# Variability in the occurence of formylated diditalose glycosides in Digitalis purpurea L.

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# Summary


INTRODUCTION and SCOPE

The mutual synergistic activity of digitoxin and verodoxin has in recent years led to an interest in *Digitalis purpurea* L. races with a high content of the verodoxin complex. In order to select such races, answers were required to the following questions:
Is the total verodoxin complex (glucoverodoxin + verodoxin) genetically determined, as it is the case with other glycosides in the plant, and/or does it depend on the individual development of the plant?
In order to be able to answer these questions it was necessary to determine the content of the primary glycoside glucoverodoxin and the secondary glycoside verodoxin in the plant. A method was therefore developed for the quantitative determination of the most important cardiac glycosides in *D. purpurea*, among which are those of the verodoxin complex.

CHAPTER 1. *Cardiac glycosides in Digitalis purpurea L.*

The most important cardiac glycosides occurring in *D. purpurea* are reviewed. The methods which have been in use up till now for the quantitative determination of these glycosides are discussed.

CHAPTER 2. *Materials and methods*

The method worked out for the routine quantitative determination comprises the following steps: isolation of the above-mentioned glycosides from the plant material; separation of the constituents of this glycoside mixture by means of thin-layer chromatography; visualization of the spots with fuming hydrochloric acid; and measurement of the fluorescence intensity of the spots on stimulation with UV light.
These fluorescence measurements were carried out with a Zeiss TLC spectrofluorimeter by comparison with a reference mixture of the individual glyco-

sides to be measured as internal standard.
On adding up the results of these measurements, they were found to be in agreement with those obtained by colorimetric determinations for the total glycosides of the A and B series.

## CHAPTER 3. *Plant materials, cultivation, and processing*

*D. purpurea* plants of different origins were investigated. The plants were grown in a greenhouse near the laboratory or in the gardens at Buitenpost (Friesland).
An experiment in which the leaves were dried at different temperatures showed that the glycoside yield was highest when the leaves were dried at room temperature. Drying at $50^{o}$ and $70^{o}$ gave a lower yield. The loss, however, was small; it was spread evenly over all the glycosides and thus did not affect the results of the investigation. The effect of different conditions of storage was also studied. It was found that the composition of the glycoside mixture present in the material dried at $70^{o}$ remained unchanged.

## CHAPTER 4. *Application of the results of the preliminary experiments*

### Section 4.1. *Distribution of the glycosides in the mature rosette*

The distribution of the different glycosides in the mature rosette was investigated, partly in order to determine which part of the plant could be taken as a representative sample in the later selection work. The results obtained led to the following conclusions:
- Although the total glycoside content is highest in the young leaves, this has little effect on the amount of glycosides in the whole rosette which is determined chiefly by the larger middle leaves.
- Older leaves contain relatively more of B- and E-series glycosides than of A-series glycosides.
- Proportionately, the largest amount of digitalose glycosides occurs in the young leaves.
- Relatively, formylation takes place to the greatest extent in the older

leaves

- The presence of the formylated digitalose glycoside verodoxin is determined by the influence of these last two factors, which act in opposite directions. It is for this reason that relatively the greatest amount of verodoxin is nevertheless found in the middle leaves.
- The first-mentioned conclusion indicates that the middle leaves are the most important in searching for chemical races with a high verodoxin content.
- Juvenile forms of the plant, artificially maintained by close planting, could provide a greater yield of digitalose glycosides.

Section 4.2. *Production of glycosides at different stages in the development of the plant, and the effect of certain planting techniques*

Apart from the effect of the cultivation methods, it was intended here to determine the most favourable moment for ensuring maximum yield of cardiac glycosides. From the results the following conclusions were drawn:

- Provided conditions are favourable, sowing from seed gives rise to a greater yield of leaf material, and of glycosides, per unit area than does setting out young plants.
- The amount of A-series glycosides, expressed as weight per unit area, increases during growth more than does that of the other glycosides; however, the relative amounts, expressed as percentages, indicate a diminution in the A-series glycosides.
- Mature leaves have a relatively high percentage of gitaloxin. This is to be seen particularly in the high content in those plants which have been planted out and the low content in those plants which have been raised from seed. In the rosette of plants which have been planted out, these mature leaves predominate.
- During growth of the rosette, compared with the digitoxose glycosides, the digitalose glycosides show the smallest increase.
- The percentage of C-16 hydroxy glycosides increases during growth of the rosette.

Section 4.3. *The glycosides during the growth of plants from different origins*

As a start to the selection procedure, a comparison was made between *D. pur-*

*purea* plants of different origins. For the sampling, the findings described in section 4.1 were applied and mature leaves were taken for the purpose. The investigation resulted in the following conclusions:

- Of the populations studied, the leaf yield of two of them, Krakow and Bonn, was least affected by the external conditions.
- There are populations with high and populations with low contents of certain glycosides.
- For each population there is a relative increase in the content of glycosides during growth. This increase is due largely to an increase in the B-series glycosides.
- The populations Bonn and Wageningen were called A types and those from Krakow and Surrey B types. The A types are characterized by a percentage of A-series glycosides which is greater than that of the B- or E-series glycosides.
- Although the different populations have different levels, during growth the total amount of E-series glycosides in a population is maintained at a more or less constant level.
- With only slight variations between the different populations, the percentages of the B- and E-series glycosides were found to be in the ratio 1 : 1.
- The populations Wageningen, Krakow, and Surrey contain more digitalose glycosides at the beginning of the season than do the other populations.
- At the beginning of the season, for the population Krakow the ratio of bound digitalose to bound digitoxose is in favour of digitalose. For the other populations, this ratio is equal or against digitalose.
- At the end of the season, the ratio of digitalose to digitoxose C-16 hydroxy glycosides tends to the same value for all the populations.
- A cross between the populations from Krakow and from Wageningen or Surrey could lead to very good results.

Finally, with regard to the questions posed in the introduction, the following points may be made:
There are distinct differences between the levels of verodoxin accumulation in the seven different populations. It is also clear that the total amount of glycosides belonging to the verodoxin complex is partly determined by the individual development of the plant.