THE MORPHOLOGY AND ANATOMY OF THE LEAF OF *PODOPHYLLUM HEXANDRUM* ROYLE

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A brief review of the history and medicinal uses of *Podophyllum hexandrum* Royle (= *P. emodi* Wall.) is given, together with an illustrated account of the macroscopy and anatomical structure of the leaf. The diagnostic characters of the powdered leaves are recorded and illustrated.

Podophyllum hexandrum Royle, although esteemed by the natives of India for its "bile expelling properties", was not introduced into Western Medicine until the late nineteenth century. Dunstan and Henry (1898), Thompson (1890) and Umney (1892) had reported a resin content equal to, or greater than, that of the American plant, *P. peltatum* L., and Hooper (1913) recommended the cultivation of *P. hexandrum* in India in view of its inclusion in the *Indian and Colonial Addendum to the British Pharmacopoeia* (1900). In recent years, interest in podophyllum as a purgative and cholagogue has declined although it still occurs as an active ingredient in Compound Tablets of Colocynth and Jalap, B.P.C. and various proprietary "liver pills". During this same period, interest has grown because of its reported activity as a mitotic poison and carcinoclastic, and from Kaplan (1942), who pioneered the use of podophyllin in condyloma acuminata, to Kern and Franger (1950), who investigated its use in cutaneous carcinoma, numerous successful treatments of dermatological conditions have been reported and reviewed by Nelson (1953). Further clinical applications have been reviewed by Kelly and Hartwell (1954).

Hussain, Chandri, Muhammad and Wahhab (1954) reported on the resin content of various plant organs of *P. hexandrum*, and recorded a resin content of 7–9 per cent w/w for the leaves, and indicated that they may serve as a more economic source of the resin than the rhizome and root. We have made preliminary estimations of the podophyllin content of the leaves of *P. hexandrum* grown in England and Norway, by the method of the *British Pharmaceutical Codex* (1959) modified so that the final purification by filtration and solution in alcohol was replaced by solution of the precipitated resin in chloroform and treatment with dilute ammonia solution to separate the resin from the chlorophyll—the resin was obtained by evaporation of the ammoniacal solution.

The present investigation was undertaken to describe the anatomical structure of the leaf and to note the diagnostic characters necessary for the identification of whole or powdered leaf and to distinguish it from the leaf of *P. peltatum*, to be described later.

MATERIAL

All the material was supplied as *P. emodi* Wall. but the literature suggests that *P. emodi* and *P. hexandrum* Royle are synonymous and comparison

with material at the Natural History Museum, London, S.W.7, confirmed its identity as *P. hexandrum* Royle. The leaves were obtained from three sources: (*a*) various nurserymen supplied plants which were transplanted to a shady position in a light peaty soil in West Yorkshire; (*b*) the botanical garden of Bergen University and (*c*) plants grown by Dr. T. E. Wallis at Mill Hill, London.

Methods of Investigation

Chloral hydrate solution was used to prepare fragments for examination of epidermises, but no heating was necessary with material which had been previously stored in 60 per cent ethanol and the epidermis of the petiole was best mounted directly in 50 per cent v/v glycerol solution. Serial sections were prepared from material embedded in polyethylene glycols 4,000, 20 per cent and 1,500 GEN, 80 per cent w/w (Fell and Rowson, 1955) and were mounted in 50 per cent v/v glycerol solution. Fresh material was used in attempts to locate the resin, which is soluble in ethanol and in polyethylene glycols. Phloroglucinol and hydrochloric acid were used to detect lignified material and chloral iodine and 0·02N iodine solution to establish the presence of starch in the endodermis. The aceto-carmine staining technique (Brown, 1951) was used to establish the presence of chromosome material in the commonly occurring granular bodies and hence to confirm their identity as nuclei. The transient bright orange-red colour produced by 50 per cent v/v nitric acid with the resinous material was characteristic. The extracted resin and commercial podophyllin behaved similarly.

Macroscopy

The characters of the leaf of *P. hexandrum* vary according to the age of the plant. The cotyledons, which are epigeal (Fig. 1, *A–F*) and persist until the first-year leaf emerges through the cotyledonary stalk, are fused along their bases and are continuous with the cotyledonary stalk. The plumule emerges from a slit at one side of the base of the cotyledonary stalk and stands opposite to the cotyledons. There is no hypocotyl, and, since the cotyledons may, in these circumstances, be regarded as a combined "cotyledon", *P. hexandrum* provides a good example of pseudo-monocotyledonous germination, similar to certain other species within the Berberidaceae and Ranunculaceae (McLean and Ivimey-Cook, 1956). The cotyledons are ovate, with a single main vein running from the stalk to the emarginate apex and anastomosing secondary veins (Fig. 1, *C*). The first-year leaf has three broadly ovate, or obovate, irregular lobes with five main veins emerging from the petiole apex and anastomosing secondary veins. The margin is irregularly serrate (Fig. 1, *D*). The leaves fall each winter; the leaves in the succeeding two years differ from their predecessors only in their increased size. Growth proceeds sympodially, and the flowering shoot is produced in the fourth or fifth year, emerging enclosed in a sheath which persists for several weeks. On bursting it reveals a terminal flower-bud surmounting two coiled leaves (Fig. 1, *B*). The pedicel arises from the petiole of one leaf, or less frequently from the junction of the two petioles, but the leaves do not

uncoil until the petals have fallen. Only the flowering shoot has two leaves on a common stalk, all other leaves occur singly and are radical. The leaves of the flowering shoot are often more divided than the radical ones and the lobes are more lanceolate (Fig. 1, *A*). Both types of leaf are mottled red-brown and green, the amount of red decreasing as the leaf matures.

