

If analogous to the complexes of bismuth tri-iodide with various organic amines described by Kraut (3), one mole of amine halide or of base might be combined with one or two or more moles of bismuth tri-iodide. It was found that the formula  $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$  gave calculated bismuth and nitrogen percentages closely approximating the analytical values. Formulas constructed with the amine base, or with potassium bismuth iodide, did not fit as well. The compound would appear quite analogous chemically to quinine iodobismuthate which is cited by François and Seguin (4) as having the formula  $C_{20}H_{24}N_2O_2.2HI.2BiI_3$ . If the precipitate of the quinine complex and its mother liquor were heated with excess hydrochloric acid, François and Blanc (5) obtained a crystalline compound with the formula  $C_{20}H_{24}N_2O_2.HI.3BiI_3$ . Variable composition of quinine iodobismuthates was noted by Vita and Bracaloni (6) according to the amount of washing of the precipitate. We have obtained precipitates of thiamin and bismuth tri-iodide which, when less thoroughly washed than the preparations cited as to analysis below, contained 21.0-21.1% bismuth. If the conditions of washing were fixed, however, the batches were quite uniform in composition.

The substance was insoluble in water. Like sodium iodobismuthite (Hanzlik *et al.*, 7) and quinine iodobismuthate (Picon, 8) the thiamin bismuth complex was found to be somewhat soluble in ethylene glycol or propylene glycol or glycerol. It was slightly soluble in acetone, insoluble in ethyl acetate.

For convenience in biological work, the compound was given the short name of bismothiamin. Pharmacological data on the toxicity and rate of mobilization of this preparation after intramuscular injection in animals will be published elsewhere.

*Analysis.* Calcd. for  $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$ : Bi, 27.6%; N, 3.69%.

Found: Bi (Leonard's (9) colorimetric method), 27.3%, 27.7%; N (micro-Kjeldahl<sup>2</sup>), 3.28%.

#### SUMMARY

The insoluble, red addition compound formed by the precipitation of thiamin chloride with Dragendorff's reagent was analysed as to content of bismuth and nitrogen, and was found to correspond to the formula:  $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$ , containing 27.6% bismuth. For biological work it is called bismothiamin. In view of its chemical analogy to quinine iodobismuthate, the pharmacological properties of bismothiamin are being studied.

<sup>2</sup> I am indebted to Professor C. E. Braun for the N analyses.

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## A Pharmacognostic Study of *Digitalis Ambigua*

By Barbara Jacobs\*

#### INTRODUCTION

In view of the fact that various reliable sources have reported *Digitalis ambigua* to be as efficient therapeutically, as *Digitalis purpurea*, it seems timely to collect and organize the information available, and to complete the anatomical picture for this potentially useful species of the *Digitalis*.

In their whole state, the leaves of the various species of *Digitalis* are sufficiently distinctive to prevent confusion. In the powdered state, however, they are very difficult to distinguish. Although they are all similar with respect to the nature of their physiological action, they vary in strength, and it is therefore essential to establish characteristics so that acceptable species may be differentiated from undesirable species.

In this report I have confined myself strictly to the pharmacognosy of the subject. The chemistry, pharmacology and therapeutics involved are only cited to justify the value of the work.

All drawings have been made directly from fresh material obtained by request from the University of Minnesota College of Pharmacy.

\* Prize winning paper; Kilmer award.

## NOMENCLATURE

There have been so many names applied to this species of *Digitalis* that there is considerable confusion in recognizing them all as the same plant.

According to Maheu and Chartier (1) the following names have been applied:

*Digitalis grandiflora* All.  
*Digitalis grandiflora* Rchb.  
*Digitalis ochroleuca* Lindl.  
*Digitalis ochroleuca* Jacq.  
*Digitalis ochroleuca* Rchb.  
*Digitalis ambigua* Murr.  
*Digitalis ambigua* Lindl.  
*Digitalis ambigua* Sturm.  
*Digitalis orientalis* Mill.  
*Digitalis flava* Georgi  
*Digitalis lutea* Maja  
*Digitalis lutea* Pole  
*Digitalis purpurascens* Roth.  
*Pentstemon frutescens* Lamb.

## REVIEW OF LITERATURE

The first scientific article devoted to this species of digitalis appeared in the literature in 1888. Paschkis (2) in Germany determined the constituents and reported them as being identical to those found in *Digitalis purpurea*.

In 1893 Goldenberg (3) of France was experimenting with some unknown species, one of which proved to be *Digitalis ambigua*. He reported that it had the same type of action as *Digitalis purpurea*, but varied in energy of action.

The next report by Hans Benyschek (4) in 1900 was concerning this variance in energy. He claimed that the yield of proximate principles in the various species depended inversely on the amount of water storage, which in turn, varied with the locality and the time of year during which the plants were collected. In this he infers that actually there may be little difference in energy if all species were grown and collected under the same conditions.

