several groups [1–9]. Pregnenolone and progesterone are precursors and Tschesche [9] has proposed the biosynthetic routes shown in Scheme 1 for the formation of digitoxigenin, digoxigenin and gitoxigenin.

In order to obtain more information about cardenolide production in *Digitalis lanata*, ^{3}H-progesterone and ^{3}H-pregnenolone were applied as precursors using the method of Caspi [5].



Scheme 1. Possible biosynthetic routes for cardenolide production in *Digitalis lanata*.

RESULTS AND DISCUSSION

Progesterone-[^{3}H] and pregnenolone-[^{3}H] were applied to the leaves and after few weeks the leaves were extracted with MeOH–H_2O. The extract was freed from coloured material and protein and the cardenolide components were separated by preparative TLC. More than 10% of the progesterone radioactivity and 3% of pregnenolone radioactivity initially applied to the leaves was recovered in the cardenolide fractions. The distribution of radioactivity between the several components is shown in Tables 1–3. Data in the Tables are the results of several experiments carried out over a period of three years.

Progesterone-[^{3}H] was applied to leaves of plants of different ages (Tables 1 and 2), since it was found previously that the quantities of the three principal lanatoside glycosides constantly changed in the first year of the plant's life [10]. In the progesterone-[^{3}H] feeding experiments, the appearance of free genins was observed.

In those leaves that were not treated with radioactive precursors, the glycosides were not labelled. Thus progesterone and other intermediate products of glycoside biosynthesis were apparently not transported from the site of application under the experimental conditions employed. However a small amount of radioactivity did appear in unidentified compounds in the untreated leaves, indicating that some degradation and transfer does occur but only to a minor extent.

In the autumn incubation, labelling of gitoxigenin and gitoxin was greater than that of the

Table 1. Metabolites of progesterone applied to *Digitalis lanata* in June and July

Compound	Dry plant material (μg/g)	Radioactivity cpm/g dry plant material ($\times 10^{-2}$)	Distribution of radioactivity (%)	In corp. (%)
Lanatoside A	259	2·20	0·71	0·088
Lanatoside B	449	2·12	0·68	0·084
Lanatoside C	250	5·35	1·72	0·214
Digitoxin	143	81·19	26·19	3·247
Gitoxin	183	88·61	28·58	3·544
Digoxin	133	82·09	26·48	3·823
Digitoxigenin		3·51	1·13	0·140
Gitoxigenin		40·62	13·10	1·624
Digoxigenin		4·26	1·37	0·170

other genins and glycosides respectively. This difference is in accord with the theory of Tschesche, who has suggested that the synthesis of gitoxigenin follows a different pathway from that of digitoxigenin and digoxigenin [9].

Comparison of the results obtained in summer and in autumn (Tables 1 and 2) indicates that, in the summer, glycoside synthesis occurs more slowly and labelling of digitoxin, gitoxin and digoxin is greater than that of the lanatoside glycosides. The high radioactivity in the digitoxin, gitoxin and digoxin may be due to the fact that their transformation into lanatoside glycosides is relatively slow, or alternatively, that the degrada-

Table 2. Metabolites of progesterone applied to *Digitalis lanata* in August and September

Compounds	Dry plant material (μg/g)	Radioactivity cpm/g dry plant material ($\times 10^{-2}$)	Distribution of radioactivity (%)	In corps. (%)
Lanatoside A	1161	48·0	11·08	1·96
Lanatoside B	911	50·0	11·33	2·00
Lanatoside C	360	40·0	9·05	1·60
Digitoxin	171	28·0	6·33	1·12
Gitoxin	256	41·0	9·27	1·64
Digoxin	214	34·0	7·69	1·36
Digitoxigenin		65·0	14·70	2·60
Gitoxigenin		90·0	20·36	3·60
Digoxigenin		46·0	10·40	1·84

