

THE ANATOMY OF THE LEAF OF *SYMPHYTUM OFFICINALE* L.

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The history and uses of the leaves of *Symphytum officinale* L. are given together with an illustrated account of the macroscopy of the upper and lower leaves of the plant and of the anatomical structure of the leaf. The diagnostic characters of the powdered leaves are also recorded and illustrated.

COMFREY has been used in medicine for many centuries. It was used for healing broken tissue and bone, hence its Greek name *Συμφυτίου* derived from *δύμωω*, to unite (Barton and Castle 1877). The brief morphology and medicinal uses of two species of *Symphytum* (*S. petraeum* and *S. alterum*) are described in Dioscorides' Herbal (ca. A.D. 100) (Gunther, 1959). Parkinson (1640) stated that the plant is *S. petraeum*, whereas Turner (1548) and Matthioli (1598) used the specific name *S. alterum*. The modern name, Comfrey, derives from the mediaeval Latin *Comfria*, which in turn replaced the Latin *Conferva* of Pliny (Grigson, 1955). It was also known as *Consolida maior* and, by the Romans, *Solidago* (Dodoens, 1586; Salmon, 1610). The Romans took the plant to northern Europe (Kamm, 1938), and it was used by the Saxons as a vulnerary (Martindale, 1924). By A.D. 1000 it had appeared in the monastery lists and leech-books (Kamm, 1938). From the Middle Ages to the middle of the nineteenth century, there was a belief that both the roots and leaves had the power to heal wounds and bones (Dodoens, 1586, Salmon, 1610). This resulted in the use of names such as knit-back, bone set, nit-bone, healing blade, bruisewort (Kamm, 1938) and blackwort (Gerard, 1633). It was widely used for quinsy, whooping cough, in poultices for bruises and open wounds and also as a styptic or a pectoral (Kamm, 1938).

In 1912 it was found to be of value in the formation of epithelial tissue in external ulceration and in ulcers of the stomach and duodenum, due to the presence of allantoin (Bramwell, 1912; Wood and Lawall, 1926). The infusion is still used as a fomentation for reducing inflammations associated with sprains and bruises and as a medicine for chest complaints. It is widely used in country districts, particularly Warwickshire (Min. Agric. Fish., 1941), and is generally available commercially.

The British Pharmaceutical Codex of 1934 introduced a monograph for the leaf, but current and recent textbooks refer to the drug only as an adulterant of the leaf of *Digitalis purpurea* (Perrot, 1943; Trease 1957, Wallis, 1960). The only detailed work on the leaf is that of Bider (1935); this is not illustrated and deals only with macroscopical features together with histological epidermal features, particularly the trichomes. Kay (1938) has briefly described and illustrated the covering trichomes.

LEAF OF *SYMPHYTUM OFFICINALE* L.

In view of the fact that no detailed anatomical work is evident, and the well-known use of the plant in medicine and agriculture, this communication describes the detailed structure of the leaf, to establish the diagnostic characters necessary for its identification in the whole or powdered condition.

MATERIAL

The material used for the work was collected from wild plants growing in areas near Bradford. Further material was obtained from plants by transplanting roots from Yorkshire and propagated vegetatively in Stapleford, Nottinghamshire. All plants used in the present work possessed the characters typical of the species.

METHODS OF INVESTIGATION

Chloral hydrate solution proved satisfactory for epidermal preparations; systematic serial sections were prepared by methods described previously (Fell and Rowson, 1955, 1956), employing polyethylene glycols and freezing microtomy.

Sections were mounted in chloral hydrate solution or dilute glycerol when making drawings of parenchymatous tissues, in phloroglucin and hydrochloric acid when investigating the nature of the vascular tissues, and chloral-iodine to demonstrate the presence of micro-starch. Ruthenium red and methylene blue solutions gave positive reactions with mucilage in the cells of the upper and lower epidermises of the interneural lamina and midrib, also the pericyclic collenchyma of the midrib; negative reactions were given by corallin soda solution and 0.02 N iodine. A negative result for tannins was obtained on the addition of 5 per cent ferric chloride solution to sections of the leaf.