The mature leaves of *P. hexandrum* measure from 12 to 25 cm. across and are peltate with an eccentric, erect, cylindrical petiole. They are pentagonal in outline, the lamina being palmatisect with 3 to 5 lobes.

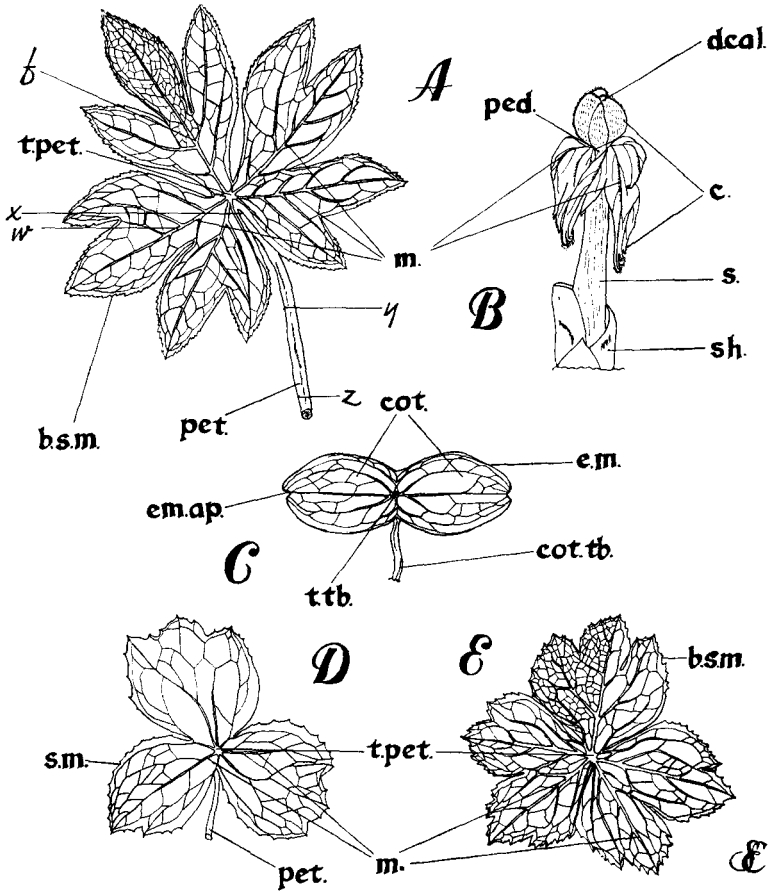

FIG. 1. Leaf of *Podophyllum hexandrum* Royle. *A*, complete mature leaf from flowering plant; *B*, flowering shoot; *C*, young seedling, showing fused cotyledons; *D*, first year, immature leaf; *E*, mature leaf from non-flowering plant. *A* and *E* × 1/5; *B* and *D*, × ½; *C* × ⅔. *b.s.m.*, biserrate margin; *c.*, covering trichome; *cot.*, cotyledon; *cot. tb.*, cotyledonary tube; *d. cal.*, deciduous calyx; *e.m.*, entire margin; *em ap.*, emarginate apex; *m.*, main vein; *ped.*, pedicel; *pet.*, petiole; *s.*, stem; *sh.*, sheath; *s.m.*, serrate margin; *t. pet.*, top of petiole; *t. tb.*, top of cotyledonary tube; w, position of sections 3, *A*, and 4, *A*; x, y and z, position of sections 4*B–D*; f, position of first main vascular junction.

Each lobe is subdivided, the lateral ones into two and the terminal ones into three ovate, or ovate-lanceolate, segments; the margin is entire in the lower half of the lobes and serrate, or irregularly biserrate, in the apical half. The venation is palmate with five main veins arising from the apex of the petiole and running to the acute tip of each main lobe. The secondary veins branch from them alternately at an acute angle and run towards the tip of the lobe segments. In each case, at the conjunction of the two lobes, or segments, a single vein, arising in the case of the lobes from the apex of the petiole and in the case of the segments from the main veins, divides and its branches run along the margins of the two lobes, or segments, formed (Fig. 1, *A* and *E*). The tertiary veins leave the secondary veins at a wide, or even obtuse, angle and terminate near the margin. In each marginal tooth three ultimate veinlets unite to form the terminal network (Fig. 2, *A*). The veins are prominent on the under-surface, are lighter in colour than the interneural tissue and are covered with long, silky, covering trichomes. In some leaves similar trichomes are visible along the margin (Fig. 2, *B*, *F* and *G*). The interneural tissue is glabrous except near the larger veins.

Anatomical Structure

Lamina

Interneural Tissue (Fig. 2, *B*, *C*, *E*, *H* and *I*; Fig. 3, *B*)

The Upper Epidermis is covered with a relatively thin cuticle which is smooth over most of the surface of the leaf but slightly granular at the margin. The epidermal cells are polygonal and measure about Lev L and B 37 to **47** to 71 μ and H 26 to **33** to 45 μ with wavy anticlinal walls; in occasional cells, red-brown pigment occurs (Fig. 2, *E*; Fig. 3, *B*). *Trichomes* are absent from this surface and *stomata* are very rare. Upon each marginal tooth 4 to 10 *hydathodes* are often present; they measure about Lev L 48 to **57** to 78 μ and Lev B 32 to **39** to 45 μ (Fig. 2, *A* and *C*).

Mesophyll. The *palisade* consists of one layer of regular cylindrical cells measuring about Lev L and B 15 to **26** to 60 μ and H 44 to **53** to 75μ, and a second layer of shorter cylindrical cells measuring about Lev L and B 22 to **38** to 67 μ and H 25 to **45** to 63μ, which is discontinuous at irregular intervals. The *spongy mesophyll* consists of 5 or 6 layers of trabeculate parenchyma cells about 18 to **41** to 74 μ in diameter, with occasional transversely elongated cells, measuring about 14 to **41** to 74 μ in diameter and 59 to **91** to 126 μ in length with large intercellular spaces (Fig. 2, *H*; Fig. 3, *B*). *Calcium oxalate* crystals occur rarely; they measured from 14 to 22 μ in diameter and were located in the spongy mesophyll, always very close to a vein.