Two years later Jaccard and Golaz (5) worked out in great detail the chemical composition of the four species best known in Switzerland and France, namely: *purpurea*, *lutea*, *lanata* and *ambigua*.

In 1904 Boudgest (6) described this plant under the name of *Digitalis grandiflora* as a yellow-flowered species of digitalis which was found abundantly in Switzerland and which possessed activity as a cardiac remedy as pronounced and equal to *Digitalis purpurea*. He claims that this has been demonstrated by previous investigation, and that there is no longer any ground for the rejection of the leaves of *Digitalis ambigua* as a medicinal agent.

Dr. James Burmann (7) completed in 1912 an assay conducted over a period of five years. Among other drugs it included fresh leaves from wild growing plants of *Digitalis ambigua* and *Digitalis purpurea* collected at flowering. They were assayed for digitoxin by the gravimetric method of Keller and the following results obtained.

	<i>D. ambigua</i> , %	<i>D. purpurea</i> , %
1907	0.134	0.078
1908	0.120	0.063
1909	0.067	0.033
1910	0.069	0.037
1911	0.148	0.070

In 1916 researches conducted in the Pharmacognostic Institute of the Vienna University (8) indicated that *Digitalis ambigua* was an acceptable substitute for *D. purpurea*.

A year later at the University of Minnesota, in this country, R. E. Morris (9) made a pharmacological comparison of twenty-three species and numerous varieties. He reported *D. ambigua* as equal to *D. purpurea*, and made the general statement that if care in use, standardization and accurate dosage were practiced that the species or variety was of little importance.

*D. ambigua* was not again mentioned in the literature for over ten years. The next article appeared in 1929 and concerned standardization. *D. ambigua* was cited as the only spontaneous species and very common in Italy. The determination of digitalin was carried out by the Keller-Ecolle method and showed 0.078% digitalin. Since the medicinal activity is not due entirely to this principle another assay by the Foke's method (modified) was conducted. Conclusions were that *D. ambigua*

was less active than *D. purpurea* in the ratio of 0.75 to 1 (10).

In 1934, Maheu and Chartier (1) of France did the only extensive pharmacographical work that has been done on this species. They worked out the morphologic and anatomic characters of whole leaves and powder. Their descriptions have been used in this paper as a guide during actual observations.

This same year, Hocking (11) gives us his viewpoint on the relative strength of the active principles. He opposes some of the earlier reports by stating that according to composition, fertilizer and treatment, the soil exercises a predominant influence on the actual strength of the active principles. He does agree, however, that the climate and frequency of cutting have great influence on both the yield and the strength.

About this time there was considerable experimenting with hybrids of *D. ambigua* and *D. purpurea*. Giant plants were bred but have not proven of any great value (12).

The last important reference to this species was in the "Quarterly Cumulative Index Medicus" for 1937, where it is definitely reported that *D. ambigua* has been used in medicine successfully.

#### HABITAT

*Digitalis ambigua* is indigenous to Europe and Western Asia. It is especially abundant in the woods and rocky mountain areas of Switzerland, France, Austria and Italy. It has been found to some extent anywhere across central Europe from Spain to Russia and western Siberia.

It has been naturalized in the United States and has for some years been successfully grown at the University of Minnesota.

#### EXPERIMENTAL

*Examination of the Entire Plant.*—*Digitalis ambigua* is a perennial herb growing from 50 cm. to a meter in height. The stems are cylindrical, green, pubescent and sometimes ribbed. The leaves are numerous, simple, alternate, exstipulate and sessile or clasping.

The inflorescence is a simple raceme of yellowish campanulate flowers. The calyx is hairy, usually with five linear-lanceolate lobes which are recurved