An investigation was made into the nature of numerous crystals present in the bases of many trichomes, epidermal cells, phloem and, to some extent, in the remaining parenchymatous tissue. They did not dissolve in phloroglucin and concentrated hydrochloric acid, or on the addition of 66 per cent w/w sulphuric acid. They were found to be birefringent when examined in polarised light after mounting in dilute glycerol or chloral hydrate solutions, or even after the addition of strong acid. Since, therefore, the crystals were obviously not calcium carbonate or oxalate, it was thought that they may be silica or allantoin. Mothes and Engelbrecht (1954) have proved that allantoin occurred in the leaves. Vogl (1918) found monoclinic crystals of allantoin in the rhizome of *S. officinale*. He mounted sections of the rhizome in 20 per cent acetic acid in alcohol, covered, and sealed the glass with paraffin, when further recrystallisation was effected. This could not be demonstrated in the leaves examined; since these had been preserved in ethanol, it was assumed that all the allantoin present had already crystallised out.

Schlepegrall (1892) and Titherley and Coppin (1912) identified allantoin in the root of *S. officinale*. On the addition of mercuric nitrate solution, a white precipitate was produced; the same method was used with scarlet runner beans by Power and Salway (1913). When sections and surface

preparations were treated with this reagent, a white precipitate and occasionally a red colour was produced with some crystals, particularly those in the base of the trichomes, indicating that a proteinous substance was present.

Comfrey root contains a little tannin. An aqueous extract of the leaves was treated with 95 per cent ethanol saturated with sodium chloride, and the filtrate was tested with 5 per cent ferric chloride solution and 1 per cent iron and ammonium citrate solution, but no positive results

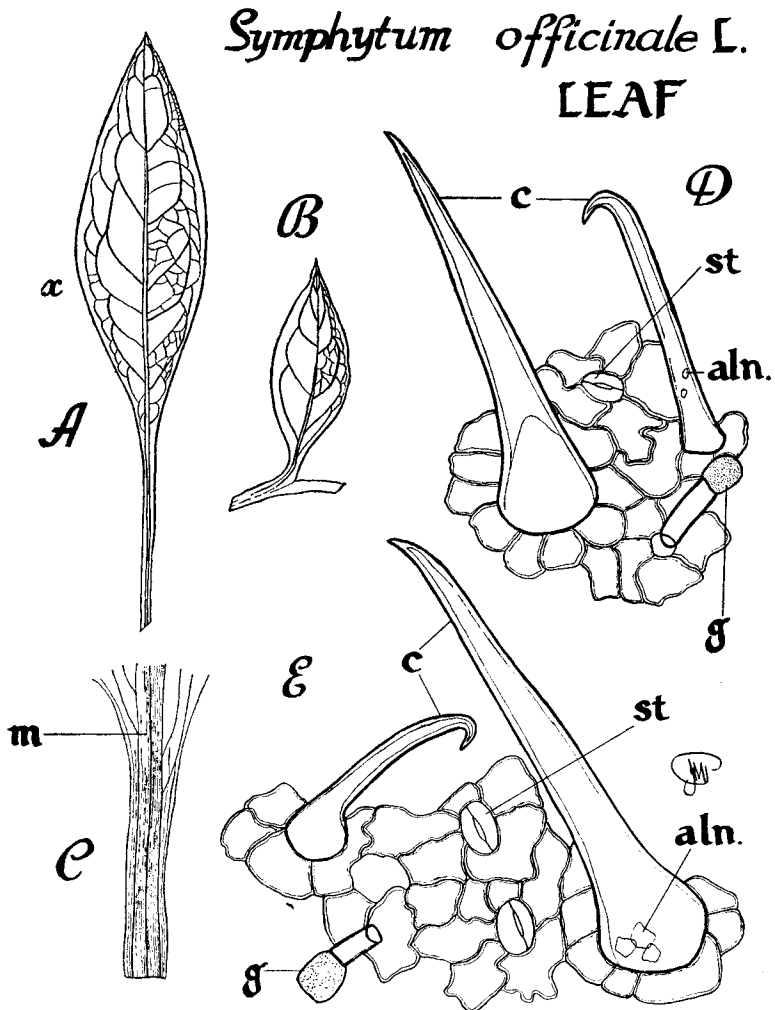


FIG. 1. Leaf of *Symphytum officinale* L. *A*, complete lower petiolate leaf. *B*, Complete upper sessile leaf. *C*, enlargement of petiole, showing decurrent lamina and lateral veins. *D*, upper epidermis of lamina. *E*, lower epidermis of lamina. *A* $\times \frac{1}{4}$; *B* $\times \frac{1}{4}$; *C* $\times 1$; *D* and *E* $\times 200$. aln., allantoin crystals; c, covering trichome; g, glandular trichome; m, midrib; st, stoma. x, position at which transverse section illustrated by Fig. 2, *A*, was made.

