ANATOMICAL STUDIES IN THE GENUS *RUBUS*

PART I. THE ANATOMY OF THE LEAF OF *Rubus idæus* L.

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THE gross morphology of the leaves of the raspberry plant, *Rubus idæus* L., Family Rosaceæ, has been described in several standard botanical works[1–3] and in certain textbooks of pharmacognosy[4–6]. These descriptions provide sufficient information for the identification of the entire drug. Much of the material now supplied commercially is, however, in the chopped condition or in very coarse powder. At present, only relatively meagre information is available concerning the microscopical structure of the leaflets; a brief and partially illustrated account is given by Brandt and Wasicky in Thoms' Handbuch[5], and by Jane Béguin[7]; these are summarised in the monograph upon Raspberry Leaf in the British Pharmaceutical Codex, 1949[8]. These works provide no description or illustrations of the rachis and stipules. Moreover, since recent work[9–11] has shown that the leaf possesses pharmacological activity, it seemed desirable to present a detailed and illustrated anatomical description of the leaf, in order to show those characters by which the genuine drug can be recognised and distinguished from the leaves of related plants, such as the blackberry and loganberry.

MATERIAL

The material used throughout this present work consisted of the leaves obtained from stands of *Rubus idæus* growing wild in woodlands of Boxhill, Surrey. Further material, propagated vegetatively from the Boxhill wild plants, was obtained from the Museum Experimental Gardens at Mayfield, near Ashbourne, Derbyshire. All plants used possessed the characters typical of the species.

METHODS OF INVESTIGATION

The structure of the two epidermises was difficult to observe in preparations made by heating pieces of the lamina in chloral hydrate solution. This was due firstly to the dark colour of the leaflets and secondly to the dense tomentum of trichomes on the lower epidermis. Portions of the leaflets were bleached by macerating them overnight in Dakin's Solution (Surgical Solution of Chlorinated Soda, B.P.), after first shaving off the trichomes of the lower epidermis, and then mounted in 50 per cent. v/v glycerol. The walls of the epidermal cells were more clearly defined when mounted in glycerol than in chloral hydrate solution. The glandular trichomes, however, were not properly expanded or cleared by the dilute glycerol and for their examination a chloral mount was necessary.

Three methods of preparation of the material for sectioning were

investigated. The first, paraffin embedding as modified by Johansen[12], was considered unsatisfactory, because of shrinkage and distortion of cells. The bulk of the sections prepared for the purpose of this investigation were made by a second method, using polyethylene glycol. This method has been described in a separate communication by ourselves[13], and is satisfactory for sections of 2 μ in thickness and upwards. It is also superior in that virtually no shrinkage occurs in the cells during preparation[13–15]. The third method, carbon dioxide freezing, was also found satisfactory for sections of 8 μ and over in thickness. Thinner sections usually broke and it became almost impossible to remove them intact from the microtome knife. In the carbon dioxide freezing method very small oblong pieces were cut from the lamina, one-third of the way along the midrib from the base, the midrib lying in the median line. These pieces were first soaked in water for four hours, to remove the fixing fluid. The Reichert sledge microtome with freezing stage, freezing cap and knife cooler was employed. The microtome knife was of wedge section and used in the position at right-angles to the carriage-slide. A square of blotting paper of 6 mm. edge was placed in the centre of the freezing stage, and a single, large drop of Mucilage of Acacia, B.P., was placed on top of the blotting paper; the water-soaked piece of lamina was held vertically in the drop of mucilage by means of forceps, and carbon dioxide was admitted in spurts until the whole was frozen satisfactorily. By adjusting the orientation of the material on the freezing stage, both transverse and longitudinal sections were cut as required and both temporary and permanent mounts were prepared. Temporary preparations were conveniently made by mounting at once in 50 per cent. v/v glycerol, avoiding the use of chloral hydrate solution, which led to disintegration of the phloem and other delicate tissues.

Permanent mounts were made by transferring the sections, immediately after cutting, to a dish containing saturated crystal violet solution; after immersion in this stain for about 25 minutes they were passed successively through 25, 50, 75 and 90 per cent. ethanol, 2 minutes being allowed in each, and then counterstained in a solution of Bismarck brown (1 per cent. in 95 per cent. ethanol) for 3 minutes. Final dehydration was effected in absolute ethanol, followed by clearing in clove oil; the latter was removed by xylene, and the sections were mounted in Canada balsam. Safranin and fast green, also safranin and light green were unsuitable for making permanently mounted, double-stained preparations of this material, as it proved impossible to differentiate the safranin.

