Pergamon

PII: S0031-9422(96)00809-6

Phytochemistry, Vol. 45, No. 3, pp. 473–476, 1997

0031-9422/97 $17.00 + 0.00

VARIATION OF CARDENOLIDES WITH GROWTH IN A *DIGITALIS LANATA* BRAZILIAN CULTIVAR

FERNÃO CASTRO BRAGA,* WOLFGANG KREIS,† RUBENS ALMEIDA RÉCIO‡ and ALAÍDE BRAGA DE OLIVEIRA§

*Departamento de Química, Instituto de Ciências Exatas, UFMG, Av. Antônio Carlos 6627, CEP 31270-010 Belo Horizonte, MG, Brazil; †Lehrstuhl für Pharmazeutische Biologie, Friedrich-Alexander Universität, Staudtstraße 5, D-91058 Erlangen, Germany; ‡Fundação Ezequiel Dias (FUNED), R. Conde Pereira Carneiro 80, CEP 30510-010, Belo Horizonte, MG, Brazil; §Faculdade de Farmácia, UFMG, Av. Olegário Maciel 2360, CEP 30180-112 Belo Horizonte, MG, Brazil

(*Received in revised form* 22 *August* 1996)

Key Word Index—*Digitalis lanata*; Scrophulariaceae; cardenolides; Brazilian cultivar; cardenolides growth content variation.

Abstract—The content of the main cardenolides in *Digitalis lanata* harvested in Brazil was determined by HPLC, in two different stages of growth. The analysed plants, totaling 49, presented great variation in the contents of lanatoside C and digoxin (jointly quantified), lanatoside A, lanatoside B, glucoevatromonoside, odorobioside G, glucogitoroside, glucoverodoxine, glucodigifucoside and digitalinum verum. The sum of the analysed cardenolides in the 12-month-old plants was higher than the determined concentrations in plants collected later (18 months) ($P < 0.0001$). Lanatoside C and digoxin were the major cardenolides in both groups, followed by glucodigifucoside. Lanatoside A content decreased in the older plants ($P < 0.0001$), whereas lanatoside C showed an opposite trend ($P < 0.05$). The estimated average dry leaf content of lanatoside C and digoxin was 1820 ± 900 nmol g^{-1} in the younger plants, and 2040 ± 840 nmol g^{-1} in the older plants. Some individual plants have shown a leaf content above 3500 nmol g^{-1} of these cardenolides and will be selected for improvement of the cultivar.

INTRODUCTION

Digitalis lanata is the industrial source of digoxin and lanatoside C, used in the treatment of congestive heart failure. Agricultural production is the only economically feasible process to obtain these cardenolides and thus, investigations on new cultivars are of interest. The great variation in the cardenolides contents of *D. lanata* from different regions and a remarkable diversity in individual plants from a determined place have been reported [1–3]. By parental selection, strains of *D. lanata* with a high content of the desired cardenolides have been obtained [1, 2, 4, 5].

It is recognised that variations in environmental conditions induce changes in primary production, besides leading to complex alterations in secondary metabolism of *D. lanata* [6]. The content of cardenolides in *Digitalis* at various stages of plant growth has been reported. Such studies are primarily concerned with the accumulation of the cardiac glycosides in different plant organs and the distribution of these compounds in distinct regions of the leaves and inside the rosette [1, 4, 7, 8].

The scope of the present work was to analyse the growth variation of the main cardenolides (lanatoside A, B and C, digoxin, glucoevatromonoside, odorobioside G, glucogitoroside, glucoverodoxine, glucodigifucoside and digitalinum verum) in a Brazilian experimental cultivar of *D. lanata*. This will allow the selection of plants with higher contents of the pharmacologically meaningful cardenolides for improvement of the cultivar.

RESULTS AND DISCUSSION

The cardenolides concentration differed significantly between the 12-month-old and 18-month-old *D. lanata* cultivated in the Maciço do Itatiaia, in terms of their total sum of cardenolides ($P < 0.0001$), as well as in the majority of the individually estimated compounds (Table 1). Lanatoside C and digoxin, jointly estimated, were found to be the major cardenolides in both stages of development (Table 2). An enhancement in the content of these cardenolides in the older plants was significant, despite the decrease in the concentration of the total cardenolides (Table 1). On the other hand, lanatoside A concentration diminished from the first to the second group (Table 2). Lan-

§Author to whom correspondence should be addressed.

Table 1. Age variation of average cardenolides contents (nmol g dry wt^{-1}) in leaves of *Digitalis lanata* cultivated at Maciço do Itatiaia, Brazil

Cardenolide	12-month-old plant*	18-month-old plant*	*P*-value
Lanatoside A	1080 ± 710	630 ± 630	$P < 0.0001$
Lanatoside B	290 ± 220	190 ± 200	$P < 0.0001$
Lanatoside C + digoxin	1820 ± 900	2040 ± 840	$P < 0.05$
Glucoevatromonoside	240 ± 200	90 ± 140	$P < 0.0001$
Odorobioside G	90 ± 90	140 ± 110	$P < 0.0001$
Glucogitoroside	1860 ± 690	1340 ± 520	$P < 0.0001$
Glucoverodoxine	280 ± 130	320 ± 140	$P > 0.05$
Glucodigifucoside	2170 ± 740	1780 ± 640	$P < 0.0001$
Digitalinum verum	580 ± 340	690 ± 360	$P < 0.05$
Digitoxin	60 ± 60	100 ± 110	$P > 0.05$
Total	8450 ± 1640	7240 ± 1830	$P < 0.0001$

*Data presented are the average results of 49 plants, each one being the mean of three replicates.