LEAF OF *SYMPHYTUM OFFICINALE* L.

were obtained. The mucilage obtained by precipitation was washed with water to free it from the salt; treatment with ruthenium red and methylene blue gave positive results, whereas corallin soda and 0.02 N iodine gave negative reactions.

MACROSCOPY

Symphytum officinale L. is an erect hispid perennial, producing erect stems 2–3 ft. in height; both cauline and radical leaves are present. The plant grows in damp places especially near rivers and streams.

The leaf is simple, broadly lanceolate in shape, about 10–20 cm. in length and 3–4 cm. wide, with an acute apex and an entire or slightly wavy margin. The lateral veins anastomose towards the margin of the leaf; this feature shows more prominently on the under surface (Fig. 1, A).

The lower leaves are petiolate. The petiole is winged and the lateral veins of the leaf run parallel with the midrib, as far as the junction of the petiole with the stem (Fig. 1 A and C).

The upper leaves are sessile, the base being decurrent with the stem. They are similar in shape and features to the lower leaves, but are about half their size (Fig. 1, B).

The leaves have a brownish-green colour, no characteristic odour, a slightly astringent taste and the surface is hispid.

ANATOMICAL STRUCTURE

LEAVES

Lamina, interneural region (Fig. 1, D and E; Fig. 2, B)

The UPPER EPIDERMIS is covered with a thin smooth cuticle and consists of one layer of polygonal cells having sinuous anticlinal walls. They measure about *H 22 μ and Lev L and B 36 μ to 94 μ . *Stomata* occur fairly frequently and are of the anisocytic type. They are level with the epidermis, circular to oval in outline and have a prominent central pore. The circular stomata are about 25 μ in diameter and the oval ones about 25 μ to 36 μ long, 14 μ to 25 μ wide (Fig. 1, D; Fig. 2, B). *Hydathodes* are absent, mucilage is present in the cells, also numerous crystals of allantoin which are irregularly shaped but measure about 5 μ in any diameter (Fig. 2, B).

Covering trichomes are numerous, may be of two types and arise from both veins and interneural epidermises. The more common type has unicellular, straight, thick, cellulose walls, tapering to an acute apex. The lumen is visible throughout the entire length; the base is swollen and often contains several crystals of allantoin. The trichomes measure about 365 μ to 880 μ in length and 43 μ to 90 μ wide at the base, which

* The symbols H, Lev, Lev L and Lev B are suggested for the purpose of describing organs showing bilateral symmetry by Moll and Janssonius (1923a). The symbol H = height in a direction perpendicular to the surface of the organ; Lev = in the direction of the surface of the organ; Lev L and Lev B = parallel to the surface at the same time in a longitudinal or transverse direction respectively.

is surrounded by about 8 to 12 small epidermal cells (Fig. 1, *D*; Fig. 5). The second type of covering trichome which is characteristically hooked occurs infrequently and may even be absent. They are unicellular, straight except for the hooked acute apex and have thick cellulose walls. They measure about 115 μ to 240 μ in length and 29 μ to 36 μ wide at