Table 3. Metabolites of pregnenolone applied to *Digitalis lanata*

Compound	Dry plant material (μg/g)	Radioactivity cpm/g dry plant material ($\times 10^{-2}$)	Distribution of radioactivity (%)	In corp. (%)
Lanatoside A	566	5·9	16·66	0·522
Lanatoside B	219	4·5	12·71	0·398
Lanatoside C	252	4·9	13·84	0·433
Digitoxin	80	1·5	4·23	0·132
Gitoxin	91	1·5	4·23	0·132
Digoxin	138	2·3	6·49	0·203
Digitoxigenin		6·0	16·94	0·531
Gitoxigenin		3·6	10·16	0·318
Digoxigenin		5·2	14·68	0·460

tion of lanatoside glycosides is a relatively rapid process. It is very probable that the lanatoside glycosides are synthesized from digitoxin, gitoxin and digoxin and one of the main degradative paths leads through these glycosides. It is also known that lanatoside glycosides are formed in the presence of UDP-glucose from digitoxin, gitoxin and digoxin.

Other investigations [11] show that the concentrations of glycosides and their transformations in *Digitalis lanata* are significantly influenced by acetone and other solvents, and this effect must be considered when evaluating the present results.

EXPERIMENTAL

Methods. TLC was on Kieselguhr-G (Merck) impregnated with Me_2CO–formamide (9:1). The lanatoside glycosides were separated in system 1: C_6H_6–dioxane–$CHCl_3$–formamide (10:50:80:1), lanatoside A R_f 0·35, lanatoside B R_f 0·21, lanatoside C R_f 0·12. Secondary glycosides and genins were separated with system 2: MeCOEt–toluene–formamide (25:25:1), digitoxin R_f 0·35, gitoxin R_f 0·20, digoxin R_f 0·16, digitoxigenin R_f 0·60, gitoxigenin R_f 0·40, digoxigenin R_f 0·26. Quantitative determination of the glycosides after repeated chromatographic purification was by the xanthydrol method. Radioactivity of glycosides and genins was measured after several chromatographic purifications. Genins were eluted with $CHCl_3$–MeOH from the plates after separation with system 2, and then purified by TLC on Si gel developed with C_6H_6–EtOAc (1:4) and with C_6H_6–MeOH (50:1) [12]. The radioactivity of the chromatographically pure glycosides and genins was determined by liquid scintillation counting using 10 ml. of scintillation fluid (5 g 2,5-diphenyloxazole and 100 mg 1,4-di-2, 5-phenyloxazolyl-benzene/l. toluene).

Materials. Digitalis lanata plants were obtained from Gyógynövénykutató Intézet (Research Institute of Medicinal Plants) Budapest.

Tritium labelling of progesterone and pregnenolone was performed by the BF_3 method with HOAc [13, 14]. With progesterone the tritiation reaction was carried out at + 20°, but because of the sensitivity of pregnenolone the reaction was conducted at −10°. This resulted in a great difference in their specific activities (progesterone 8·71 m Ci/mmol; pregnenolone 22·15 uCi/mmol). After tritiation the products were purified by TLC on Si gel, developed with EtOAc–$CHCl_3$–H_2O (90:10:1) and then crystallised from EtOH–H_2O (1:1) [14].

Application of the precursors to the plants. Plants used were cultivated in soil and were 3 (Table 1) and 5 (Table 2) months old respectively. Leaves were rinsed with Me_2CO and Me_2CO solns of progesterone ($2·5 \times 10^5$ cpm) or pregnenolone ($1·13 \times 10^5$ cpm) were applied.

Extraction. Four weeks after administration of the labelled precursors, the leaves were extracted with MeOH–H_2O (4:1). The extract was deproteinized with 10% ethanolic lead acetate. Coloured substances were extracted with CCl_4 and from the remaining soln the genins and glycosides were extracted with $CHCl_3$. The $CHCl_3$ extract was dried, evaporated and solid residue subjected to TLC to obtain the cardenolides [15].

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