

## MORPHOLOGICAL, ANATOMICAL AND CHEMICAL ANALYSIS OF CARDIAC GLYCOSIDES CONTAINING MEDICINAL PLANTS AND HERBAL DRUGS

Cardiac glycosides are natural compounds, whose aglycones are derivatives of steroids or cyclopentanoperhydrophenantrene, containing at the 17-position unsaturated 5-membered (cardenolid) or 6-membered (bufadienolid) lactone ring. They have the highly specific and powerful action that they exert on the cardiac muscle. Because of their action on the heart muscle, they are named cardiac glycosides.

### ***Chemical tests for identification of cardiosteroids***

#### **1. Test for a 5-membered lactone ring:**

Reaction by Balie. Add 0,5 ml of 1 % picric acid solution and 5 drops of 10 % sodium hydroxide to 1 ml of investigated solution.

Reaction by Legal. Place 1 ml of the obtained extract into a test tube add the equal volume of 1 % sodium nitroprusside solution and 2-3 drops of 10 % sodium hydroxide. Mix content of the test-tube by shaking and watch the change of colour. Dark- brown colouring should be observed

#### **2. Test for determination of a steroid nucleus.**

Reaction by Libermann-Burchard. Evaporate 1 ml of the extract to dryness on a water bath. Dissolve the residue in 0,5 ml of acetic anhydride, and transfer into a dry test-tube and add **carefully** (!) 0,5 ml of 98 V % sulphuric acid down the test-tube walls, thus two layers are not mixed. Observe the colour ring on the border of two layers of liquids. Dark- blue colouring should be observed

#### **3. Test for determination of deoxysugars**

Reaction by Keller-Killiani. Evaporate 1 ml of the extract to dryness on a water bath. Dissolve the residuum in 0,5 ml of acetic acid containing 3 % of sol. FeCl<sub>3</sub> and transfer into a dry test-tube. Add carefully (!) 0,5 ml of 98 % sulphuric acid containing 3 % sol. of FeCl<sub>3</sub> thus two layers are not mixed. Dark-blue colouring should be observed .

### **The method of thin-layer chromatography for determination of cardiac glycosides**

(according to British Pharmacopoeia)

Carry out thin-layer *chromatography*, using *silica gel* as the coating substance and a mixture I of 7.5 volumes of *water*, 10 volumes of *methanol* and 75 volumes of *ethyl acetate* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply I separately, as bands 2 cm x 3 mm, 20 ml of each of the following solutions. For solution (1) add 20 ml of *ethanol (50%)* and 10 ml of *lead acetate solution* to 1.0 g in *No. 180 powder*, boil for 2 minutes and allow cooling. Centrifuge and extract the clear supernatant liquid with two 15-ml quantities of *chloroform*, separating the layers, if necessary, by centrifugation. Dry the combined extracts over *anhydrous sodium sulphate* and filter (solution A). Evaporate 10 ml of solution A to dryness on a water bath and dissolve the residue in 1 ml of a mixture of equal volumes of *chloroform* and *methanol*. For solution (2) dissolve 5 mg of *purpureaglycoside A*, 1 mg of *purpureaglycoside B*, 5 mg of *digitoxin* and 2 mg of *gitoxin* in a mixture of equal volumes of 1 *chloroform* and *methanol* and dilute to 10 ml with the same solvent. After removal of the plate, allow the solvents to evaporate, spray with a mixture of 8 volumes of a 25% w/v solution of *trichloroacetic acid* in *ethanol (96%)* and 2 volumes of a 1% w/v solution of *chloramme*, heat I at 100° to 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The chromatogram obtained with solution (2) shows a light blue fluorescent band in the lower part due to purpureaglycoside B and just above it a brownish yellow fluorescent band due to purpureaglycoside I A. In the middle pan there is a band of light blue fluorescence due to gitoxin and above it a band of brownish yellow fluorescence due to digitoxin. The bands in the chromatogram obtained with I solution (1) correspond in position, size and colour to those in the chromatogram obtained with I solution (2), other fluorescent bands may also be present.

