

# *Scopolia lurida*, Dunal; the structure of the leaves and stem

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The aerial parts of *Scopolia lurida* contain large quantities of tropane alkaloids and are a possible source of hyoscyamine and hyoscyne. The leaves and stems are very similar to those of *Atropa belladonna* but they can be distinguished by certain microscopical characters; these include the presence of branched covering trichomes on the leaves and stems of *Scopolia lurida* and the absence of idioblasts containing microsphenoidal crystals of calcium oxalate from the leaf lamina.

Reports from Eastern Europe on the high alkaloid content of *Scopolia lurida* (family Solanaceae), have directed attention to this plant as a possible source of the tropane alkaloids, particularly hyoscyamine and hyoscyne. Vysotskaya (1956) found a total alkaloid content of 1.2% in the leaves and Libisov (1959) reported that plants grown in the U.S.S.R. contained up to 1.5% of alkaloids in the aerial parts. Gritsaeva & Prozorovskii (1960) stated that the plant contained "about three times as much alkaloid as belladonna, there being about 1% present in the leaf." Similar reports from Polish workers (Szymańska, 1953, 1956; Sowicka & Szymańska, 1966), have indicated that the aerial parts contain up to 0.8% of atropine and hyoscyne.

*Scopolia lurida* is a perennial herb, indigenous to the Himalayas but readily cultivated in Europe. The leaves and stems show a remarkable similarity to belladonna in their general morphology, and whilst the detailed structure of belladonna is well documented, that of *Scopolia lurida* has not been fully investigated. The plant was first described by Dunal (De Candolle, 1852) and the morphological characters were also briefly outlined by Semlinskii (1958). Schlepegrell (1892) recorded the presence of branched, multicellular trichomes on the leaf and a superficial examination of the leaf microscopy was made by Fedde (1896) and more recently by Szymańska (1956). The detailed structure of the plant has not, however, been described.

## LEAF

### *Morphological characters* (Fig. 1.1)

Leaves simple, ovate to ovate-lanceolate, 14 to 30 cm long and 6 to 15 cm wide; margin entire, slightly sinuous and ciliate. Base acute, decurrent and slightly asymmetric, apex acute. Both surfaces bright green, the upper darker than the lower; the upper with scattered small, whitish hairs and the lower densely pubescent, especially over the veins. Midrib and lateral veins very prominent on the lower surface, slightly raised on the upper surface; lateral veins (about 8 on each side) leaving the midrib at an angle of about 50° and anastomosing near the margin; subsidiary venation reticulate. Texture thin and papery.

Petiole 2 to 6 cm long, flattened on the adaxial surface with two raised ridges,

one on each side, running longitudinally; abaxial surface densely pubescent. Pale green or, on mature leaves, slightly reddish.

### *Histological characters*

*Lamina.* The upper epidermis (Fig. 1.5) consists of a layer of cells with thin, wavy anticlinal walls except near the margins and over the veins, where the walls are straight or only slightly sinuous. The cuticle is thin and, where it covers the straight-walled cells at the leaf margins, it is strongly striated. Stomata, mainly anisocytic but occasionally anomocytic, are fairly numerous; they measure about  $33\text{--}45\ \mu\text{m} \times 22\text{--}34\ \mu\text{m}$ . Scattered clavate glandular trichomes are present, about  $75\text{--}170\ \mu\text{m}$  long, each consisting of a thin-walled, unicellular stalk and an ovoid head composed of from four to six (sometimes up to 12) cells with brown contents (Fig. 2.6). Covering trichomes are absent. The single layered palisade is not continuous over the veinlets, but is replaced in these regions by groups of small, closely-packed, rounded parenchymatous cells. The layer of spongy mesophyll adjacent to the palisade is modified to form collecting cells, which are sometimes elongated and appear as a second palisade layer; several of these cells contain cluster crystals of calcium oxalate up to about  $50\ \mu\text{m}$  in diameter and prisms about  $7\text{--}25\ \mu\text{m}$  long (Fig. 1.7, Fig. 2.1, 2.2). Similar crystals occur in the underlying mesophyll layer. Groups of vascular tissue of the veinlets occur at frequent intervals, each consisting of a small number of vessels surrounded by sieve tissue and thicker-walled parenchymatous cells.