The Lower Epidermis is covered by a thin cuticle, striated in small patches near some stomata (Fig. 2, *I*). The epidermal cells are polygonal, measuring about Lev L and B 30 to **55** to 94 μ and H 15 to **25** to 38 μ, with very wavy anticlinal walls. Anomocytic *stomata* are numerous, raised slightly above the level of the epidermis, elliptical, or circular, in outline, and measure about 35 to **45** to 52 μ in length and 29 to **37** to 45 μ in breadth, or 35 to **39** to 45 μ in diameter (Fig. 2, *I*; Fig. 3, *B*).

Covering trichomes occur on this surface over, and near, the veins and in the marginal region. They are thin-walled, cellulosic and unicellular in both regions with a blunt and rounded apex. Near the veins, the trichomes are of one type only with a smooth cuticle and measuring about

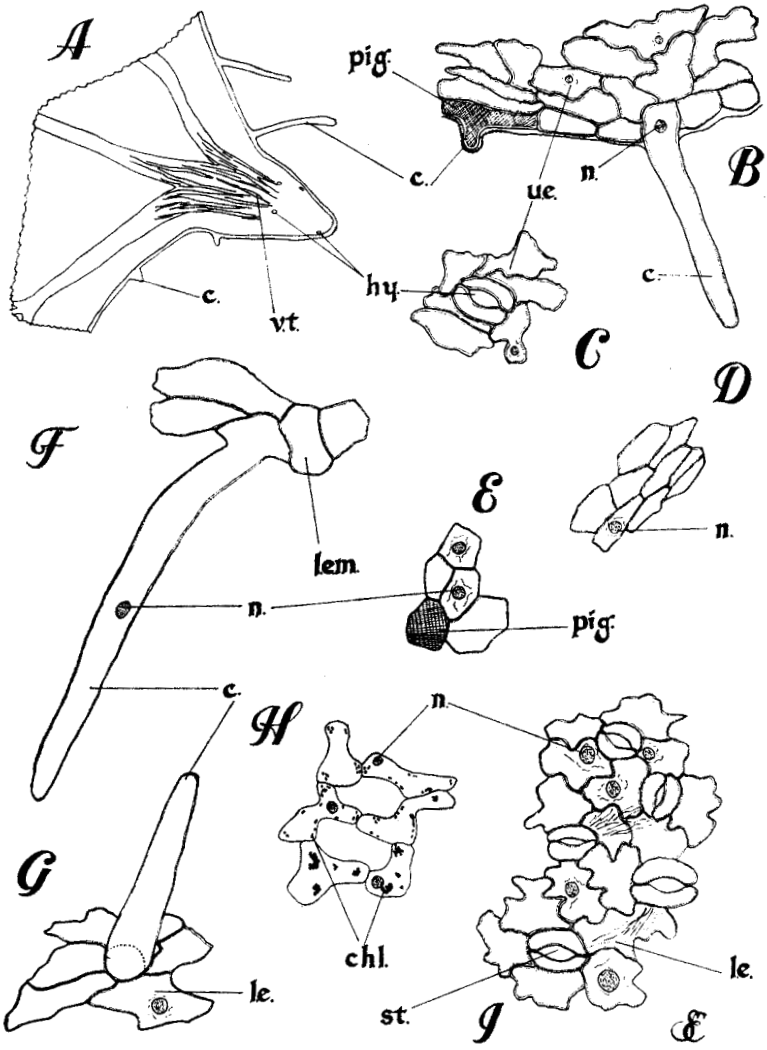

FIG. 2. Leaf of *P. hexandrum* Royle. *A*, marginal tooth of leaf; *B*, upper epidermis of leaf, marginal region; *C*. upper epidermis of leaf with hydathode; *D*, upper epidermis of main vein; *E*. upper epidermis of lamina; *F*, lower epidermis of main vein; *G*, lower epidermis of lamina, neural region; *H*, spongy mesophyll; *I*, lower epidermis of lamina, interneural region; *A* × 30; *B–I*, × 150. *c.*, covering trichrome; *chl.*, chloroplast; *hy.*, hydathode; *l.e.*, lower epidermis; *l.e.m.*, lower epidermis of main vein; *n.*, nucleus; *pig.*, pigment, *st.*, stoma; *u.e.*, upper epidermis; *v.t.*, veinlet termination.

150 to **425** to 825 μ in length and about 22 to **40** to 60 μ in diameter at the base. In the marginal region the number of trichomes varies, being very rare in some leaves and forming a dense covering in other specimens examined. The majority resemble those of the neural region in appearance (Fig. 2, *B*), but are slightly smaller, being about 150 to **272** to 520 μ in length and 26 to **35** to 53 μ in diameter at the base. In addition, a small number of shorter trichomes with a slightly warty wall occur near the teeth (Fig. 2, *A* and *B*). These are of the same width but are only about 20 to **36** to 55 μ in length.

The lamina has a serrate margin, individual teeth being acutely pointed. The tertiary veins extend to within about 0·3 mm. of the teeth apices and terminate in several small spiral elements. Three ultimate veinlets unite to form this terminal network about 0·6 mm. from the apex (Fig. 2, *A*).

