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BIOCONVERSIONS LEADING TO MINOR CARDIAC GLYCOSIDES IN *CONVALLARIA MAJALIS**

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Key Word Index—*Convallaria majalis*; Liliaceae; lily of the valley; biosynthesis; cardenolides; sarhamnoloside; tholloside.

Abstract—The biosynthesis of two minor cardenolides in *Convallaria majalis*, sarhamnoloside and tholloside, was investigated by application of labelled cardenolide precursors to leaves. Evidence for the concomitant occurrence of alternative pathways differing in the sequence of hydroxylation reactions at C-11 and C-19 was obtained.

INTRODUCTION

Convallaria majalis L. contains about 40 different glycosides of the cardenolide type, which are synthesized in the green tissues only [1]. The biogenetic pathways of the major cardiac glycosides involve different types of hydroxylation reactions at C-19 (Fig. 1: R^1) and C-11 (Fig. 1: R^2), and dehydrogenation at C-19 [2, 3].

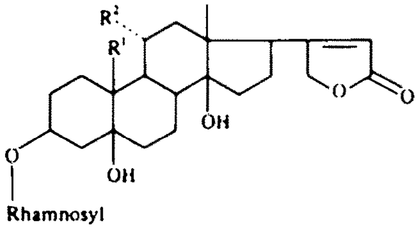
RESULTS AND DISCUSSION

HPLC was found to be an excellent method for isolation of very small amounts of cardiac glycosides [4, 5]. Thus, ^{14}C-labelled cardenolides obtained under photosynthetic conditions [5] became available for investigation of biogenetic routes, leading to minor cardiac glycosides. Among these the metabolism of two cardenolides, sarhamnoloside (**5**) and tholloside (**6**), which are C-11 hydroxylated derivatives of convallatoxol (**2**) and convallatoxin (**3**), respectively, was examined. Our aim was to elucidate the sequence of bioconversions leading to **5**: does hydroxylation at C-19 precede that at C-11 or vice versa? In the case of **6**: is dehydrogenation at C-19 the last step or does it occur before the C-11 hydroxylation? It seems also possible that all combinations of hydroxylation and dehydrogenation exist *in vivo*.

In the first experiments we used a chemotype [6] of *C. majalis* (origin: Debrecen, Hungary) with a high content of lokundjoside (**4**). Within this plant we expected a high level of C-11 hydroxylation activity. For comparison, plants originating from Paris, France, with a low content of **4** were also investigated. The results are summarized in Table 1.

Using **4** as precursor, **5** was formed by C-19 hydroxylation analogous to the known biosynthetic route: periplorhamnoside (**1**) → convallatoxol (**2**) [3]. Equally, **5** appeared labelled after application of **2** (hydroxylation at C-11). In both cases the incorporation rate was higher by a factor of about two with the Debrecen plants. However, **6** could be identified as product after application of **5** (dehydrogenation at C-19), but not when **3** was tried as precursor. Compound **6** can be considered as the end product of this line of minor glycosides. The incorporation rate is higher than in the case of the hydroxylations leading to **5**. Accordingly conversion of **2** into **3** occurs at a higher rate than **1** → **2** [2, 3]. A more extended scheme of cardenolide metabolism is outlined in Fig. 2.

With the exception of **3** → **6** all precursor → product transformations did work. The C-11 hydroxylated cardenolides sarhamnoloside (**5**) and lokundjoside (**4**) are synthesized via dual pathways, whereas tholloside (**6**) seems to be formed by C-19 dehydrogenation only.



	R^1	R^2	
1	Me	H	Periplorhamnoside
2	CH_2OH	H	Convallatoxol
3	CHO	H	Convallatoxin
4	Me	OH	Lokundjoside
5	CH_2OH	OH	Sarhamnoloside
6	CHO	OH	Tholloside

The following interconversions have been observed [2,3]:
$4 \leftarrow 1 \rightarrow 2 \rightarrow 3$

Fig. 1. Structures of selected cardenolides of *Convallaria majalis*.

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*This paper is dedicated to Prof. Dr. Max Wichtl on the occasion of his 60th birthday.