The cells of the lower epidermis (Fig. 1.6) resemble those of the upper epidermis but the anticlinal walls are more markedly wavy. As on the upper epidermis, the cells over the veins and near the margins are straight-walled and, in the marginal region, the cuticle is strongly striated (Fig. 2.4, 2.7). Anisocytic and, occasionally, anomocytic stomata occur more frequently than on the upper surface. Glandular trichomes, similar to those on the upper epidermis, are present but are less abundant; they are found mainly over the veins. Numerous covering trichomes are also present; these occur scattered over the whole of the lamina and are particularly abundant over the veins. They are uniseriate and irregularly branched; the branches develop unequally so that some may be unicellular and others have up to five cells; repeated branching may take place resulting in a many-armed structure composed of 30 or more cells and measuring up to  $850\ \mu\text{m}$  long. The cells have thin, smooth walls and the terminal cell of each branch has a bluntly rounded apex (Fig. 2.5).

Similar covering trichomes are present on the margins of the leaf. They arise from the apices of projections which occur at frequent intervals; each projection is composed of from one to eight rounded cells with thin walls and a striated cuticle. One trichome, or occasionally two, arises from each projection (Fig. 2.4, 2.8).

### *Midrib (Fig. 1.3)*

Over the midrib the cells of the upper epidermis are longitudinally elongated with straight or slightly sinuous anticlinal walls. The periclinal walls are slightly thickened and convex and covered by a finely striated cuticle. A few glandular trichomes and very occasional anomocytic stomata are present. The cells of the lower epidermis are similar to those of the upper epidermis but the cuticle is not striated; stomata and glandular trichomes are present, also numerous covering

