

# CHEMICAL STUDIES OF THE LEAVES AND INFLORESCENCES OF *DIGITALIS PURPUREA* L. AND OF ALLIED SPECIES

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The total glycoside content and the nature of the individual glycosides in the leaves and inflorescences of *Digitalis purpurea*, *D. lanata*, *D. lutea*, *D. thapsi* and *D. ambigua* have been studied. A similarity in the chemical composition of the leaves and inflorescences of each species has been demonstrated. The yield of glycosides from *D. purpurea* and from *D. thapsi* would be increased by harvesting the entire aerial parts of the second year plants.

AFTER the discovery of floral organs in commercial samples of the leaves of *Digitalis purpurea* and *D. lanata*, Cowley and Rowson (1958 and 1960), the total glycoside content of the inflorescences of *D. purpurea*, *D. lanata*, *D. lutea*, *D. thapsi* and *D. ambigua*, has been investigated and compared with that of corresponding leaves.

Extensive references are available on the constituents of the leaves of *D. purpurea* and *D. lanata*; less is known however about the constituents of the leaves or inflorescences of any of the species investigated in the present paper.

## EXPERIMENTAL AND RESULTS

### *Material*

(i) A commercial sample of first year leaves of *D. purpurea* with a declared potency of 14.26 I.U./g. (ii) First year leaves from wild plants collected at the same time as second year leaves and inflorescences from wild plants. (iii) Second year leaves and inflorescences of the other species were kindly provided by Messrs. Burroughs Wellcome, the Royal Botanic Gardens, Kew and the Botanic Gardens at Oxford and Edinburgh. All samples were dried to constant weight at 55–60°.

### *Total Glycosides*

The first and second year leaves, the inflorescences and the individual morphological members of the inflorescence of *D. purpurea*, also the second year leaves and inflorescences of the other species were assayed for total glycosides. The method used was that of Rowson (1955): the results are shown in Tables I and II.

### *Individual Glycosides*

The nature of the individual glycosides present in the leaves and inflorescences of each species was investigated by the chromatographic technique of Fuchs, Wichtl and Sachs (1958) and Kaiser (1955).

The filtrate (40 ml.), obtained in the total glycoside assay after addition of the sodium sulphate solution (Rowson, 1955), was extracted with chloroform (20 ml.) and then successively with 4 × 20 ml. of a chloroform:methanol (3:1) mixture. The combined extracts were dried with

TABLE I

TOTAL GLYCOSIDE CONTENT OF LEAVES AND INFLORESCENCES OF *Digitalis* SPECIES EXAMINED

Species	Source	Total percentage glycosides (calculated as digitoxin)		
		Leaf		Inflorescence
		1st year	2nd year	
<i>D. purpurea</i>	Commercial (14.26 I.U./g.) ..	0.38		
	Wild plants .. ..	0.35	0.23	0.33
<i>D. lanata</i>	Messrs. B. W. & Co. .. ..	1.02		
"	Kew .. ..			0.26
<i>D. lutea</i>	Oxford .. ..		0.30	0.21
<i>D. thapsi</i>	Edinburgh .. ..		0.80	0.40
"	Messrs. B. W. & Co. .. ..		0.76	0.31
<i>D. ambigua</i>	Messrs. B. W. & Co. grown in Kent from—			
	(a) French seed .. ..		0.47	0.27
	(b) Russian seed .. ..		0.39	0.17
	(c) German seed .. ..		0.91	
"	Oxford .. ..		0.40	0.38
"	Kew .. ..		0.51	0.26
"	Kew .. ..		0.86	0.42

TABLE II

*Digitalis purpurea*INDIVIDUAL MORPHOLOGICAL MEMBERS OF INFLORESCENCE  
(MEANS OF SEVEN DETERMINATIONS)

Total percentage glycoside (calculated as digitoxin)					
Flower	Corolla with stamens	Corolla without stamens	Stamens	Stamens	Calyx with gynaecium
0.45	0.41	0.33	0.78		0.48
Fruit with calyx	Mature calyx	Axis (lower)	Axis (upper)	Seeds	Bract (green)
0.53	0.36	0.20	0.12	0.57	0.49

exsiccated sodium sulphate and filtered. The filtrate was evaporated to dryness below 40° *in vacuo*. The residue was dissolved in the minimum volume of a chloroform:methanol (1:1) mixture, transferred to another flask and evaporated to dryness as above. This residue was dissolved in sufficient chloroform:methanol:formamide (2:2:1) mixture to give an approximately 2 per cent solution of the glycosides, calculated from the assay results already recorded.

The components were run on formamide impregnated paper (Whatman No. 1) using equal parts of xylene and methyl ethyl ketone to separate the secondary glycosides, and chloroform:tetrahydrofuran:formamide (50:50:6.5) to separate the primary glycosides. Pure reference substances alone and in admixture were also run under the same conditions as the extracts. Ascending solvent technique was used; the temperature was 20 ± 2° and the running time was 3 hr.

After running, the papers were dried at 100° for 1 hr., sprayed with a 25 per cent solution of trichloroacetic acid in ethanol, containing one part in 15 of freshly prepared 3 per cent aqueous chloramine, and dried at 120° for 4 min. They were examined in ultra-violet light.