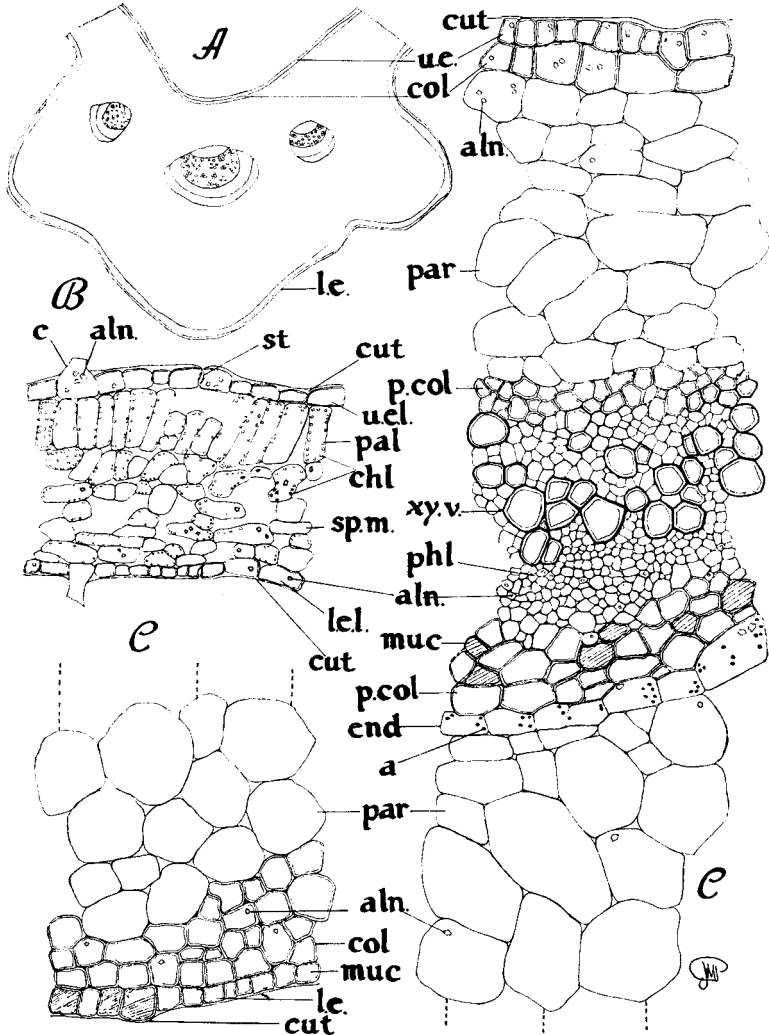


FIG. 2. Leaf of *Symphytum officinale* L. *A*, transverse section of midrib, cut at position *x* (see Fig. 1, *A*). *B*, transverse section of lamina, interneural region. *C*, central region of *A*. *A* $\times 25$; *B* and *C* $\times 200$. *a*, starch; aln., allantoin crystals; *c*, covering trichome; chl, chloroplast; col, collenchyma; cut, cuticle; end, endodermis; l.e., lower epidermis; l.e.l. lower epidermis lamina; muc, mucilage; pal, palisade; par, parenchyma; p.col, pericyclic collenchyma; phl, phloem; sp.m., spongy mesophyll; st, stoma; u.e., upper epidermis; u.e.l., upper epidermis of lamina; xy.v., xylem vessel.

LEAF OF *SYMPHYTUM OFFICINALE* L.

the base; the base is swollen and may contain crystals of allantoin (Fig. 1, D).

Glandular trichomes are also present, but are less numerous than the covering type. They consist of a unicellular stalk, and a unicellular, sub-spherical glandular head. They are about $57\ \mu$ to $90\ \mu$ long and the head has a diameter of about $21\ \mu$ to $36\ \mu$ and occur on both veins and interneural epidermis (Fig. 1, D).

The MESOPHYLL is clearly differentiated. The *palisade* consists of a single layer of cells, which is not continuous across the midrib. The individual cells are cylindrical and measure about H $36\ \mu$ to $61\ \mu$, Lev L and B $11\ \mu$ to $22\ \mu$ and contain chloroplasts (Fig. 2, B). Immediately below the palisade are four to six rows of *spongy mesophyll*. They are loosely arranged, are both rounded and elongated in surface view and measure about H $14\ \mu$ to $29\ \mu$ and Lev L and B $22\ \mu$ to $47\ \mu$. They contain chloroplasts, and many crystals of allantoin (Fig. 2, B; Fig. 3, A).

The LOWER EPIDERMIS also has a thin smooth cuticle. Its cells measure about H $11\ \mu$ to $18\ \mu$ and Lev L and B $18\ \mu$ to $43\ \mu$. The anticlinal walls are more strongly wavy than those of the cells of the upper epidermis. *Stomata* are very numerous, of the anisocytic type and oval in shape. They measure about $22\ \mu$ to $26\ \mu$ in length and $18\ \mu$ to $25\ \mu$ wide (Fig. 1, E). Mucilage and numerous crystals of allantoin are present in these cells (Fig. 2, B).

Covering trichomes are numerous and are similar to those present on the upper interneural epidermis. The straight conical covering trichomes on the lower surface are shorter than those of similar type on the upper surface. They measure about $190\ \mu$ to $560\ \mu$ in length and $36\ \mu$ to $90\ \mu$ wide at the base (Fig. 1, E). The hooked covering trichomes are similar in character to those on the upper interneural surface, but are more numerous (Fig. 1, E).

Glandular trichomes are rare, occasional ones are found on the veins; they have similar structures to those found on the upper interneural epidermis (Fig. 1, E).