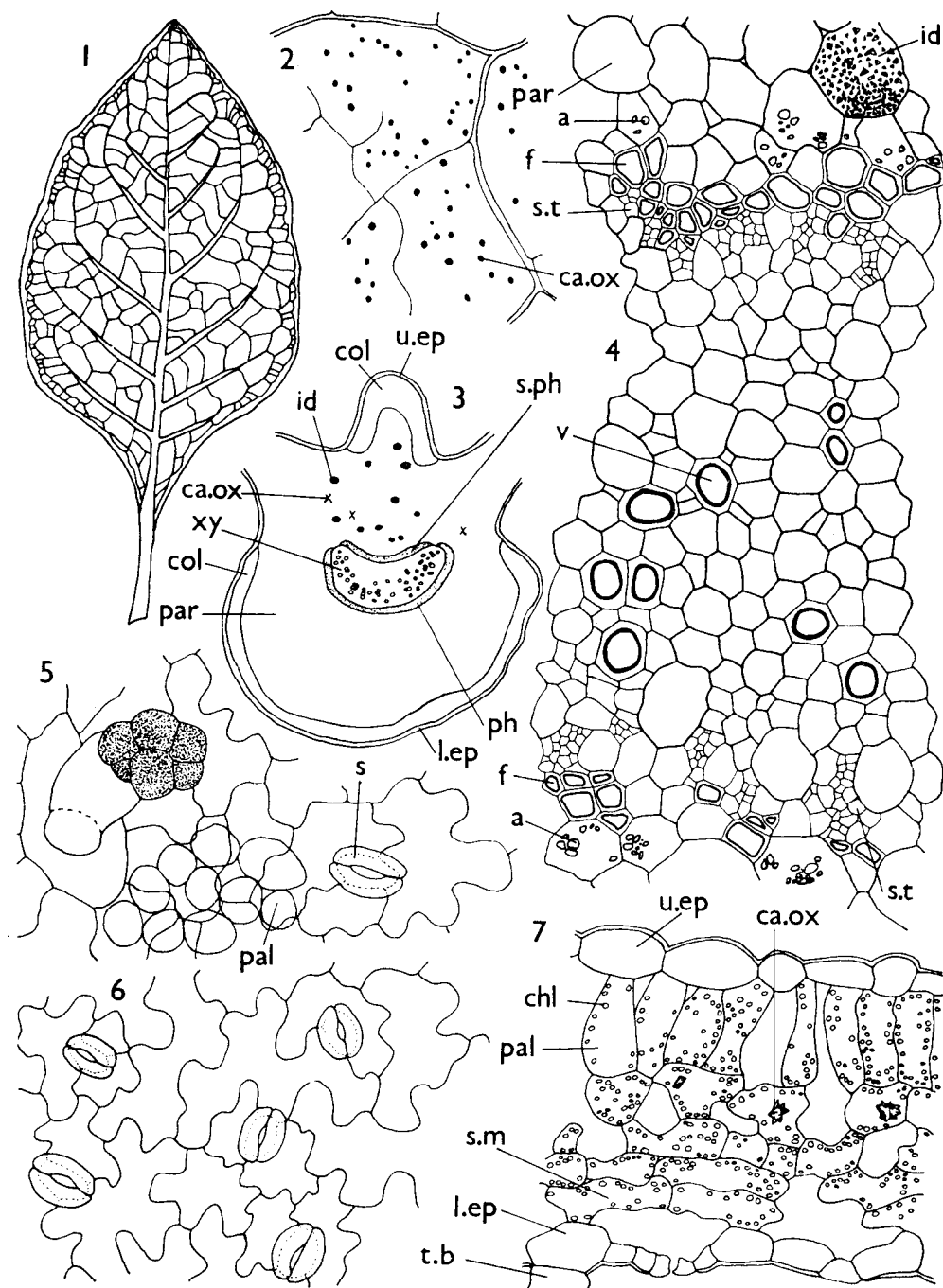


FIG. 1. Leaf of *Scopolia lurida* Dunal. 1, leaf  $\times \frac{1}{2}$ ; 2, part of the lower surface showing vein islets and distribution of calcium oxalate idioblasts  $\times 30$ ; 3, diagram of a transverse section of the midrib  $\times 20$ ; 4, details of the central region of a transverse section of the midrib; 5, upper epidermis in surface view; 6, lower epidermis in surface view; 7, details of a transverse section of the lamina. 4 to 7,  $\times 225$ . a, starch granules; ca. ox, calcium oxalate cluster crystal; chl, chloroplast; col, collenchyma; f, fibre; id, idioblast; l. ep, lower epidermis; pal, palisade; par, parenchyma; ph, phloem; s, stoma; s. m, spongy mesophyll; s. t, sieve tube; s. ph, supernumerary phloem; t. b, trichome base; u. ep, upper epidermis; v, vessel; xy, xylem.