Table 2. Age variation of cardenolides composition (per cent of analysed cardenolides*) in leaves of *Digitalis lanata* cultivated at Maciço do Itatiaia, Brazil

Cardenolide	12-month-old plant	18-month-old plant
Lanatoside A	15.5	10.5
Lanatoside B	4.3	3.1
Lanatoside C + digoxin	26.4	34.6
Glucoevatromonoside	2.4	1.0
Odorobioside G	0.9	1.6
Glucogitoroside	18.8	15.7
Glucoverodoxine	3.0	4.0
Glucodigifucoside	22.5	21.3
Digitalinum verum	5.9	8.0
Digitoxin	0.3	0.2

*The quantified compounds represent about 70% of total cardenolides, according to spectroscopic results provided by the Baljet reaction [9].

atosides A and C concentration data, in percentage terms, was statistically analysed for correspondence and correlation coefficients of −0.465 and −0.535 were obtained for the 12-month and 18-month-old plants, respectively. Such inverse correlation indicated a biosynthetic conversion of lanatoside A to C, as previously suggested [4].

The compounds of the A-series, lanatoside A, glucoevatromonoside, odorobioside G, glucodifucoside and digitoxin, are the major cardenolides in both stages of development (Tables 1 and 2), similar to the reported data for green house cultivated *D. lanata* [4]. Glucodigifucoside and glucogitoroside presented just a small variation in their percentual composition and showed high contents in the 12-month-old and 18-month-old plants (Tables 1 and 2). A similar regular concentration for these biosides was described for green house cultivated *D. lanata* [4].

A direct correlation between the concentrations of lanatoside C and total cardenolides was found when these results were statistically analysed, for both groups of plants. Correlation coefficients of 0.263 and 0.507 were obtained for the 12-month-old and 18-month-old plants, respectively.

The adaptation of a plant to a new environment can operate at different levels in the metabolism and it may affect secondary production. Therefore, *D. lanata* plants cultivated in Egypt usually flower in the first year of their growth, while those grown in the U.S.A. and in Europe usually flower in their second year of growth [7]. Under weak natural light conditions the dense rosette morphological stage was never reached by *D. lanata* cultivated in France [10]. Similarly to indigenous plants, *D. lanata* cultivated at Maciço do Itatiaia have flowered in the second year of growth and the morphological stage of dense rosette was reached in the first year. Furthermore, the cardenolides contents determined for the 12-month-old plants were similar to those of greenhouse cultivated and indigenous plants [3–5], thus suggesting an acclimatisation of the species to the new environment. Previous studies covered the development of the cardenolide profile in young plants only [1, 4, 7] while the present investigation was focused on the two stages of the plant growth.

The data displayed in Fig. 1(a) and (b) represent the summed contents of lanatoside C and digoxin in the *D. lanata* Brazilian cultivar. These histograms indicate a normal distribution of these cardenolides, at both stages of plant development. The 12-month-

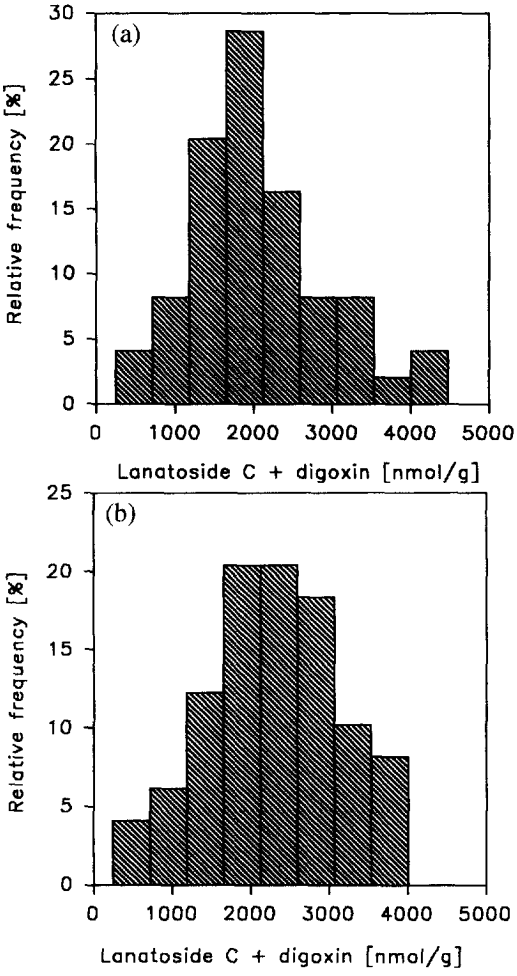

Fig. 1. Frequency distribution of lanatoside C and digoxin contents (jointly quantified) in leaves of *Digitalis lanata* cultivated at Maciço do Itatiaia, Brazil ($n = 49$ in both groups). Cardenolides are expressed in nmol g^{-1} dry leaf. (a) 12-month-old plants; (b) 18-month-old plants.