Midrib (Fig. 2, A and C; Fig. 3, B to F)

The midrib has a typically dicotyledonous structure, the only variation being the number of individual bundles present in transverse sections from apex to the base of the leaf. Serial sections showed that one bundle occurs at the apex and about 12 at the base, this increase being due to the entrance of the successive lateral veins into the central area of the meristele.

The UPPER EPIDERMIS is covered by a thin cuticle and consists of a single layer of straight walled, elongated polygonal cells measuring about H $25\ \mu$ to $50\ \mu$, Lev B $25\ \mu$ to $50\ \mu$ and Lev L $65\ \mu$ to $180\ \mu$. Numerous crystals of allantoin are present in the cells, also mucilage (Fig. 2, C; Fig. 3, B).

Stomata, when present, measure about $28\ \mu$ to $33\ \mu$ in length and $25\ \mu$ to $28\ \mu$ wide, but were absent in about one-half of the specimens examined.

Covering trichomes are fairly frequent and similar in character to those of the upper interneural epidermis. Hooked trichomes are either absent or infrequent, and are similar to those on the upper interneural epidermis.

Glandular trichomes are numerous, measuring about $79\ \mu$ to $115\ \mu$ in length. They consist of a unicellular or occasionally bicellular stalk and a unicellular subspherical glandular head about $25\ \mu$ to $32\ \mu$ in diameter (Fig. 3, B and D).

The LOWER EPIDERMIS has a thin, smooth cuticle and consists of straight walled, elongated polygonal cells measuring about H $11\ \mu$ to $36\ \mu$, Lev B $11\ \mu$ to $36\ \mu$ and Lev L $75\ \mu$ to $180\ \mu$. Numerous crystals of allantoin and mucilage are present in the cells of this layer (Fig. 2, C; Fig. 3, C).

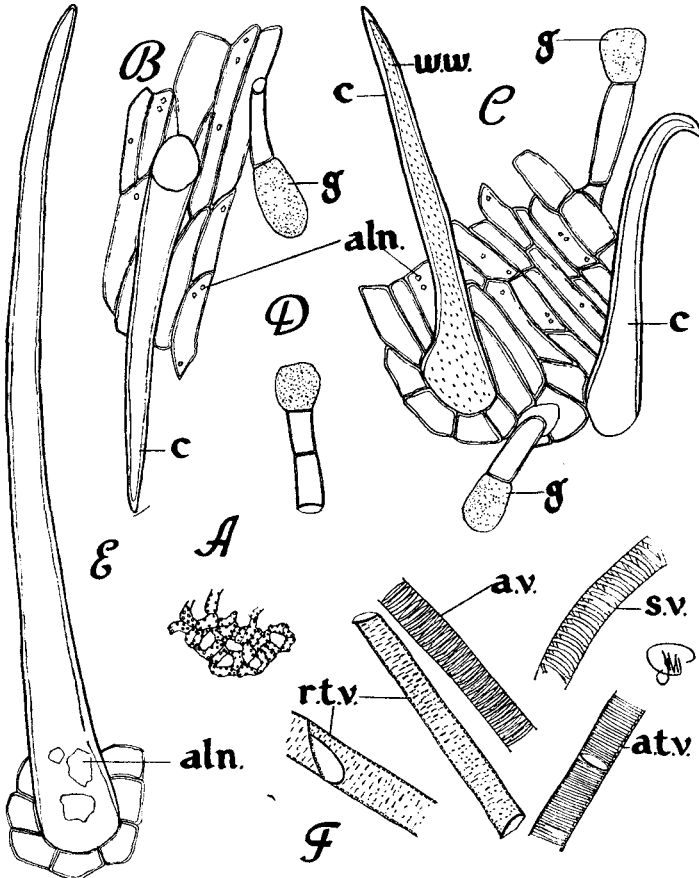


FIG. 3. Leaf of *Symphytum officinale* L. A, spongy mesophyll in surface view. B, upper epidermis of midrib. C, lower epidermis of midrib. D, glandular trichome with a bicellular stalk from upper epidermis of midrib. E, covering trichome from lower epidermis of midrib. F, isolated elements obtained by maceration of the midrib. A, B, C, D and F $\times 200$; E $\times 100$. aln., allantoin crystals; a.t.v., annular tracheidal vessel; a.v., annular vessel; c, covering trichome; g, glandular trichome; r.t.v., pitted tracheidal vessel; s.v., spiral vessel; w.w., warty wall.