**NB! Do not taste cardiac glycosides containing herbal drugs because of highly poisoning!**

## MORPHOLOGICAL AND ANATOMICAL ANALYSIS OF MEDICINAL PLANTS AND HERBAL DRUGS, CONTAINING CARDIAC GLYCOSIDES

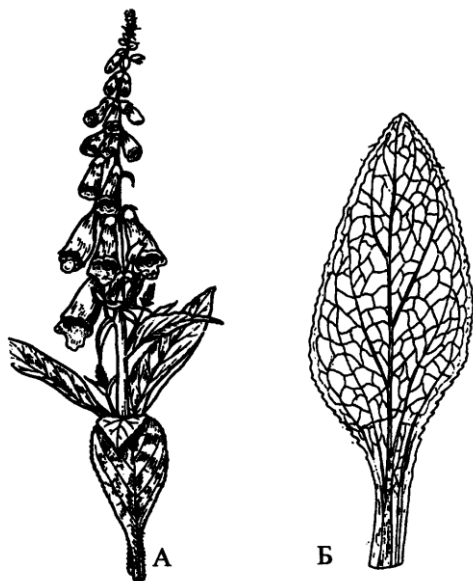
**Digitalis leaf (Purple Foxglove leaf) - *Folium Digitalis***

**Purple Foxglove - *Digitalis purpurea L.***

## Cardiac glycosides containing medicinal plants and herbal drugs

### Family Scrophulariaceae

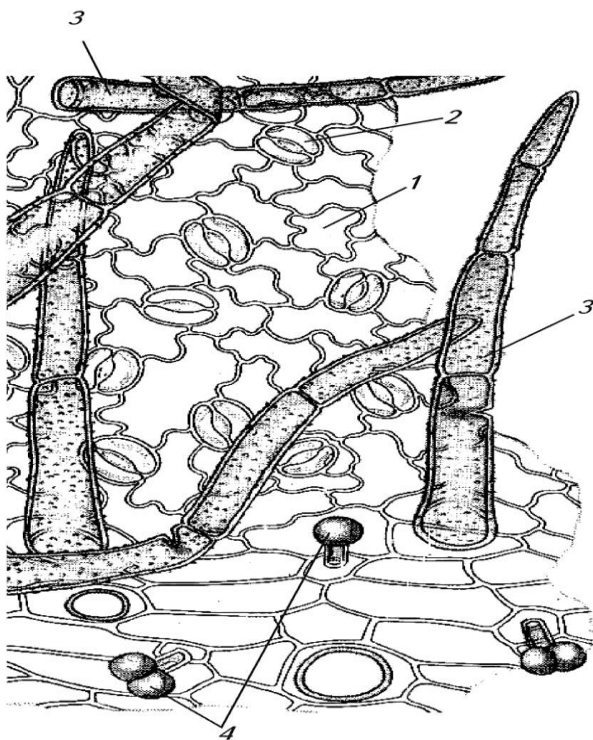
**Definition** Digitalis leaf consists of the dried leaf of *Digitalis purpurea* L. It contains not less than 0.3 per cent of cardenolic glycosides, expressed as digitoxin ( $M_r$  765), and calculated with reference to the drug dried at 100 °C to 105 °C.



**Description.** Digitalis leaves are usually ovate-lanceolate to broadly ovate in shape, from petiolate to sessile and about 10-30 cm long and 4-10 cm wide. The lamina is decurrent at the base; apex is subacute. The margin is crenate or dentate. Both surfaces are hairy, particularly the lower one and a fringe of fine hairs is found on the margin. The veins are depressed on the upper surface, but very prominent from the lower one. The main veins leave the midrib at an acute angle, afterwards branching and anastomosing repeatedly. The leaves are of a dark greyish-green colour. The drug has no marked odour, but a distinctly bitter taste.

**Fig. 13.1. A - Flowering plant of Purple Foxglove, B- leaf of Purple Foxglove**

### Anatomical characteristics



Surface preparations show that the upper epidermis consists of polygonal, relatively straight-walled cells (1), and bears both simple (3) and glandular hairs (4). The cells of the lower epidermis are wavy and the stomata (2) and hairs much more numerous than on the upper surface of the leaf. The stomata (2) are small and slightly raised above the surrounding cells. The simple hairs (4) are uniseriate, 2-7 celled, bluntly pointed, smooth or finely warty with cells often collapsed alternately at right angles. The glandular hairs (3) have a unicellular or occasionally a short uniseriate pedicel with a unicellular or bicellular terminal head