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Results are given in Tables III and IV. Several unidentified spots, belonging to the "A" series of glycosides, were found in the leaves of *D. lutea*, and in the leaves and inflorescences of *D. lanata*, *D. thapsi* and *D. ambigua*.

TABLE III  
CONSTITUENTS FOUND IN THE SECOND YEAR LEAVES AND IN THE INFLORESCENCES OF *Digitalis* SPECIES EXAMINED

Constituent	<i>D. purpurea</i>		<i>D. lanata</i>		<i>D. lutea</i>		<i>D. thapsi</i>		<i>D. ambigua</i>	
	L.	Infl.	L.	Infl.	L.	Infl.	L.	Infl.	L.	Infl.
Purpurea Glycoside A .. ..	+		+		+		+	+	+	+
Acetyl Digitoxin .. ..							+	+		
Digitoxin .. ..	+	+	+	+	+		+	+	+	+
Purpurea Glycoside B .. ..	+	+			+		+	+		
Gitoxin .. ..	+			+	+	+		+		+
Glucogitaloxin* .. ..		+								
Gitaloxin* .. ..		+	+		+		+	+		
Gitaloxigenin* .. ..		+								
Digitalinum verum .. ..	+	+		+		+		+	+	+
Strospeside .. ..	+	+	+	+	+	+	+	+	+	+
Lanatoside A .. ..			+	+					+	
Desacetyl Lanatoside C .. ..			+	+			+			
Acetyl Digoxin .. ..			+	+						
Digoxin .. ..			+	+						
Lanatoside D .. ..			+	+						

\* Identified by reference to Kaiser (1955), Van Os (1960) and Witchl (1960).

TABLE IV  
CONSTITUENTS FOUND IN THE INDIVIDUAL MEMBERS OF THE INFLORESCENCE OF *Digitalis purpurea*

Constituent	Flower	Corolla with stamens	Corolla without stamens	Stamens	Calyx with gynaeceum
Purpurea Glycoside A .. ..	+	+	+	+	+
Digitoxin .. ..	+	+	+	+	+
Digitoxigenin .. ..	+				
Purpurea Glycoside B .. ..	+	+	+	+	+
Gitoxin .. ..	+	+	+		+
Gitoxigenin .. ..	+				
Glucogitaloxin* .. ..	+	+		+	
Gitaloxin* .. ..	+	+	+	+	+
Digitalinum verum .. ..	+	+	+	+	+
Strospeside .. ..	+	+	+	+	

Constituent	Fruit with calyx	Mature calyx	Axis (lower)	Axis (upper)	Seeds	Bract
Purpurea Glycoside A .. ..	+	+	+	+		+
Digitoxin .. ..	+	+	+	+		+
Digitoxigenin .. ..			+	+		+
Purpurea Glycoside B .. ..		+	+	+		+
Gitoxin .. ..	+	+	+	+		+
Gitoxigenin .. ..						+
Glucogitaloxin* .. ..			+			
Gitaloxin* .. ..	+	+		+		
Digitalinum verum .. ..		+	+		+	+
Strospeside .. ..		+	+			+

\* Identified by reference to Kaiser (1955), Van Os (1960) and Witchl (1960).

DISCUSSION

*Total Glycosides*

Table I shows that the entire inflorescences of *Digitalis purpurea* contained as much total glycoside as did the first year leaves and this was significantly greater than for the leaves of the second year plants.

The morphological members of these inflorescences had a relatively consistent percentage of total glycosides: thus values for the axis were low, and for the stamens, high. For the other members the values were intermediate (Table II). Calcandi and Ciropol (1958) on the other hand found the lowest, not the highest, percentage of glycoside in the stamens.

Of the other species examined only *D. thapsi* and *D. ambigua* (Oxford) had inflorescences with a total glycoside content similar to that of the inflorescence of *D. purpurea*. None of these other species had more glycoside in the inflorescence than in the second year leaves (Table I).

#### *Individual Glycosides*

The individual glycosides in the leaves of the species examined showed that *D. lutea* and *D. thapsi* contained mixtures similar to that of *D. purpurea*. The presence of lanatoside A in *D. ambigua* and of derivatives of lanatoside C in *D. lutea* is interesting; the latter observation confirms work quoted by Hoch (1961) which is mostly confirmed by our present observations. The following facts are reported for the first time: purpurea glycoside A and purpurea glycoside B in *D. lutea* and *D. thapsi*; gitoxin in *D. lutea*; gitaloxin in *D. lanata*, *D. lutea* and *D. thapsi*; lanatoside A in *D. ambigua* and strosipeside in *D. lutea*, and in *D. thapsi*.

With the exception of *D. lanata* and *D. lutea* the constituent glycosides in the inflorescence were similar to those in the leaf of the same species. It is interesting that primary glycosides of the "A" series were not found in the inflorescences of *D. purpurea*, *D. lanata* and *D. lutea*, although they were found in the morphological members of the inflorescence of *D. purpurea* (Tables III and IV).

The individual glycosides in the inflorescence of *D. purpurea* are the same as those in the leaves: the total glycoside content of these inflorescences is similar to that of first year leaves and is greater than that of second year leaves. It is thus recommended that the official preparation should consist of the dried leaves, or leaves and flowering tops, of *Digitalis purpurea* L.

The inclusion of the inflorescence with the leaves might well apply also to *D. thapsi* and to certain strains of *D. ambigua*.

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