LEAF OF *SYMPHYTUM OFFICINALE* L.

Covering trichomes are frequent, similar in character to those of the lower interneural epidermis, but much larger. They measure about $468\ \mu$ to $1,140\ \mu$ in length and $54\ \mu$ to $108\ \mu$ wide at the base. Their walls are cellulosic in nature, staining blue with 0.02 N iodine followed by 66 per cent w/w sulphuric acid. Some trichomes have acute apices, others have blunt ones; the walls of some trichomes are warty. Crystals of allantoin may be present in the base of these trichomes (Fig. 3, C and E). Hooked covering trichomes occur in varying frequency; they are similar in structure to those on the upper epidermis of the interneural lamina (Fig. 3, C).

Glandular trichomes are frequent and are larger than those on the upper midrib epidermis. They consist of a bicellular stalk, the lower cell being much smaller than the upper, and a unicellular sub-spherical glandular head. They measure about $94\ \mu$ to $162\ \mu$ in length and the glandular head is about $25\ \mu$ to $36\ \mu$ in diameter (Fig. 3, C). Glandular trichomes of a similar type to those on the upper interneural epidermis are also present.

Stomata, similar in structure to those on the upper epidermis of the midrib, occur.

The CORTEX contains one or two rows of hypodermal *collenchyma* below the upper epidermis and one to three rows above the lower epidermis. Chloroplasts are absent, but occasional crystals of allantoin are present. The remainder of the cortex is parenchymatous (Fig. 2, C, Fig. 3).

An *endodermis* (or starch sheath) is present and is approximately horse-shoe shaped in transverse section. It consists of one layer of cells containing minute starch grains, immediately adjacent to the pericyclic region. No other layer contains starch grains (Fig. 2, C). Despite the fact that the layer does not completely surround the meristele, such an arrangement may be regarded as an endodermis (cf. the endodermis of *Althaea* (Moll and Janssonius, 1923b).

The MERISTELE is round to oval in shape and well defined in transverse section (Fig. 2, A).

The PERICYCLE consists of a well-defined area of *collenchyma* above the xylem and below the phloem. Most of the cells contain mucilage (Fig. 2, C).

The PHLOEM is composed of sieve tissue and parenchyma. The individual sieve-tube elements measure about $100\ \mu$ to $200\ \mu$ in length and about $10\ \mu$ in diameter, with transverse sieve plates. The companion cells are narrow, but quite well defined in longitudinal section. The medullary rays are not clearly defined. Numerous crystals of allantoin are present in this region (Fig. 2, C).

The XYLEM consists of vessels and tracheidal vessels occurring singly or in small groups. The vessels show annular and spiral thickening and measure about $15\ \mu$ to $30\ \mu$ in diameter. The tracheidal vessels have annular and pitted thickening. There is also a large amount of lignified parenchyma (Fig. 2, C; Fig. 3, F).

Lateral veins exhibit similar anatomy to that of the midrib.

PETIOLE

The petiole is about 6 to 10 cm. long and 4 to 6 mm. wide; the lamina is decurrent on both sides throughout its entire length (Fig. 1, A and C). A transverse section through the petiole shows an arc of up to 12 bundles, some of which have arisen by the anastomosis of two to four small bundles from the lateral veins in the leaf. These run parallel with the mid-rib in the central stele. Only two or three small veins run down either side of the decurrent lamina, again parallel with the midrib.