MAIN VEIN (Fig. 2, *D* and *F*; Fig. 3, *A* and *C*; Fig. 4, *A*)

The leaf, which is deeply lobed, has palmate venation and hence the following description applies to the central vein of each lobe, which runs from the petiole to the apex (Fig. 1, *A*). The veins from each lobe, from several leaves, were examined and showed no significant variation from the following description. The transverse section shows three unequal bundles embedded in a central, cordate shaped mass of collenchyma (Fig. 4, *A*). The extreme tip of each leaf segment shows one bundle only, where a side vein enters there are two bundles, and there are three, 2 cm. from the tip. The amount of central collenchyma increases proportionately. At the first main junction (Fig. 1, point *f*), the main vein contains three bundles and side vein two. These unite to produce five bundles which are gradually rearranged to form three once more. The entrance of further secondary veins disturbs the pattern temporarily but it always returns to three. No unification of the bundles of the primary veins occur before they enter the petiole.

The UPPER EPIDERMIS is composed of polygonal, straight-walled cells, elongated along the axis of the lobe (Fig. 2, *D*). They measure about Lev L 36 to **74** to 104 μ, Lev B 12 to **33** to 56 μ and H 20 to **36** to 60 μ. The cuticle is thicker than on the interneural tissue; *trichomes* and *stomata* are absent.

The CORTEX is divided into two main regions. The upper hypodermal region is composed of thick-walled collenchyma, the cells measuring about L 100 to **187** to 260 μ and R and T 20 to **29** to 45 μ, but the lower hypodermal region becomes collenchymatous only in the basal half of the lobe, where it is large-celled, measuring about L 75 to **135** to 225 μ and R and T 26 to **63** to 108 μ. The remaining cortex is parenchymatous, the cells measuring about L 92 to **142** to 203 μ, R and T 8 to **15** to 24 μ above the stele and about L 80 to **154** to 240 μ, R and T 12 to **40** to 75 μ below the stele. The outer cortical cells contain a few elliptical chloroplasts, similar to those of the interneural tissue, and rare rosette crystals of *calcium oxalate*; only four of these were measured, the diameters were between 30 and 45 μ (Fig. 3, *A*).

The *endodermis* is not well differentiated, but a continuous band of

cells, which contain starch grains measuring about 4 to 6 to 12 μ in diameter, surrounds the collenchyma of the pericycle. The cells do not differ in size, shape or structure of the cell wall, from the surrounding cortical parenchyma, but in view of their contents may be regarded as a starch sheath (Jackson, 1953).

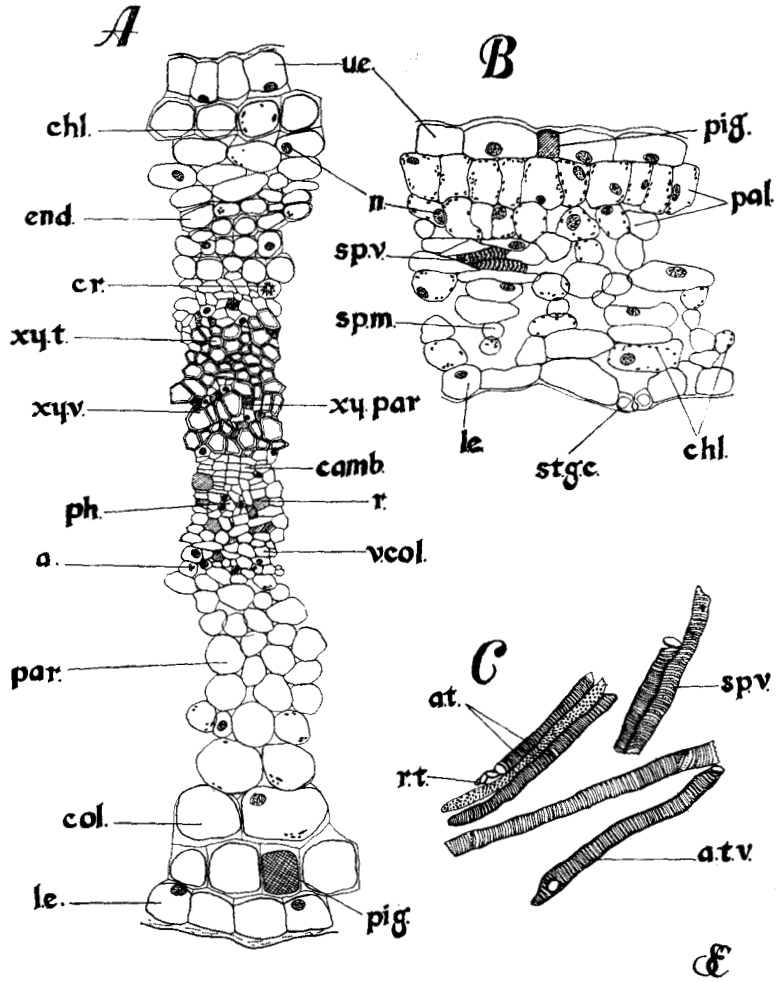

FIG. 3. Leaf of *P. hexandrum* Royle. *A*, tranverse section of the main vein cut at position *w* (see Fig. 1, *A*); *B*, transverse section of the lamina, interneural region; *C*, isolated elements obtained by maceration. *A* and *B*, × 150; *C* × 75. *a.*, starch; *a.t.*, annular tracheid; *a.t.v.*, annular tracheidal vessel; *camb.*, cambium; *chl.*, chloroplast; *col.*, collenchyma; *cr.*, crystal of calcium oxalate; *end.*, endodermis; *l.e.*, lower epidermis; *n.*, nucleus; *pal.*, palisade; *par.*, parenchyma; *ph.*, phloem; *pig.*, pigment; *r.*, resinous material; *r.t.*, reticulate tracheid; *sp.m.*, spongy mesophyll; *sp.v.*, spiral vessel; *st.g.c.*, stoma guard cell; *u.e.*, upper epidermis; *v. col.*, collenchyma; *xy.par.*, xylem parenchyma; *xy.t.*, xylem tracheid; *xy.v.*, xylem vascular vessel.