**Digitalis leaf (Foxglove leaf) - *Folium Digitalis lanatae***

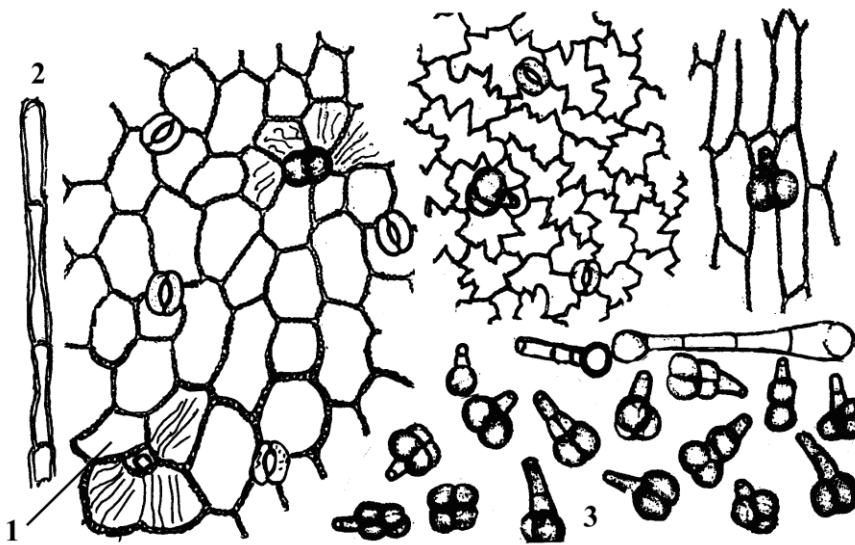
**Foxglove - *Digitalis lanata***

**Family Scrophulariaceae**



**Description.** The leaves are linear-lanceolate to oblong-lanceolate in shape, sessile, slightly leather-like and up to about 30 cm long and 4 cm broad. The margin is entire, rarer wavy. The apex is acuminate and the veins leave the midrib at a very acute angle. The surface of leaves is naked, the colour of the upper surface is green, the lower one is light-green. The odor is weak, peculiar, the taste is bitter.

*Fig. 13.2. 1 - Flowering Foxglove, 2 - Foxglove's leaf*



**Anatomical characteristics**

The upper epidermis in surface view possess distinctly beaded, the cells (1) of lower epidermis have slightly sinuous walls and the non-glandular hairs (2) are few, uniseriate and up to 14 cells in length, one or more of the cells being collapsed. There are a great number of glandular hairs (3) with one to two celled stalks and one to four celled glandular head

**Convallaria Herb, the lily-of-the-valley herb = *Herba Convallaria***

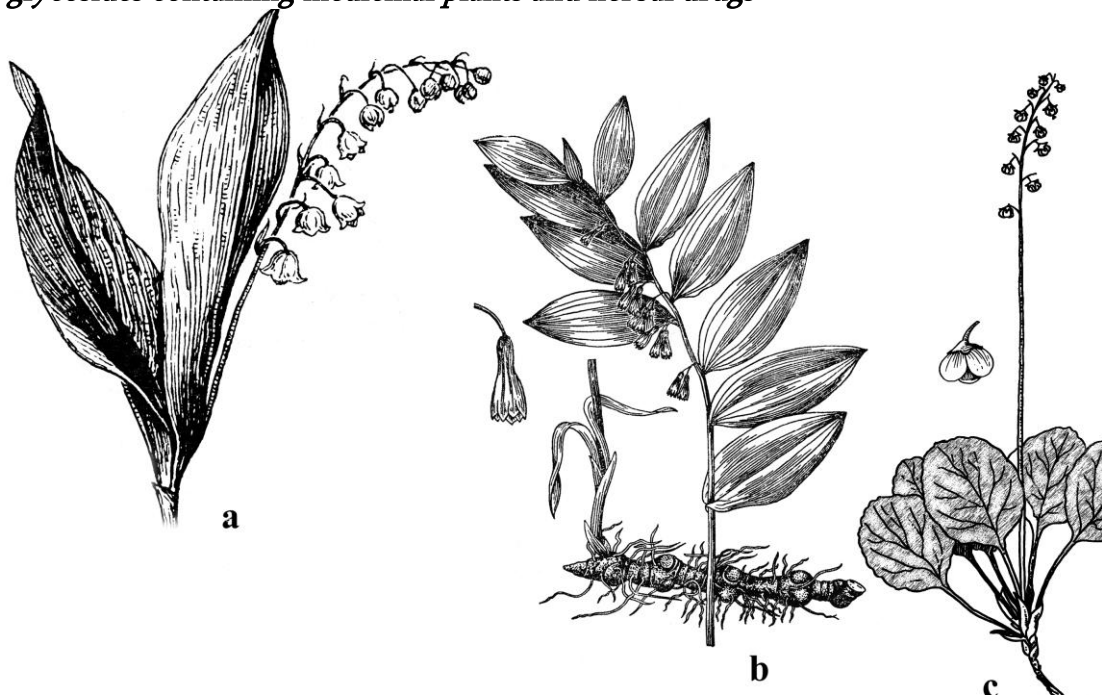
**The lily-of-the-valley, *Convallaria* = *Convallaria majalis* L.**