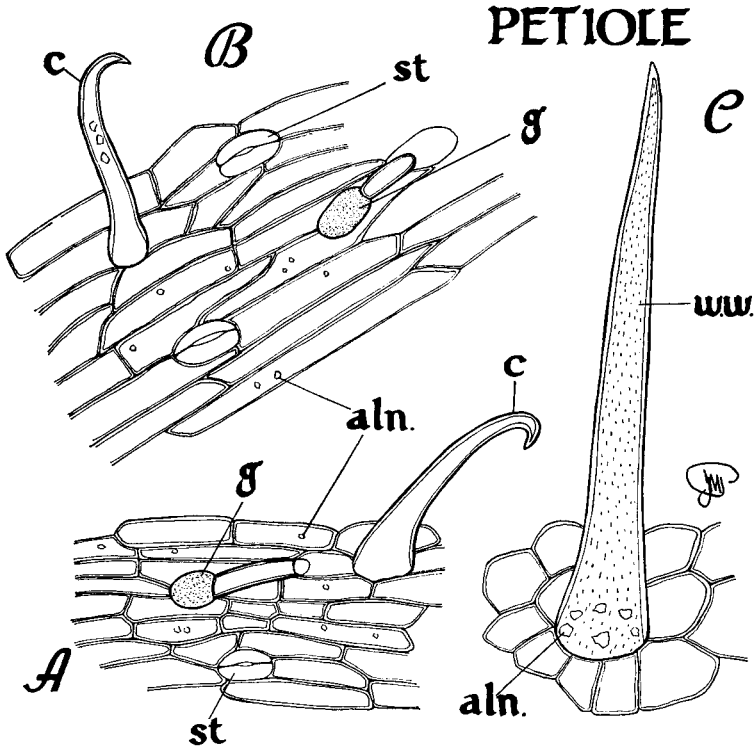


FIG. 4. Petiole of *Symphytum officinale* L. A, upper epidermis of petiole. B, lower epidermis of petiole. C, covering trichome from either lower or upper epidermis of petiole. A and B $\times 200$. C $\times 100$. aln., allantoïn crystals; c, covering trichome; g, glandular trichome; st, stoma; w.w., warty wall.

The UPPER EPIDERMIS has cells possessing a similar structure to those of the mid-rib epidermis. They contain mucilage and crystals of allantoïn (Fig. 4, A). Stomata normally occur quite frequently; they measure about 36μ to 43μ in length and are orientated in the direction of the long axis of the epidermal cells (Fig. 4, A). Covering trichomes are frequent and are of two types. Trichomes, similar to the straight covering ones present on the upper epidermis of the mid-rib, occur, but are much longer; they measure about 396μ to $1,584 \mu$ in length and

LEAF OF *SYMPHYTUM OFFICINALE* L.

about $72\ \mu$ to $127\ \mu$ wide at the base, which is usually swollen and surrounded by 8 to 12 small epidermal cells. Some trichomes have warty walls (Fig. 4, C). The hooked covering trichomes may also be present but their occurrence is variable; they are similar in dimensions to those on the mid-rib epidermis (Fig. 4, A).

Glandular trichomes are frequent on this surface and measure about $90\ \mu$ to $137\ \mu$ in length. They consist of a unicellular or occasionally bicellular stalk and have a unicellular, subspherical glandular head measuring about $22\ \mu$ to $36\ \mu$ (Fig. 4, A).

The LOWER EPIDERMIS exhibits a thin, smooth cuticle, and is composed of polygonal, elongated straight-walled cells, measuring about H $18\ \mu$ to $33\ \mu$, Lev B $14\ \mu$ to $33\ \mu$ and Lev L $97\ \mu$ to $396\ \mu$; they contain mucilage and crystals of allantoin (Fig. 4, B). *Stomata* occur frequently and are orientated in the direction of the epidermal cells; they are oval in shape and measure about $36\ \mu$ to $47\ \mu$ in length and $22\ \mu$ to $29\ \mu$ in diameter (Fig. 4, B).

Covering trichomes are frequent and are of two types, both types being equally abundant. The straight type are similar to those present on the lower epidermis of the mid-rib, some trichomes have warty walls. They have thick cellulose walls and an acute apex, and measure about $162\ \mu$ to $1,620\ \mu$ in length and about $43\ \mu$ to $151\ \mu$ at the base, which is usually swollen and may contain crystals of allantoin (Fig. 4, B). The second type of covering trichome is hooked, similar to those present on the lower epidermis of the mid-rib, but are longer and measure about $162\ \mu$ to $378\ \mu$ in length and $25\ \mu$ to $58\ \mu$ wide at the base (Fig. 4, B).

A transverse section through the petiole is similar to that through the mid-rib.

The CORTEX is composed of *collenchyma* and *parenchyma*; below the upper epidermis are two or three layers and above the lower epidermis are three or four layers of cortical collenchyma. The remainder of the cortex is parenchymatous.

Each bundle has a horse-shoe shaped endodermis immediately adjacent to the lower pericyclic region. The cells are parenchymatous and contain minute starch grains.

The PERICYCLE consists of a well defined area of collenchyma below the phloem and above the xylem.

The PHLOEM is similar in structure to that of the mid-rib.

The XYLEM consists of single or small groups of vessels and tracheidal vessels; there are no distinct medullary rays. The vessels show annular and spiral thickening, but the tracheidal vessels are almost entirely reticulately thickened. There is a large amount of unligified xylem parenchyma.