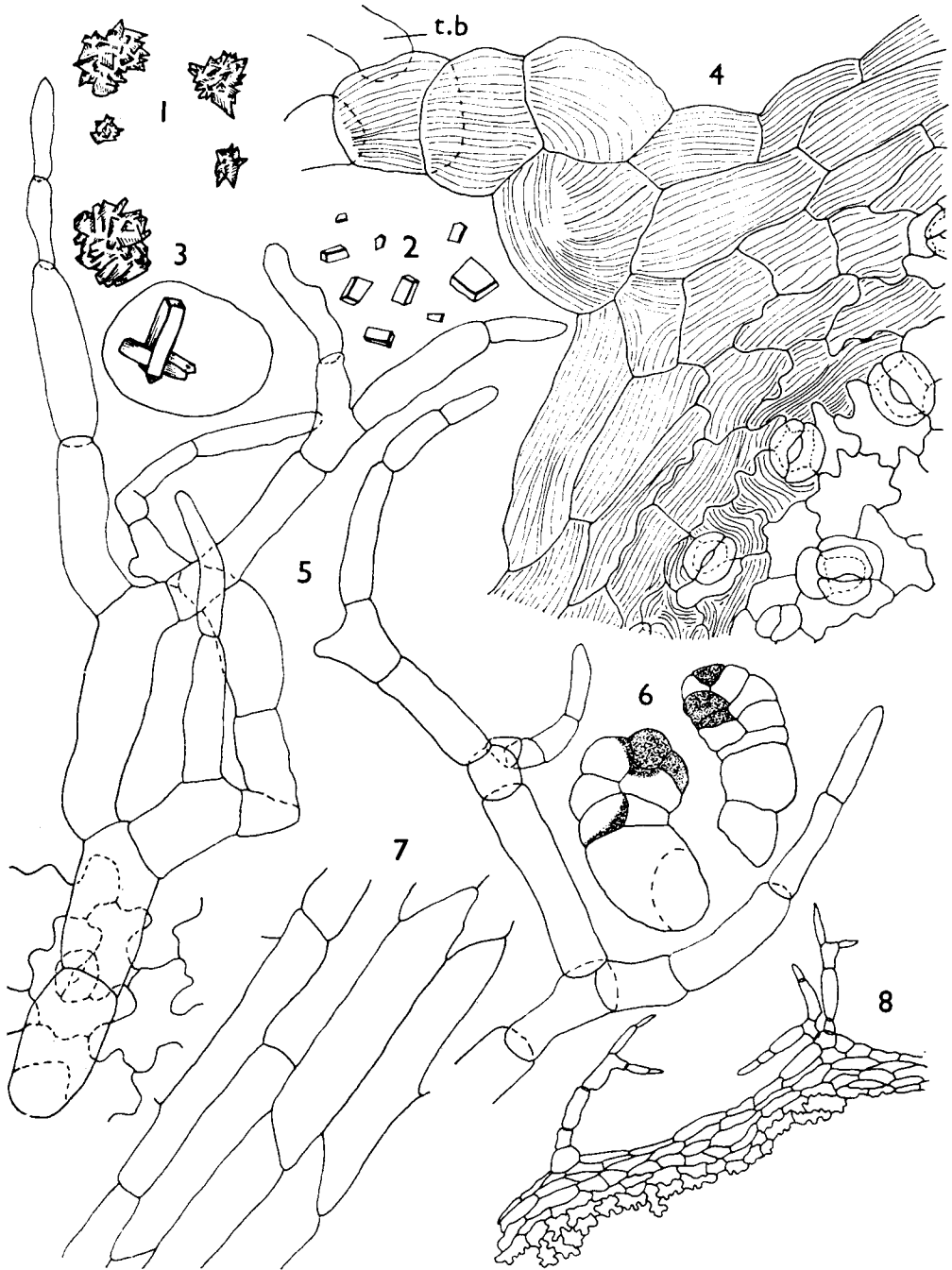


FIG. 2. Leaf of *Scopolia lurida* Dunal. 1, cluster crystals of calcium oxalate from the lamina; 2, prism crystals of calcium oxalate from the lamina; 3, cluster crystals of calcium oxalate from the midrib; 4, part of the margin from the lower epidermis, in surface view, showing a tooth and the striated cuticle; 5, covering trichomes from the lower epidermis; 6, glandular trichomes; 7, lower epidermis over a vein in surface view; 8, part of the margin showing the marginal teeth and covering trichomes. 1 to 7  $\times 225$ , 8  $\times 45$ . t.b, trichome base.

trichomes, similar to those found on the lamina, although many of the constituent cells are collapsed and some have brown contents.

Collenchyma occurs beneath both epidermises; that below the upper epidermis consists of from one to ten layers of cells, the larger amount being present at the base of the leaf and the number of layers gradually diminishing towards the apex; about two to six layers are found below the lower epidermis.