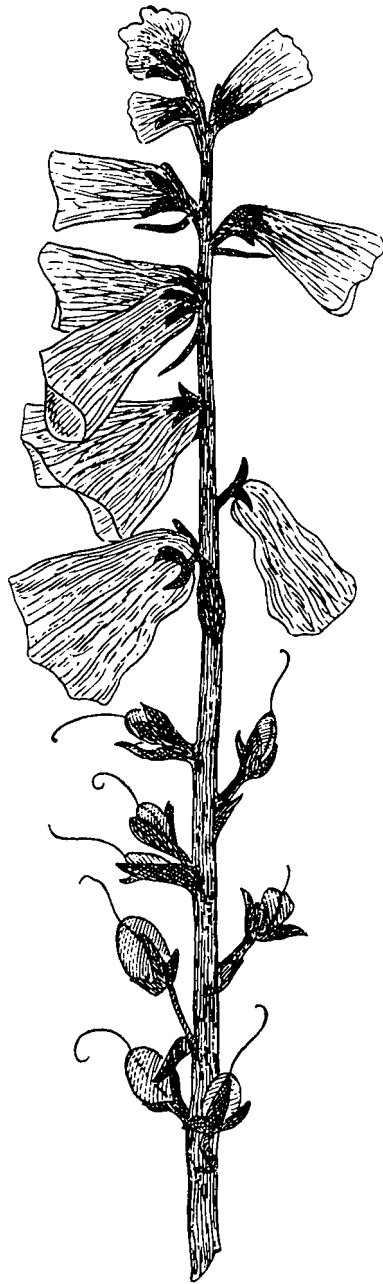


Fig. 1.—*Digitalis ambigua*. Flowering and Fruiting Branch, Natural Size.

at their ends. The corolla is from three to four cm. long, and from one and one-half to two cm. wide. The petals are yellow and are reticulately veined with brown on the inside, pubescent on the outside. Long green bracts are present at the junctions of the pedicels and rachis.

The fruit is a two celled, ovoid, pubescent capsule with central placentation and many seeds. The seeds are minute, brown to reddish brown in color, and irregular in shape. Their outer surface appears

finely reticulate with a groove-like depression on the ventral side, and there is a distinct micropyle at the apex of the groove.

*Physical Characteristics of Leaf.*—The individual leaves are dark green in color and ovate-lanceolate in shape, with a closely toothed margin. The apex is acuminate, the base tapering into a flat wide petiole which clasps the stem. The upper surface is glabrous, shiny and nearly smooth in comparison with *D. purpurea*. The lower surface is somewhat pubescent, especially along the margin and over the veins. The venation is distinct, the veins being large, brown to purple in color, and not closely reticulate. They are convex toward the lower surface, and the vein-eyelet number is from 2 to 2.5.

The odor is characteristically pungent, the taste is bitter.

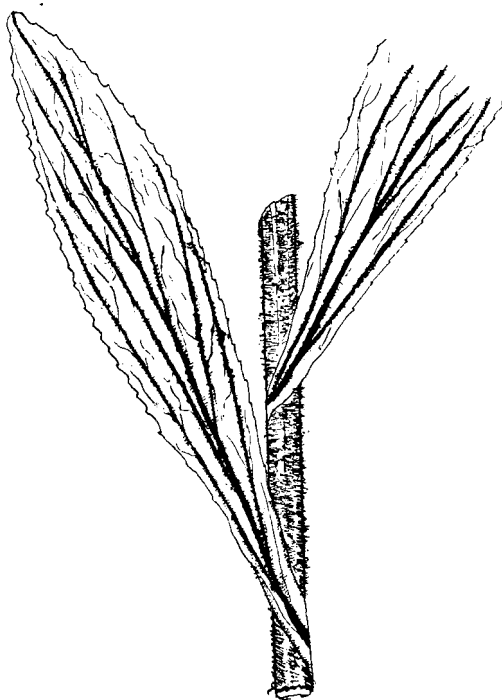


Fig. 2.—*Digitalis ambigua*. Leaves and Stem; Natural Size.

*Microscopic Examination of a Transverse Section of a Leaf.*—The upper epidermis is composed of clear cells which appear rectangular in cross section. The inner and radial walls are slightly concave and somewhat thickened, the outermost wall is convex and shows a cuticle. There are only occasional stomata on this surface, and a few hairs or hair bases from which the hairs have broken off. These hairs, both glandular and non-glandular, are described in detail under the powdered drug.

Immediately below the upper epidermis is a single layer of palisade chlorenchyma, continuous except in the region of the midrib where it is replaced by collenchyma cells thickened in their angles. The rest of the mesophyll region consists of large rec-

tangular cells and numerous intercellular air spaces. Coursing through this region are numerous fibrovascular bundles of the open collateral type. The vascular tissue of the midrib consists of an arc of xylem made up of reticulate and porous tracheæ and islets of sieve tubes.

The lower epidermis is similar to the upper in this view except that stomata and hairs are more numerous.

*Powder.*—In dilute alcohol mount numerous epidermal fragments can be clearly seen in surface view. Those from the upper epidermis are composed of subhexagonal cells with slightly wavy thickened walls, and sometimes granulations similar to those of *D. lutea* but fewer and more regularly distributed. Stomates with three to four neighboring cells are seen occasionally. Sections from over the large veins are made up of regular hexagonal cells which have thick walls and a striated cuticle. The lower epidermis is formed of hexagonal cells the walls of which are thickened and seem to vary from wavy to rectilinear. They are granulated only slightly or not at all. Stomates similar to those of the upper epidermis are numerous.

There is considerable fibrovascular material from the large veins which consists mostly of lignified reticulate and porous tracheæ.