Sections made by the freezing process were also mounted directly in 50 per cent. v/v glycerol and, like those made by the use of polyethylene glycol, showed no distortion of the cells. The permanent double-stained mounts prepared as described above showed very little shrinkage of the cells, due presumably to the much reduced exposure to ethanol compared with that required by paraffin embedding. Sections made by polyethylene glycol embedding were usually stained with hæmatoxylin or other simple stains to increase the contrast[13], thus enabling the drawings to be made more easily by means of an Abbé camera lucida, or by projection.

Macerates of prickles and rachis were prepared by using Schultz's maceration fluid.

Anatomical Structure

The leaf of *Rubus idæus* is imparipinnately compound, and may consist of three to seven leaflets, the paired lateral leaflets being sessile on the

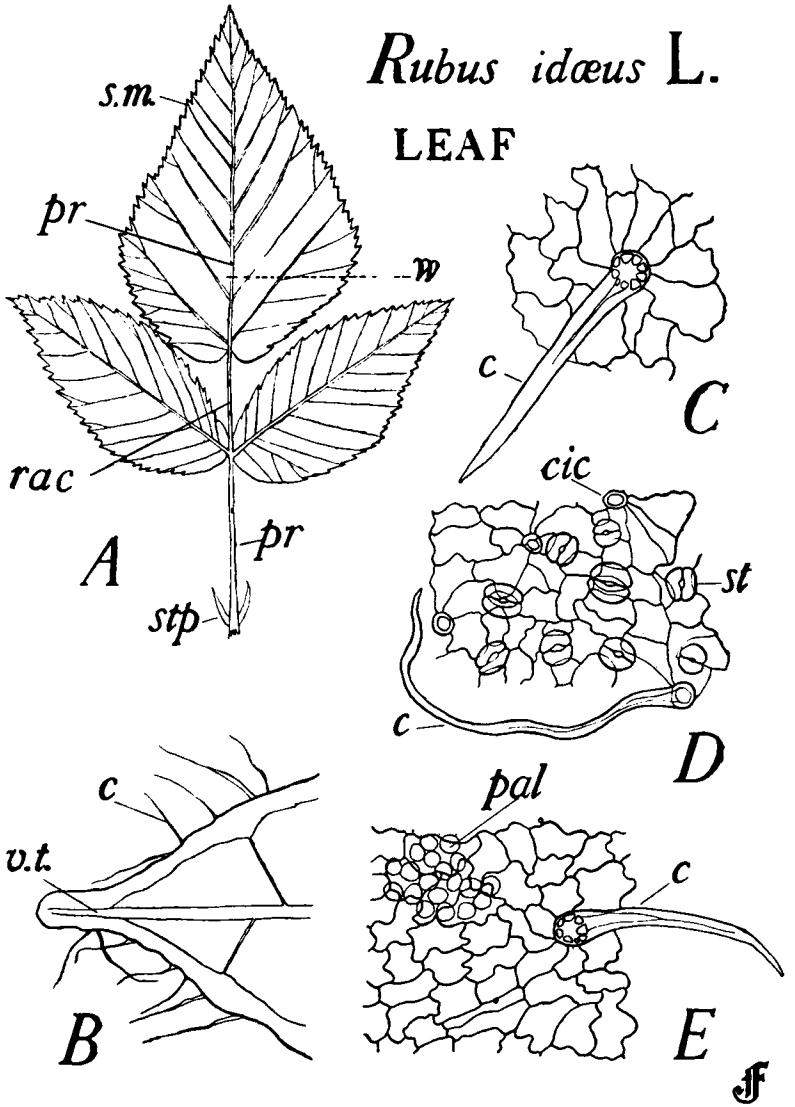

FIG. 1. Leaf of *Rubus idæus* L. *A*, complete compound leaf with terminal and two lateral leaflets and paired stipules adnate to the rachis. *B*, marginal tooth of leaflet. *C*, upper epidermis of leaflet with stiff, covering trichome. *D*, lower epidermis of leaflet. *E*, upper epidermis of leaflet. *A*, × $\frac{1}{3}$; *B*, × 25; *C*, *D* and *E*, × 200. *c*, covering trichome; *cic*, cicatrix; *pal*, palisade; *pr*, prickle; *rac*, rachis; *s.m.* serrate margin; *st*, stoma; *stp*, stipule; *v.t.*, vein termination; *w*, position at which transverse section illustrated by Fig. 2, *A* was made.

rachis. Paired stipules are adnate at the lower end of the rachis (Figs. 1, A, 3, A, and 4, A). Although the monograph of the B.P.C., 1949[8], includes only leaflets in its definition, 5 per cent. of rachis and stalk is allowed; moreover, most commercial material at present available consists of the chopped leaves, and this investigation describes, therefore, the anatomy of *a*, leaflets, *b*, rachis and *c*, stipules.

(*a*) LEAFLETS

Many leaflets, both terminal and lateral, were examined, and no anatomical differences were detected between them, or between leaflets on the non-fruiting canes and those of the fruiting canes of the following year. The following anatomical description, therefore, applies to any of these leaflets.