The MERISTELE consists of three well-defined bundles embedded in a mass of relatively thin-walled collenchyma, measuring about L 135 to **186** to 265 μ and R and T 18 to **33** to 52 μ; occasional cells contain brown contents, which do not stain with 0·02N iodine solution or 5 per cent w/v ferric chloride solution, but give a reddish-brown colour with 50 per cent v/v nitric acid (Fig. 3, *A*).

The *phloem* consists of sieve tissue with well-defined companion cells and small-celled phloem parenchyma. The sieve-tubes measure about R and T 11 to **25** to 45 μ, the individual segments being about L 90 to **150** to 200 μ with transverse or oblique sieve plates. The companion cells are narrow, being about R and T 2 to 7·5 to 18 μ and L 96 to **148** to 207 μ and usually the nuclei completely fill the width of the cells. There are two distinct medullary rays between the three bundles, composed of cellulosic parenchyma; there is no radial arrangement of cells within the bundles. Patches of phloem parenchyma, the cells measuring about L 77 to **153** to 222 μ and R and T 5 to **15** to 30 μ with brown cell contents occur which stains red with 50 per cent v/v nitric acid and orange with 0·02N iodine solution.

The *cambium* consists of a well-defined layer of thin-walled, tangentially elongated cells.

The *xylem* consists of irregularly arranged vessels and tracheids with patches of cellulosic xylem parenchyma (Fig. 3, *A*). The vessels are lignified with spiral or annular thickening and a diameter of about 5 to **12** to 22 μ. The tracheids and tracheidal vessels are similar, having spiral, annular or pitted thickening and a diameter of about 5 to **12** to 20 μ. The tracheids measure about L 120 to **280** to 430 μ and the tracheidal vessels about L 240 to **390** to 585 μ (Fig. 3, *C*); the number of tracheids increases towards the tip. The xylem parenchyma is thin-walled, cellulosic and some cells have similar brown contents to the phloem parenchyma.

The LOWER EPIDERMIS (Fig. 2, *F*; Fig. 3, *A*) is composed of large polygonal cells about Lev L 60 to **82** to 120 μ, Lev B 16 to **37** to 80 μ and H 28 to **41** to 60 μ, with slightly wavy anticlinal walls. *Stomata* are absent but numerous unicellular *covering trichomes* occur. They measure about 150 to **425** to 825 μ in length and 22 to **40** to 60 μ in diameter at the base. The walls are thin and cellulosic, and the trichomes have a blunt and rounded apex (Fig. 2, *F* and *G*).

PETIOLE (Fig. 4, *B*, *C* and *D*; Figs. 5 and 6)

The petiole is smoothly cylindrical, from 10 to 35 cm. long and from 3 to 5 mm. in diameter and sometimes showing an eccentric hollow in the basal portion of the petiole of older leaves. The vascular tissue occurs in two regions: (1) An outer ring of 15 to 25 bundles situated near the periphery and containing pericyclic fibres, phloem, cambium and xylem. Each is encircled by a mass of collenchyma bounded on its outer edge by a starch sheath. At the upper end of the petiole pericyclic fibres are few, or occasionally absent, but the amount of lignified material gradually increases towards the base and near the junction of the petiole with the stem, or rhizome, the interfascicular parenchyma in the pericyclic region

becomes lignified forming a complete ring of lignified tissue. (2) A V-shaped arrangement of larger bundles asymmetrically placed in the pith parenchyma. These bundles contain smaller numbers of pericyclic fibres, phloem, cambium and xylem all enclosed by an area of collenchyma and a starch sheath. The amount of fibrous tissue increases towards the base, but the interfascicular parenchyma does not become lignified. In the hollow petioles this V-shaped structure persists as a projection into the hollow. In some longer petioles this second arrangement of bundles tends to become circular giving two rings of bundles, the inner bundle ring