Calcium oxalate crystals are absent, and the presence of calcareous or siliceous deposits could not be demonstrated in any trichome.

POWDER

A No. 44 powder has a green colour, no odour and only a slightly astringent taste.

The powder was examined using the following reagents: dilute glycerol, chloral hydrate solution, phloroglucin and concentrated hydrochloric acid, chloral iodine, ruthenium red and methylene blue. The diagnostic features (Fig. 5) are:—

Large numbers of *covering trichomes*, either in whole or broken condition, or still attached to pieces of epidermis. Fragments up to about

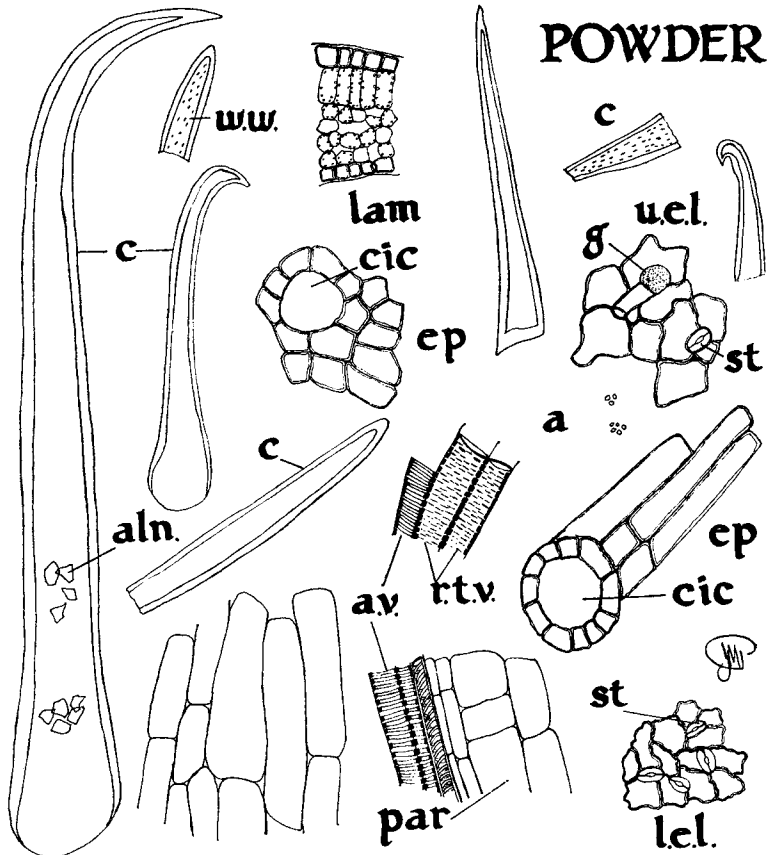


FIG. 5. Powder of *Symphytum officinale* L. All $\times 100$. a, starch; aln., allantoin crystals; a.v., annular vessel; c, covering trichome; cic, cicatrix; ep, epidermis; g, glandular trichome; lam, lamina; l.e.l., lower epidermis lamina; par, parenchyma; r.t.v., pitted tracheidal vessel; st, stoma; u.e.l., upper epidermis lamina; w.w., warty wall.

80 μ wide, of straight unicellular trichomes with acute apices, with either thick smooth or warty cellulose walls; also fragments up to 60 μ wide, of unicellular trichomes with cellulose walls and hooked, acutely pointed apices. Both types may occur on any epidermal surface. Fragments of the *upper interneural epidermis* in surface view show cells with sinuous anticlinal walls occasionally with *anisocytic stomata*, also covering and *glandular trichomes*, with a unicellular stalk and unicellular sub-spherical

LEAF OF *SYMPHYTUM OFFICINALE* L.

head; also fragments of *lower interneural epidermis* exhibit strongly wavy-walled cells with numerous anisocytic stomata and covering trichomes. Portions of *transverse sections of lamina* up to 100 μ wide, showing a single palisade and several layers of spongy mesophyll, both containing chloroplasts. Epidermal fragments of the midrib and petiole occur frequently, often showing large cicatrices. Fragments of vessels and tracheidal vessels are lignified and exhibit annular and pitted thickening. Occasional starch grains from the endodermis, allantoin crystals in the base of trichomes and mucilage from epidermal and pericyclic regions are also present. Calcium oxalate and sclerenchyma are absent.

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