old plants show greater variation in the concentrations of lanatoside C and digoxin, as evidenced by the higher standard deviation, in comparison with the older plants (1820 ± 900 nmol g^{-1} and 2040 ± 840 nmol g^{-1}, respectively). These graphics clearly show the notable heterogeneity of the analysed plant material. About 4% of the younger plants have a lanatoside C and digoxin concentration between 3770 and 4200 nmol g^{-1} and will be selected for improvement of the cultivar (Fig. 1a). Older plants showed a content distribution more centered around the mean value and an average concentration higher than the observed values for the younger plants (Fig. 1b). Eight per cent of the 18-month-old plants have cardenolides concentrations between 3300 and 3770 nmol g^{-1} (Fig. 1b).

High variation in the individual contents of lanatoside C and digoxin in native *D. lanata* has been previously reported [2, 3]. In the present study, a similar variation of cardenolides was found and the high-yield plants will be selected for improvement of the cultivar.

EXPERIMENTAL

Age variation in the cardenolides contents. Digitalis lanata Ehrh., cultivated at Maciço do Itatiaia, Brazil, was employed in this study. The plants were grown in a field, without any artificial process of irrigation. 49 specimens were selected and harvested at two stages of development: before the flowering period (12 months) and after that time (18 months). About 20 leaves from the intermediate region of each rosette were collected for analysis. After harvesting, the plants were quickly washed with H_2O, dried at 40°, for 48 hr, and then powdered and extracted for analysis of the cardenolides.

Extraction of the cardenolides. The employed procedures of cardenolides extraction and purification were reported in ref. [11]. The plants were analyzed in triplicate.

Quantitative determination of the cardenolides. Cardenolides were determined by HPLC. The analysis were carried out in a Hewlett-Packard 1090 apparatus, with a diode-array detector. An ODS C-18 column was used (Hewlett-Packard 100 × 2.1 mm i.d.) at the temperature of 40°, flow rate of 0.2 ml min^{-1} and the UV-detector wave length was 220 nm. A gradient elution of H_2O (A) and 84% CH_3CN (B) was employed: 0–5 min 85% A, 15% B; 5–10 min 80% A, 20% B; 10–12 min 80% A, 20% B; 12–25 min 73% A, 27% B; 25–35 min 55% A, 45% B; 35–50 min 40% A, 60% B; 50–52 min 5% A, 95% B. The analysis was performed by injecting 3 μl (automatic injector) of the sample and this was submitted to quantitative determination using β-methyldigoxin (100 μl, 1 mg m^{-1}) as the internal standard. Calibration graphs were constructed by plotting the ratio of the peak area of the internal standard against the weight of each glycoside [12].

Statistical analysis. The analysis of variance between plant groups (*t*-test) was performed by computer, employing SigmaPlot program, version 4.02 (Jandel Co., U.S.A.). Correlation analysis were provided by the same computer program.

Acknowledgments—F. C. B. was supported by the RHAE/CNPq, Brazil. A CNPq Researcher Fellowship (IA) to A. B. O. is also acknowledged. The experimental cultivar of *D. lanata* was initially financially supported by CEME, Ministério da Saúde, Brazil, as a part of a program for Medicinal Plant Development, at FUNED, Belo Horizonte, Brazil.

REFERENCES

1. Weiler, E. W. and Zenk, M. H., *Phytochemistry*, 1976, **15**, 1537.
2. Weiler, E. W. and Westekemper, P., *Planta Medica*, 1979, **35**, 316.

3. Wichtl, M., Bühl, W. and Huesmann, G., *Deutsche Apotheker Zeitung*, 1987, **127**, 2391.
4. Wichtl, M. and Freier R., *Deutsche Apotheker Zeitung*, 1978, **22**, 798.
5. Wichtl, M. and Mangkudidjojo, M., *Pharmakologish Zeitung*, 1984, **129**, 686.
6. Stuhlfauth, T., Klug, K. and Fock, H. P., *Phytochemistry*, 1987, **26**, 2735.
7. Balbaa, S. I., Hilal, S. H. and Haggag, M. Y., *Planta Medica*, 1970, **18**, 254.
8. Kartinig, T. and Hiermann, A., *Science of Pharmacology*, 1980, **48**, 193.
9. Baljet, H., *Schweizerische Apotheker Zeitung*, 1918, **59**, 71.
10. Brugidou, C., Jacques, M., Cosson, L., Jarreau, F. X. and Ogerau, T., *Planta Medica*, 1988, **54**, 262.
11. Wiegrebe, H. and Wichtl, M., *Journal of Chromatography*, 1993, **630**, 402.
12. Milek, F., Ph.D. thesis, University of Tübingen, Tübingen, 1994.