The remainder of the cortex is composed of rounded, thin-walled parenchyma and in the area above the meristele, a number of the cells are filled with microsphenoidal crystals of calcium oxalate measuring about 2-4  $\mu\text{m}$ ; a small number of prisms, and cluster crystals with few components, also occur (Fig. 1.3, 1.4, Fig. 2.3).

The meristele is crescent-shaped to reniform when seen in transverse section and occupies about half the diameter of the midrib (Fig. 1.3). It is surrounded by a layer of thin-walled cells forming the starch sheath. The central xylem is composed of a small number of lignified vessels, about 13 to 55  $\mu\text{m}$  in diameter, interspersed with unlignified parenchyma and separated by medullary rays from one to three cells wide. Supernumerary phloem is present and this joins with the normal phloem to form a more or less complete band surrounding the xylem. The sieve tissue occurs in groups and consists of small, thin-walled sieve tubes with associated small-celled parenchyma. In fully-mature leaves the outer part of the phloem is frequently composed of several layers of thicker-walled parenchymatous cells and these cells sometimes extend into the medullary rays and between the groups of sieve tissue.

Fibres occur immediately outside the phloem; they are found in groups or, occasionally, they form a more or less continuous band; the walls are moderately thickened and lignified (Fig. 1.4).

### *Petiole*

The structure of the petiole is very similar to that of the midrib. The collenchymatous tissue which, in the midrib, occurs in the outer cortex above and below the meristele, forms a continuous layer in the petiole and may consist of up to 25 rows of cells; this tissue also forms the ridges which occur on either side on the adaxial surface. As in the midrib, idioblasts containing microsphenoidal crystals of calcium oxalate are found in the parenchymatous tissue above the meristele and only rarely below the meristele.

## STEM

### *Morphological characters*

Cylindrical, up to 1.5 cm in diameter, pale green, sometimes with brown or purplish patches; surface with long, whitish hairs, more abundant on young stems; phyllotaxis 1/5. About half the diameter of the transversely-cut surface occupied by a yellowish-white pith; cork not developed (Fig. 3.1).

### *Histological characters*

The epidermal cells have slightly convex outer periclinal walls and are covered by a thin, smooth cuticle; in surface view they are polygonal, rather irregular in size and axially elongated.