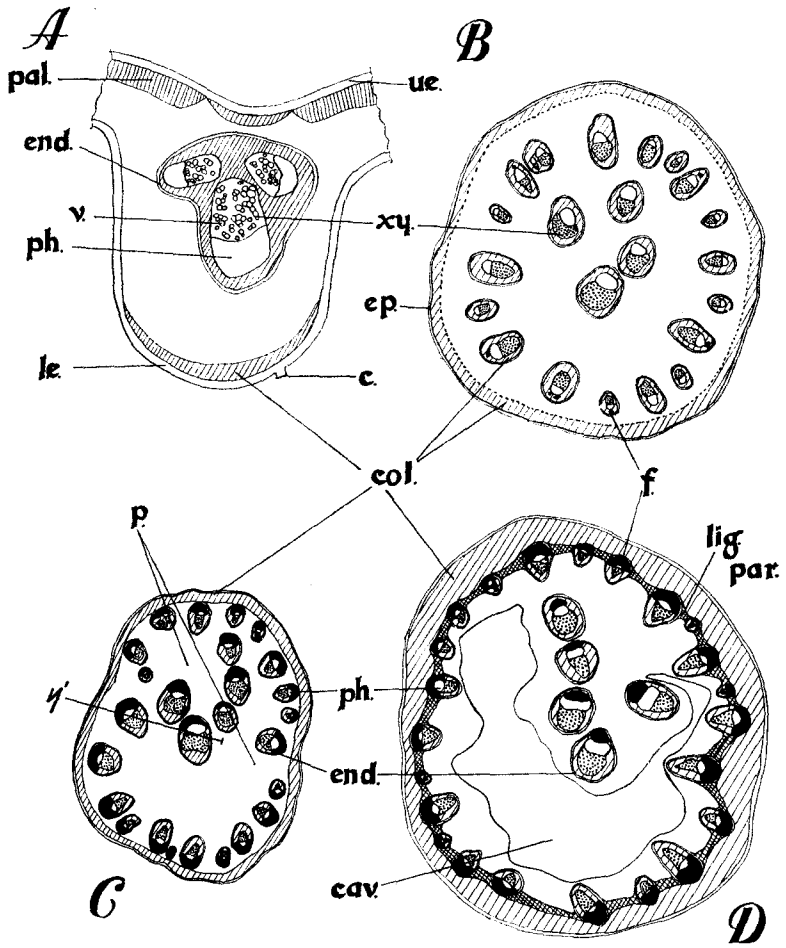

FIG. 4. Leaf and petiole of *P. hexandrum* Royle. *A*, transverse section of main vein cut at position w (see Fig. 1, *A*); *B–D*, transverse sections of petiole cut at positions *x*, *y* and *z* respectively (see Fig. 1, *A*). *A*, × 30; *B–D*, × 10. *c.*, base of covering trichome; *cav.*, cavity; *col.*, collenchyma; *end.*, endodermis; *ep.*, epidermis; *f.*, fibres; *l.e.*, lower epidermis; *lig.par.*, lignified parenchyma; *p.*, pith; *pal.*, palisade; *ph.*, phloem; *u.e.*, upper epidermis; *v.*, vessel; *xy.*, xylem; y', position and extent of section 6.

being eccentric (Fig. 4, *B*, *C* and *D*). This arrangement of the vascular system shows some affinities with some species of the Ranunculaceae (cf. *Glaucidium*, Metcalfe and Chalk, 1957); Kumazawa (1930) has suggested

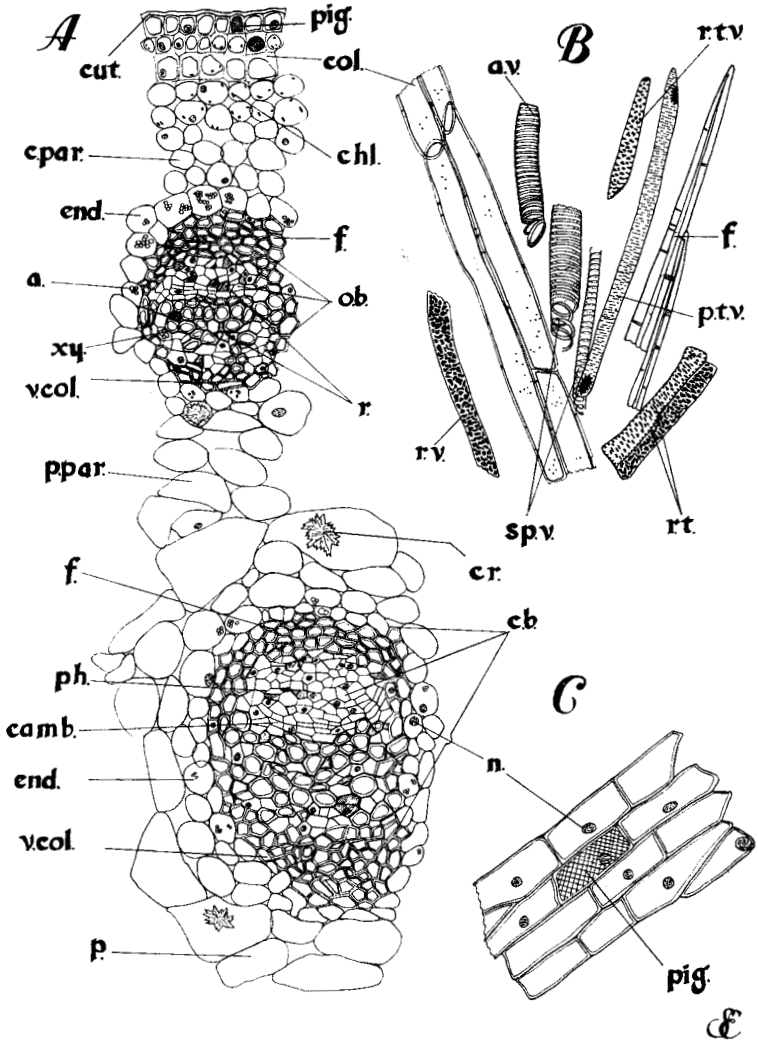

FIG. 5. Petiole of *P. hexandrum* Royle. *A*, transverse section of petiole cut at position *y* (see Fig. 1, *A*) showing an outer bundle and a central bundle; *B*, isolated elements obtained by maceration; *C*, epidermis of petiole. *A* and *C*, × 130; *B*, × 70. *a.*, starch; *a.v.*, annular vessels; *camb.*, cambium; *c.b.*, central bundle; *chl.*, chloroplasts; *col.*, collenchyma; *c.par.*, cortical parenchyma; *cr.*, crystal of calcium oxalate; *cut.*, cuticle; *end.*, endodermis; *f.*, fibre; *n.*, nucleus; *o.b.* outer bundle; *p.*, pith; *ph.*, phloem; *pig.*, pigment; *p.par.*, pith parenchyma; *p.t.v.*, pitted tracheidal vessel; *r.*, resinous material; *r.t.*, reticulate tracheid; *r.t.v.*, reticulate tracheidal vessel; *r.v.*, reticulate vessel; *sp.v.*, spiral vessel; *v.col.*, vascular collenchyma; *xy.*, xylem.

on anatomical grounds that certain genera of Berberidaceae and Ranunculaceae should be removed from their respective families and merged into a single family, the Podophyllaceae.