**Family *Liliaceae* or *Convallariaceae***

**Description.** Three kinds of herbal drugs are distinguished: flowers (inflorescence), leaves and herb. Leaves with long sheaths, separate or conjugate, oval or oblong-elliptical in shape; acuminate, entire, glabrous on both sides, with arching venation, green, petioles often yellowish. The leaf is 10-20 cm long, 3-8 cm wide. Flower scapes are naked, light green, triangular or half rounded in cross-section, terminating in a unilateral loose raceme. Flowers with a simple perianth on bent flower stems, emerging from the axils of short, filmy, lanceolate bracts.

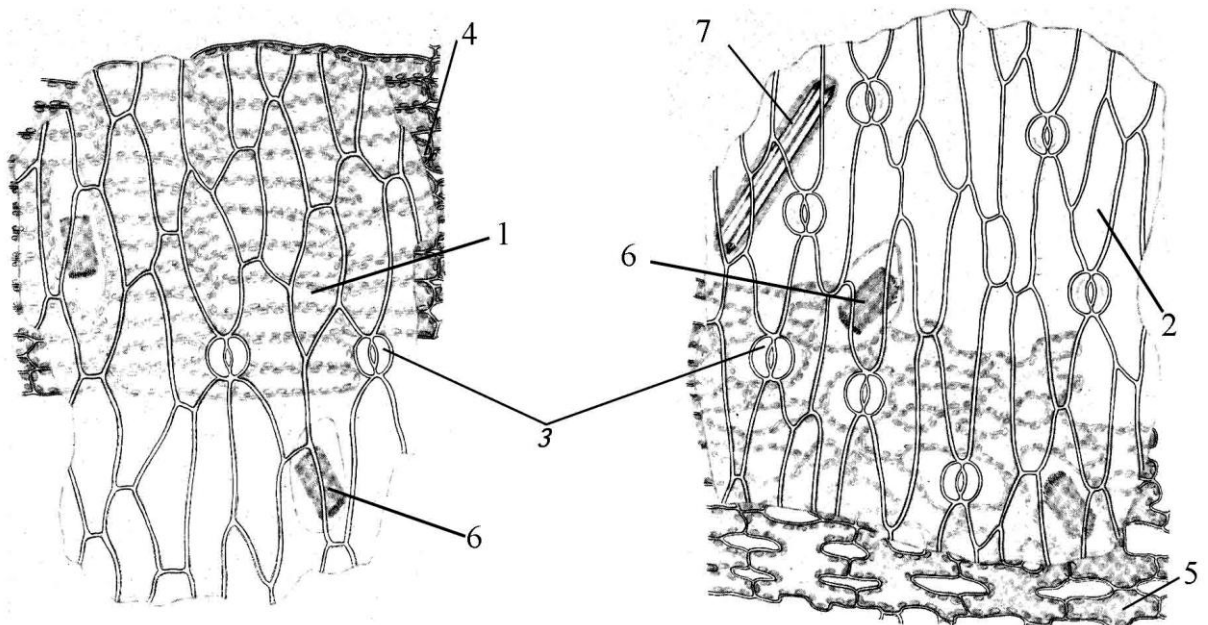
The corolla-like perianth is bell-shaped, 6 stamens on short filaments fixed at the base of the perianth. Odour is weak, faint.

*Cardiac glycosides containing medicinal plants and herbal drugs*



**Fig.13.3.** *a* - Lily-of-the-valley and its adulterants: *b* - *Polygonatum officinale*, *c* - *Pyrola rotundifolia*

**Anatomical characteristics**



Surface preparation shows on both sides epidermal cells (1) stretched along the leaf axis. Cells of the "lying" (4) palisade tissue are seen under the upper epidermis stretched horizontally and situated cross-wisely in relation to the length of the leaf, which is characteristic of the lily-of-the-valley leaves.