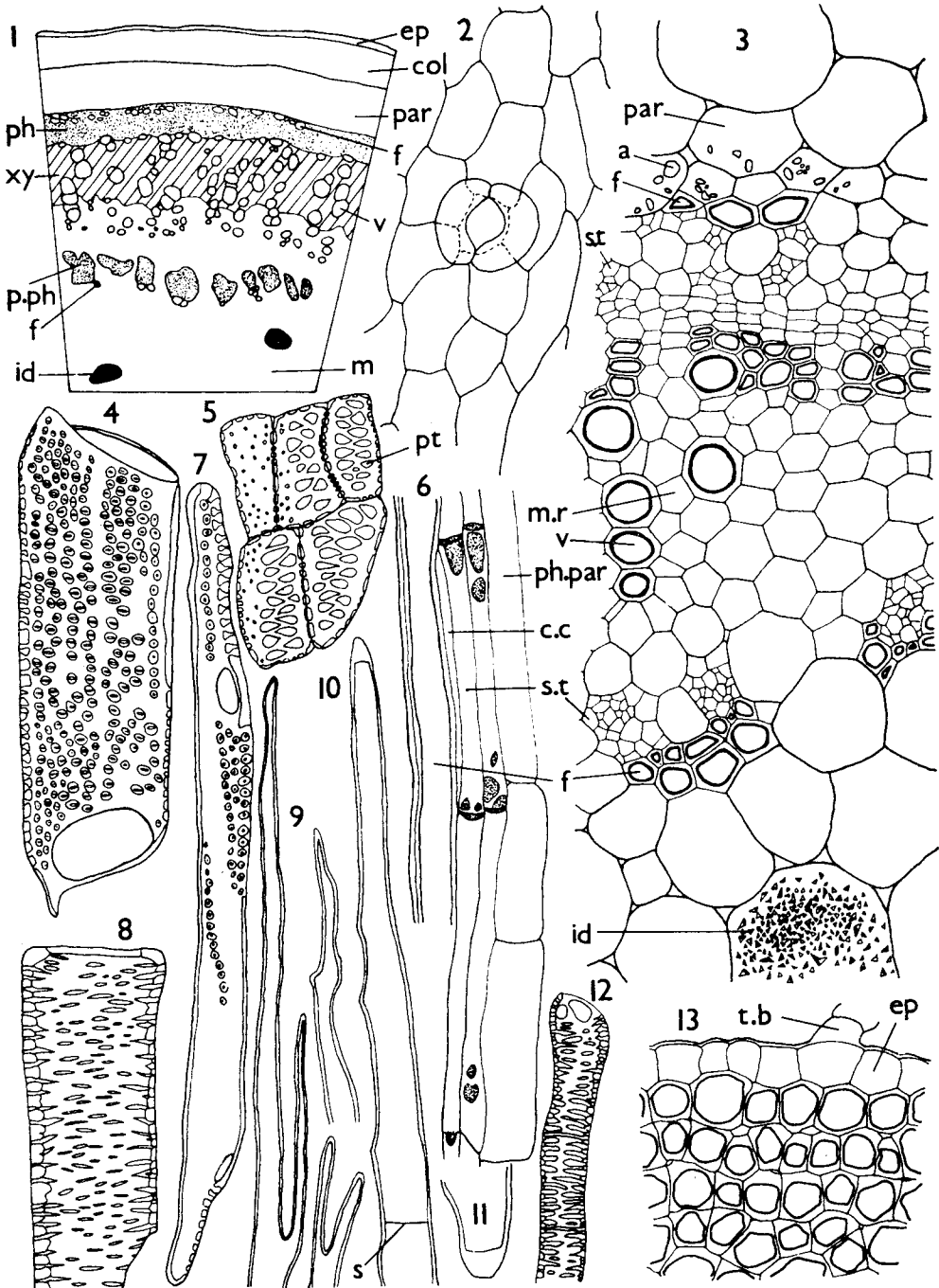


FIG. 3. Stem of *Scopolia lurida* Dunal. 1, diagram of part of a transverse section of a mature stem  $\times 20$ ; 2, epidermis in surface view; 3, details of part of a transverse section of a young stem to include the vascular tissues; 4, bordered pitted vessel; 5, xylem parenchyma; 6, longitudinal section of part of the phloem; 7, tracheidal vessel; 8, large reticulately-thickened vessel; 9, parts of xylem fibres; 10 and 11, parts of phloem fibres; 12, small reticulately-thickened vessel; 13, details of the outer part of a transverse section, showing the epidermis and underlying collenchyma. 2 to 13  $\times 225$ . a, starch granules; c.c, companion cell; col, collenchyma; ep, epidermis; f, fibre; id, idioblast; m, medulla; m.r, medullary ray; par, parenchyma; ph, phloem; ph. par, phloem parenchyma; p.ph, perimedullary phloem; pt, pit; s, septum; s.t, sieve tube; t.b, trichome base; v, vessel; xy, xylem.

Anomocytic stomata occur infrequently; they are larger than those found on the leaf lamina, measuring approximately  $52\text{--}75 \times 32\text{--}60 \mu\text{m}$  (Fig. 3.2). A few scattered, glandular trichomes are present and numerous branched covering trichomes similar to those occurring on the leaf.

The cortex is differentiated into an outer collenchymatous region composed of from five to eight rows of cells and an inner region of large-celled parenchyma; idioblasts containing microsphenoidal crystals of calcium oxalate are occasionally found in the inner region. A distinct starch sheath is present, and a few scattered starch granules also occur in the adjacent parenchyma (Fig. 3.3, 3.13).