Tracing the entry of the primary leaf veins into the petiole shows that each vein divides on entry, part of the vascular tissue forming bundles in the outer ring and part entering the central bundles.

The EPIDERMIS consists of straight-walled cells, elongated longitudinally and measuring about Lev L 80 to **172** to 280 μ, Lev B 13 to **19** to 34 μ. The cell walls are thin and cellulosic and the outer surface is covered by a thin cuticle (Fig. 5, *C*).

The CORTEX, like that of the main vein, consists of two layers of tissue. The outermost layer is a band of collenchyma, several cells wide, with very thick walls. The cells measure about L 100 to **204** to 480 μ and R and T 11 to **23** to 37 μ and contain lenticular chloroplasts. The remaining cortex is parenchymatous, frequently becoming lignified and pitted in the pericyclic region of the lower part of the petiole. The cells are similar in size to those of the collenchyma (Fig. 5, *B*).

The *endodermis* is even less distinct than in the main veins as the starch is not confined to a single layer but forms a sheath 2 or 3 cells wide around the collenchyma. The starch grains average about 6 μ in diameter.

PERICYCLIC FIBRES increase in number from apex to base of the petiole, and eventually, as seen in a transverse section, form a crescent-shaped group external to the phloem, extending on either side of the phloem tissue to the cambium. The fibres are extremely long, measuring about L 690 to **1,030** to 1,400 μ and R and T 3 to **11** to 19 μ, with thick, lignified, pitted walls and acute apices (Fig. 5, *B*; Fig. 6).

The *vascular bundles* of the outer and inner rings differ only slightly, the main difference being that the inner bundles are larger but have fewer pericyclic fibres. Both types of bundle are embedded in collenchyma, the cells of which measure about L 156 to **433** to 759 μ and R and T 7 to **19** to 34 μ. The *phloem* consists of groups of sieve tubes about 11 to **18** to 22 μ in diameter, each individual segment being about 160 to **285** to 340 μ long, associated with narrow companion cells measuring about 3 to **7** to 15 μ in diameter and 248 to **309** to 407 μ in length. There is no radial arrangement of cells within the bundles, but irregular groups of phloem parenchyma occur, the individual cells measuring about L 110 to **235** to 335 μ and R and T 11 to **22** to 37 μ, and frequently containing brown amorphous contents similar to those of the main vein. The *xylem* is well defined, the elements being irregularly arranged. The conducting elements resemble those of the main vein with a higher proportion of vessels to tracheids and some reticulate thickening (Fig. 5, *A* and *B*; Fig. 6). A little xylem parenchyma occurs close to the distinct cambium and associated with the larger vessels; some cells of the parenchyma contain brown cell-contents similar to those already described. The vessels are somewhat larger than those of the main veins, being about 7 to **26** to 60 μ in diameter. The tracheids and tracheidal vessels have diameters within this range and measure about 207 to **418** to 700 μ in length.

The PITH is composed of large-celled parenchyma, which frequently

breaks down to form an eccentric hollow at the base of the petiole. The cells are thin-walled and measure about L 110 to 275 to 420 μ and R and T 35 to 82 to 152 μ. Occasional simple starch grains measuring about 5 to 15 to 26 μ in diameter occur in the cells. In cells adjacent to the vascular bundles rosette crystals of *calcium oxalate*, measuring about 14 to 26 to 35 μ in diameter, occur (Fig. 5, *A*; Fig. 6).

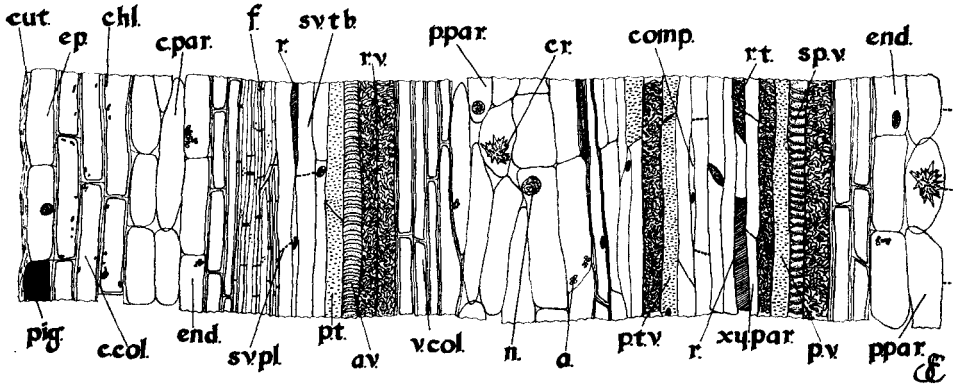

FIG. 6. Petiole of *P. hexandrum* Royle. Longitudinal section of the petiole cut at position *y'* (see Fig. 4, *C*) × 150. *a.*, starch; *a.v.*, annular vessel; *chl.*, chloroplast; *c.col.*, cortical collenchyma; *comp.*, companion cell; *c.par.*, cortical parenchyma; *cr.*, crystal of calcium oxalate; *cut.*, cuticle; *end.*, endodermis; *ep.*, epidermis; *f.*, fibre; *n.*, nucleus; *pig.*, pigment; *p.par.*, pith parenchyma; *p.t.*, pitted tracheid; *p.t.v.*, pitted tracheidal vessel; *p.v.*, pitted vessel; *r.*, resinous material; *r.t.*, reticulate tracheidal vessel; *r.v.*, reticulate vessel; *sp.v.*, spiral vessel; *sv.pl.* sieve plate; *sv. tb.*, sieve tube; *v. col.*, vascular collenchyma; *xy. par.*, xylem parenchyma.