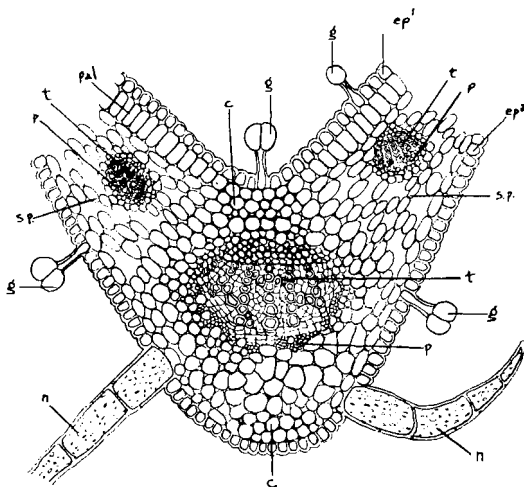


Fig. 3.—*Digitalis ambigua*. Transverse Section of Leaf: *ep*<sup>1</sup>, upper epidermis; *ep*<sup>2</sup>, lower epidermis; *pal*, palisade parenchyma; *s.p.*, spongy parenchyma; *t*, tracheæ; *p*, phloem; *c*, collenchyma; *n*, non-glandular hair; *g*, glandular hair.

There are both glandular and non-glandular trichomes present, but they are few in numbers as compared with powdered *D. purpurea*. The glandular hairs have an elongated one-celled stalk, and either a one- or a two-celled head. The non-glandular hairs are large and uniseriate up to four or five cells. They have thickened walls and numerous projections or nodules elongated in the direction of the length of the hair. These appear white

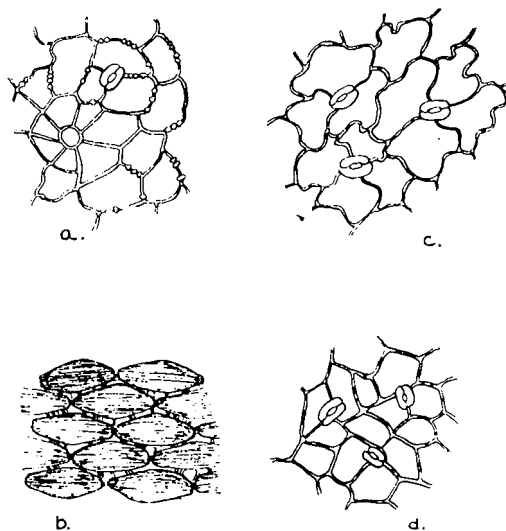


Fig. 4.—*Digitalis ambigua*. Epidermal Tissue: *a*. Upper epidermis showing granulated walls and hair base; *b*. Upper epidermis over large vein showing striations; *c*. Lower epidermis showing wavy walls and numerous stomata; *d*. Lower epidermis showing rectilinear walls and stomata.

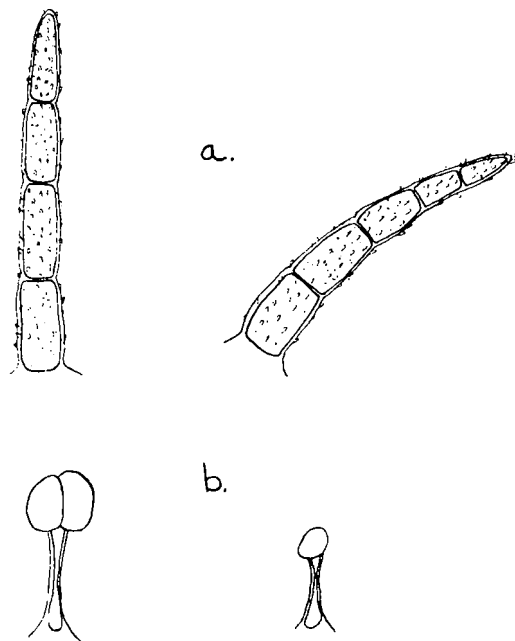


Fig. 5.—*Digitalis ambigua*. Hairs: *a*. Non-glandular,  $\times 110$ ; *b*. Glandular,  $\times 250$ .

under high magnification and give the hair a warty appearance.

The most diagnostic and distinguishing characteristics of powdered *Digitalis ambigua* are the surface sections and the non-glandular trichomes as described above.

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## The Examination of and Standards for Imported Drugs from 1790 to 1908\*

By Lyman F. Kebler†

Following the presentation of my paper on establishing the Drug Laboratory, before this section, at the Atlanta meeting, a prominent member of this Association, reminded me of the low quality of some of the imported drugs for many years and the findings of Dr. Rusby, in particular, presented at a meeting of this Association about the time the Drug Laboratory was organized, and asked why we did not start on the import work

\* Presented to the Historical Section A. Ph. A., Richmond meeting, 1940.

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