Fibres are present in the outer part of the phloem; they occur singly or in small groups and are usually associated with the groups of sieve tissue. The walls are moderately thickened and lignified with a few small, slit-shaped pits; occasional thin, unlignified, transverse septa are present and the blunt apices of the fibres are more strongly thickened (Fig. 3.10, 3.11). Individual fibres measure 1000 to 1300  $\mu\text{m}$  in length and from 30 to 160  $\mu\text{m}$  in diameter. The remainder of the phloem is composed of groups of sieve tubes and small-celled parenchyma, interspersed with larger-celled parenchyma and separated by medullary rays which are mainly uniseriate. The sieve tubes are narrow and frequently have additional sieve areas on the side walls at both ends; companion cells are only occasionally present (Fig. 3.6).

The secondary xylem in a mature stem is completely lignified. It is composed of groups of vessels, with a small amount of associated parenchyma, and numerous fibres, separated by medullary rays which are mainly uniseriate. The vessels occur in approximately radial rows and are up to about 135  $\mu\text{m}$  in diameter; the end walls are frequently oblique and occasionally two small pores may be present instead of a single perforation (Fig. 3.12). Most of the vessels occurring in the outer part of the secondary xylem have slit-shaped bordered pits (Fig. 3.4), whilst those in the inner part frequently show reticulate thickening (Fig. 3.8). Associated with the reticulate vessels are a small number of tracheidal vessels, up to about 50  $\mu\text{m}$  in diameter, with bordered-pitted walls and a single pore occurring at or near each of the bluntly-tapering ends (Fig. 3.7). The fibres of the xylem are smaller than those of the phloem and the walls are more heavily lignified with fewer pits; the ends taper more finely and are frequently forked: transverse septa are not present (Fig. 3.9).

The parenchymatous cells associated with the vessels and those forming the medullary rays are very similar. They are elongated longitudinally and have moderately thickened walls with numerous simple or bordered pits (Fig. 3.5). The medullary rays are up to about 40 cells in depth when seen in tangential longitudinal section.

A few vessels of the primary xylem, with spiral or annular thickening, occur on the inside of the secondary xylem. Perimedullary phloem is present and is composed of small groups of sieve tissue with associated fibres, separated by the large-celled parenchyma of the pith.

The fibres occur towards the inside of the groups of sieve tissue and they are similar to those found in the outer phloem but are thicker-walled and more heavily lignified (Fig. 3.3).

The remainder of the pith consists of thin-walled, parenchymatous cells, a number of which are filled with microsphenoidal crystals of calcium oxalate (Fig. 3.1, 3.3).

## DISCUSSION

The main microscopical character which distinguishes the leaves and stems of *Scopolia lurida* from those of *Atropa belladonna* L. is the presence of multicellular, branched covering trichomes on *Scopolia lurida* which are not present on belladonna. These trichomes occur on projections from the leaf margins and on the lower surface of the lamina and are very abundant on young stems.

Other important differences between *Scopolia lurida* and belladonna are:—

(a) the cuticle on *Scopolia lurida* is not striated except on the marginal cells of the leaf epidermises;

(b) the anticlinal walls of the leaf epidermises are more wavy in outline than those of belladonna;

(c) microspenoidal crystals of calcium oxalate are not present in the mesophyll of the leaf of *Scopolia lurida* although they do occur in the midrib, petiole and stem; cluster crystals and prisms are present in the mesophyll;

(d) the clavate glandular trichomes are larger in *Scopolia lurida*, measuring up to 170  $\mu\text{m}$  in length whereas those of belladonna measure up to 100  $\mu\text{m}$  (Wallis, 1967); glandular trichomes with a uniseriate stalk and unicellular head, present on belladonna leaf and stem, do not occur on *Scopolia lurida*;

(e) in *Scopolia lurida* there is a collenchymatous layer in the cortex of the stem which is not usually found in belladonna stem.

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