Stomata (3) are present on both surfaces; they are surrounded, as a rule, by 4 epidermal cells and located along the length of the leaf. The prism-crystals of calcium oxalate situated in groups of 2-4 (7) and raphides (6) are present in the mesophyll. Spongy mesophyll (5) with intercellular airspaces situated under palisade tissue along leaf.

Adonis herb, Spring Pheasant's Eye herb = *Herba Adonidis vernalis*

Spring Pheasant's eye = *Adonis vernalis* L.

Family *Ranunculaceae*



**Description.** Raw material is represented by densely-leaved shoots about 35 cm in length, with flowers or without them, sometimes with flower-buds or fruits of various stage of development. The leaves are naked, green, alternate, and sessile. The leaves are wide-ovate in shape, palmati-sected in 5 linear segments; two lower of them are shorter than the others.

Flowers are arranged at the apex of the stem and branches; they have 10-20 oblong-elliptical goldish-yellow petals. Calyx is green, downy; it has 5-8 calyx lobes, ovate in shape. Fruit is oval in shape, consisting of numerous, fine greenish nutlets. The odour is weak, characteristic, the taste is bitter.

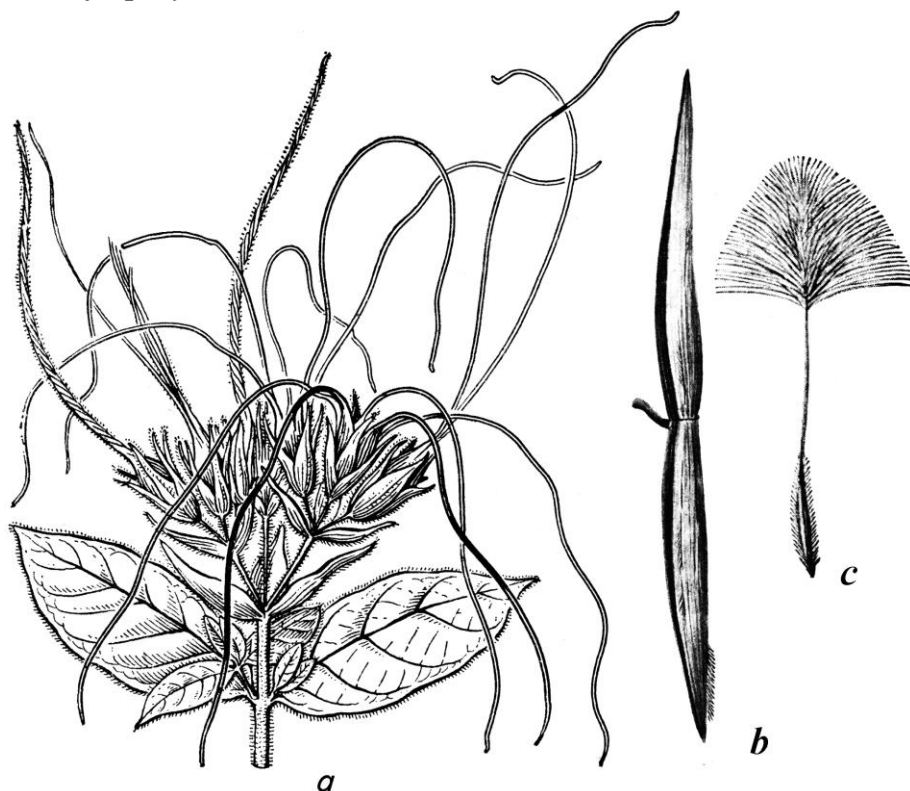
*Fig. 13.4. Flowering Spring Pheasant's Eye*

*Adulterants:* *A. wolgensis* Stev., *A. chrysocyathus* Hook. f. et Thoms., *A. appenina* L.

Strophanthus Seeds - *Semen Strophanthi*

Strophanthus, Strophanthus Kombe, S. gratus, S. hispidus

Family *Apocynaceae*



**Description.** The seeds are lanceolate or linear-lanceolate in shape, somewhat flattened, 12 to 18 mm long, 3 to 5 mm broad. The testa is densely covered with greyish-green or fawny silky hairs, which are directed towards the acuminate apex. On the ventral surface a small ridge, the raphe, runs from a point near the centre of the seed to its apex.