Table 1. Applied [U-^{14}C]-labelled and resulting cardenolides (incorporation rates corrected for label recovered in the cardenolide fraction)

Precursor	dpm · 10^4 (dpm · 10^4/mg)	Product	dpm · 10^2	dpm · 10^4 recovered in cardenolide fraction	Incorporation rate (%)
Lokundjoside (4)*	23.7 (33.3)	sarhamnoloside (5)	19.8	15.4	1.28
Lokundjoside (4)†	23.7 (33.3)	sarhamnoloside (5)	10.6	18.2	0.58
Convallatoxol (2)*	26.7 (41.8)	sarhamnoloside (5)	12.6	16.2	0.78
Convallatoxol (2)†	26.7 (41.8)	sarhamnoloside (5)	5.2	15.8	0.33
Sarhamnoloside (5)‡	4.3 (7.2)	tholloside (6)	2.7	1.0	2.78
Convallatoxin (3)†	25.2 (18.4)	tholloside (6)	n.d.	15.3	—

Origin of plant material: *Debrecen/H; †Paris/F; ‡mixed; n.d., not detectable.

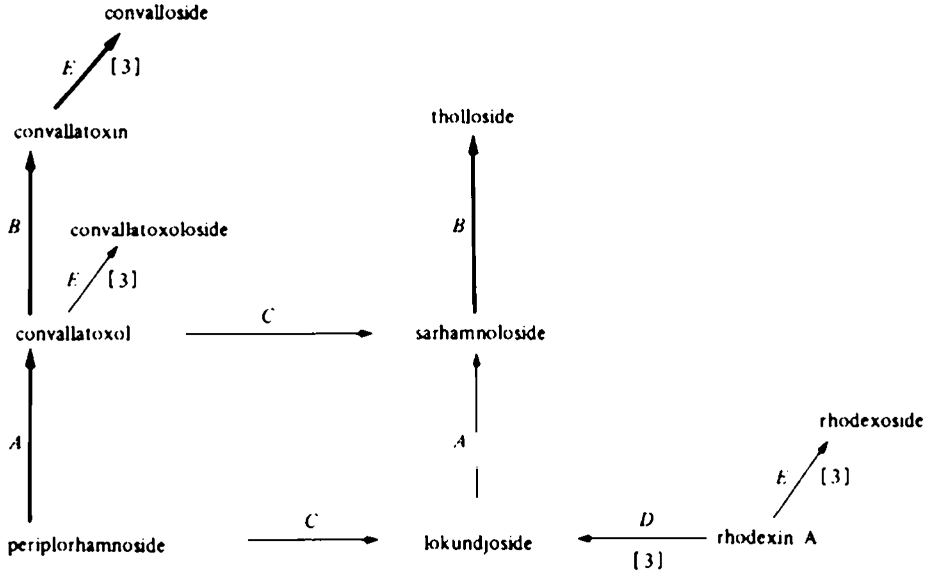

Fig. 2. Interconversions of cardenolide glycosides in *Convallaria majalis*. Reaction types: *A*, hydroxylation of C-19: -Me → -CH$_2$OH; *B*, dehydrogenation of C-19: -CH$_2$OH → -CHO; *C*, 11α-hydroxylation; *D*, 5β-hydroxylation; *E*, glucosylation (1β → 4).

EXPERIMENTAL

Plant material. Plants with high (origin: Debrecen, Hungary) and low lokundjoside (4) content (origin: Paris, France) [6] were taken from the collection of *Convallaria majalis* L., grown in the garden of the Institute of Pharmacognosy, at the stage of full flowering.

Labelled compounds. [U-^{14}C]-Labelled cardenolides were isolated from leaves of *C. majalis* after photo-assimilation of $^{14}CO_2$ as described in ref. [5]. Radioactivity parameters: see Table 1.

Application of labelled cardenolides to the leaves. As published elsewhere [5].

Identification of in vivo *conversion products at the cardenolide level.* The cardenolide fraction was obtained as described in ref. [1], and examined for labelled glycosides by TLC, silica gel 60 F$_{254}$ Merck, using three solvent systems [I: $CHCl_3$–MeOH–H_2O, 7:3:1, lower phase; II: MeCOEt–toluene–H_2O–MeOH–HOAc, 80:10:6:5:2; III: EtOAc–MeOH–H_3BO_3 (4.6% aq. soln)–HOAc, 55:20:15:10] (detection: 2.5% vanillin in EtOH–conc. H_2SO_4, 1:4). Radioactivity was detected using a 2π-TLC scanner. In addition, the cardenolides were isolated by HPLC [4], and their radioactivity was determined in a liquid scintillation counter.

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