(*i*) *Lamina, interneural region* (Fig. 2, A and C)

The UPPER EPIDERMIS is covered with a relatively thick, smooth cuticle, and consists of a layer of polygonal cells having slightly wavy anticlinal walls (Fig. 1, E); they measure about H 8 to 20 μ, and Lev L and B 10 to 30 μ*; stomata are absent; frequent *covering trichomes* occur, about 200 to 500 μ long, and about 18 to 35 μ wide at their bases. They are unicellular, with thick, lignified walls, tapering and acutely pointed, with heavily thickened bases frequently exhibiting linear pits. The lumen is wide at the base, but it narrows towards the apex of the trichome, becoming obliterated in the upper half. The bases of the trichomes are usually surrounded by about 8 radiating epidermal cells (Fig. 1, C). The spiral markings reported by other authors[6,7] were only indistinctly seen on some of the trichomes on the upper surface of the lamina. They were observed more clearly on the trichomes of older leaves after prolonged boiling with chloral hydrate solution, and on the trichomes of the stipules—*vide infra*.

The MESOPHYLL is clearly differentiated; the *palisade* consists of a single layer of cells, frequently becoming doubled near the midrib. Individual cells are cylindrical, moderately elongated, measure about H 28 to 40 μ, Lev 5 to 14 μ and contain numerous chloroplasts, about 1 to 4 μ in diameter. Scattered irregularly in the palisade are large, rounded idioblasts, each containing a cluster or rosette crystal of *calcium oxalate* about 8 to 30 μ in diameter. The *spongy mesophyll* consists of 2 to 4 layers of rounded or elongated cells about H 3 to 15 μ, Lev L and B 4 to 18 μ, also containing numerous chloroplasts, about 1 to 4 μ in diameter.

The LOWER EPIDERMIS has a smooth, thin cuticle. Its cells measure about H 7 to 20 μ, Lev L and B 9 to 38 μ, their anticlinal walls are slightly more wavy than those of the upper epidermis (Fig. 1, D); *stomata* are numerous, are of the anomocytic (ranunculaceous) type, and are usually surrounded by radiating epidermal cells; they are slightly raised above the level of the epidermis and vary in outline from nearly circular to

* The symbols H, Lev, Lev L and Lev B are suggested for the purpose of describing organs showing bilateral symmetry by Moll and Janssonius[17]. 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 and at the same time in a longitudinal or transverse direction respectively.