*Fig. 13.5. A - The upper part of strophanthus, B - fruit of strophanthus, C - seed*

## Cardiac glycosides containing medicinal plants and herbal drugs

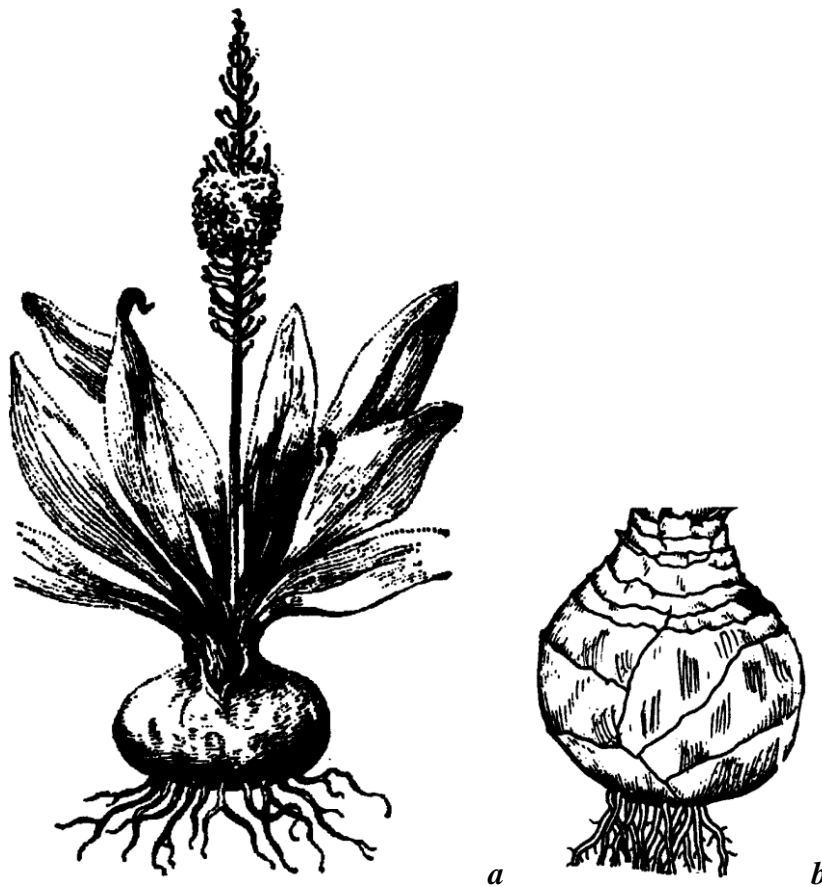
**Squill Bulb = *Bulbus Scillae***

**Squill = *Urginea maritima* (syn. *Scilla maritima*, *Drimia maritima*)**

**Family *Liliaceae***

**Definition.** Squill consists of the bulb of *Drimia maritima* (L.) Stearn, collected soon after the plant has flowered, divested of its dry, outer, membranous coats, cut into transverse slices and dried. It is known in commerce as white squill.

**Description.** Transverse slices, about 5 to 8 mm thick, occurring as straight or curved triangular pieces about 5 to 50 mm long and 3 to 8 mm wide at mid-point, tapering towards each end, yellowish white, texture horny, somewhat translucent, breaking with an almost glassy fracture when quite dry, but readily absorbing moisture when exposed to the air and becoming tough and flexible; transversely cut surface showing a single row of prominent, vascular bundles near the concave edge and numerous smaller bundles scattered throughout the mesophyll. Odorless or almost odorless.



**Fig. 13.6. a - Flowering Squill, b - Squill Bulb**

**Anatomical characteristics.** Epidermis: cells polygonal and axially elongated, 1 to 2 times longer than wide, cuticle thick, stratified; stomata very rare, *anomocytic*, and nearly circular in outline, about 50 to 60  $\mu\text{m}$  in diameter; mesophyll of colourless, thin-walled parenchyma containing very occasional starch granules, many cells containing bundles of acicular crystals of calcium oxalate embedded in mucilage, crystals up to about 1 mm long and about 1 to 15  $\mu\text{m}$  wide; other cells containing sinistrin; vascular bundles collateral, scattered throughout the mesophyll; xylem vessels with spiral and annular wall thickening; trichomes absent.