POWDER

The colour of a No. 40 powder varied from yellowish-green to dark-green, but all had a heavy odour and bitter taste. When a small amount of the powder is mixed with a 5 per cent w/v solution of ferric chloride a gradual darkening of the particles of tissue is observed and when mixed with a 5 per cent w/v solution of copper acetate an emerald-green colour gradually develops on the particles.

To examine the structural features of the powder mounts were prepared using 50 per cent v/v glycerol solution, solution of chloral hydrate or phloroglucinol and hydrochloric acid. The diagnostic characters of the Powder (Fig. 7) are:

1. Numerous whole and fragmented unicellular *covering trichomes*, from the lower epidermis of the lamina, with thin cellulosic walls and blunt and rounded apices.

2. Fragments showing, in surface view, the sinuous walled cells of the *upper interneural epidermis* and usually the underlying palisade and, in the marginal region, infrequent covering trichomes.

3. Fragments of the *lower interneural epidermis* showing, in surface view, the wavy-walled epidermal cells and anomocytic *stomata*.

4. Less frequent particles showing the straight-walled cells of the *upper epidermis of the main vein* or the elongated straight-walled cells of the *lower epidermis of the main vein*, with numerous trichomes or cicatrices.

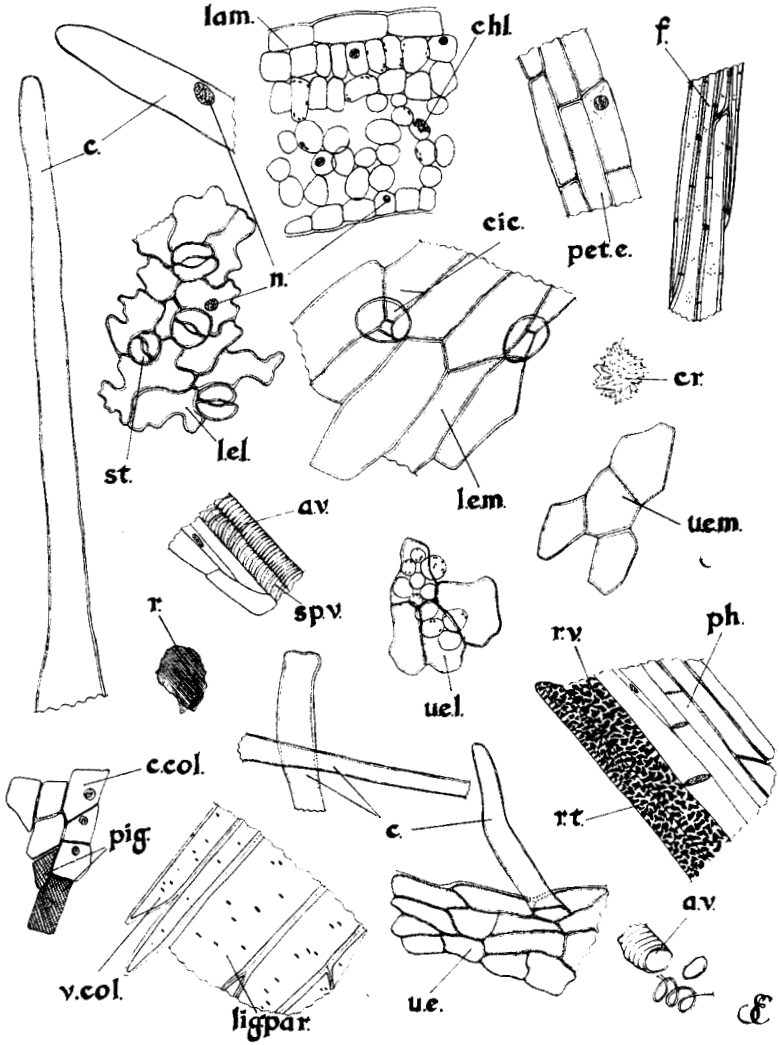

FIG. 7. Powdered leaf and petiole of *P. hexandrum* Royle. All × 150. *a.v.*, annular vessel; *c.*, covering trichome; *c.col.*, cortical collenchyma; *chl.*, chloroplast; *cic.*, cicatrix; *cr.*, crystal of calcium oxalate; *f.*, fibre; *lam.*, lamina; *l.e.l.*, lower epidermis of lamina; *l.e.m.*, lower epidermis of main vein; *lig. par.*, lignified parenchyma; *n.*, nucleus; *pet.e.*, petiole epidermis; *ph.*, phloem; *pig.*, pigment; *r.*, resinous material; *r.t.*, reticulate tracheid; *r.v.*, reticulate vessel; *sp.v.*, spiral vessel; *st.*, stoma; *u.e.*, upper epidermis; *u.e.l.*, upper epidermis of lamina; *u.e.m.*, upper epidermis of main vein; *v.col.*, vascular collenchyma.

5. Fragments of lamina in transverse sectional view, about 200 μ wide with a double, or single, palisade.

6. Small spiral and annular vessels from the veins; reticulate vessels associated with large sieve-tubes, and large annular and spiral vessels from the petiole.

7. Lignified, acutely pointed *fibres* with pitted walls; collenchyma with simple pits associated with *lignified parenchyma* from the inter-fascicular region of the petiole.

8. Cortical collenchyma from the main veins or petiole, with thick cellulosic walls and frequently containing nuclei and red pigment.

9. Infrequent cluster crystals of *calcium oxalate* from main veins or petiole, and occasionally from the spongy mesophyll.

10. Occasional brown *resinous fragments*, non-cellular in nature, which gradually dissolve to give a green solution in 5 per cent w/v copper acetate solution and an orange-red colour with 50 per cent v/v nitric acid.

Proportion of petiole. Investigations on samples available showed that the percentage of petiole present was about 14 to 22 per cent by weight of the dry leaf.

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