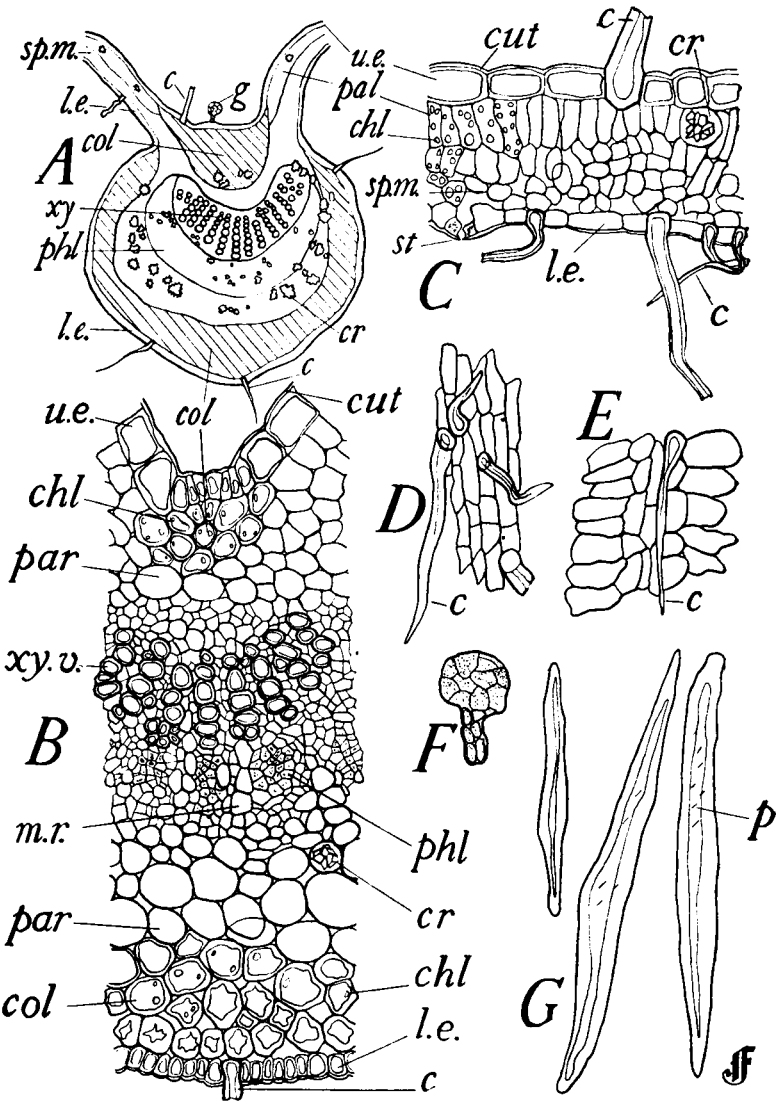

FIG. 2. Leaflet of *Rubus idæus* L. *A*, transverse section of midrib of terminal leaflet, cut at the position *w* (see Fig. 1, *A*). *B*, central region of Fig. *A*. *C*, transverse section of lamina, interneural region. *D*, lower epidermis of midrib. *E*, upper epidermis of midrib. *F*, glandular trichome from upper epidermis of midrib. *G*, isolated sclereids of prickle. A, × 45; B–G × 200. *c*, covering trichome; *chl*, chloroplast; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *g*, glandular trichome; *l.e.*, lower epidermis; *m.r.*, medullary ray; *p*, pit; *pal*, palisade; *par*, parenchyma; *phl*, phloem; *sp.m.*, spongy mesophyll; *st*, stoma; *u.e.*, upper epidermis; *xy*, xylem; *xy.v.*, xylem vessel.

elliptical. The circular stomata are about 16 to 20 μ in diameter and the elliptical ones are about 12 to 18 μ wide and 18 to 22 μ long. Long *covering trichomes* are present in very great numbers, forming a tomentum

or "felt" by their mutual intertwining; they are unicellular, about 150 to 500 μ long and 6 to 18 μ wide at the base; their walls are thickened but unlignified, remaining unstained in mounts of phloroglucin and hydrochloric acid or of aniline sulphate, and the lumen is not usually so extensively obliterated as in the case of the covering of hairs of the upper epidermis; they are curled, tapering with blunt apices and thickened but smooth bases (Figs. 1, D, and 4, F).

The lamina has a serrate margin, the teeth being acutely pointed. The minute ends of the secondary and tertiary veins, which extend to within about 60 μ of the teeth apices, terminate in a few very small, spiral vessels. Two fine veinlets, one on either side, converge towards the central veinlet and unite with it about 0·3 mm. from the tip of the tooth (Fig. 1, A and B).

(*ii*) *Midrib* (Fig. 2, A and B)

Histologically, the midrib shows a typically dicotyledonous structure, moreover, no significant variation was noted in transverse sections cut at twelve different positions between base and apex of the leaflet.

The UPPER EPIDERMIS consists of a single layer of elongated, straight-walled cells measuring about H 15 to 20 μ, Lev B 5 to 12 μ and Lev L 20 to 40 μ; they are heavily cuticularised. *Covering trichomes* are present in moderate number and are similar in character to those of the upper interneural epidermis (Fig. 2, B and E). Occasional *glandular trichomes* about 65 to 110 μ long occur on the upper epidermis of the midrib and to a lesser extent on the upper epidermis of main lateral veins; they comprise a multiseriate or biseriate, multicellular stalk, about 3 to 6 cells long, frequently with granular contents, and a subspherical, multicellular, glandular head about 36 to 54 μ in diameter (Fig. 2, F).

The LOWER EPIDERMIS consists of small, longitudinally elongated straight-walled cells measuring about H 6 to 18 μ, Lev B 5 to 10 μ and Lev L 30 to 52 μ. *Covering trichomes* arise frequently, similar in character to those of the upper interneural epidermis (Fig. 2, B and D).

Laterally compressed, conical or comma-shaped *prickles* are commonly found on the lower surface of the midrib. These consist of elongated, interlocking sclerotic cells measuring about 50 to 160 μ long and 10 to 38 μ wide, which possess lignified, much thickened walls, traversed by occasional oblique and linear pits. Towards the apex of the prickle, they have pointed ends and some cells have granular contents. The lumen may be wide or narrow (Figs. 1, A, 2, G, and 4, F and G).

The CORTEX contains abundant supporting hypodermal *collenchyma* arranged in several rows towards both surfaces of the midrib, that towards the lower surface being greater in extent. These cells are heavily thickened in the angles, and measure about L 40 to 110 μ, R and T 8 to 28 μ; chloroplasts are commonly present, measuring about 2 to 4 μ in diameter. The interior of the cortex is of *parenchyma*; individual cells measure about L 56 to 106 μ, R and T 10 to 38 μ. Idioblasts occur fairly frequently in this tissue, each containing a large cluster or rosette crystal of *calcium oxalate*, about 12 to 45 μ in diameter (Fig. 2, A and B).

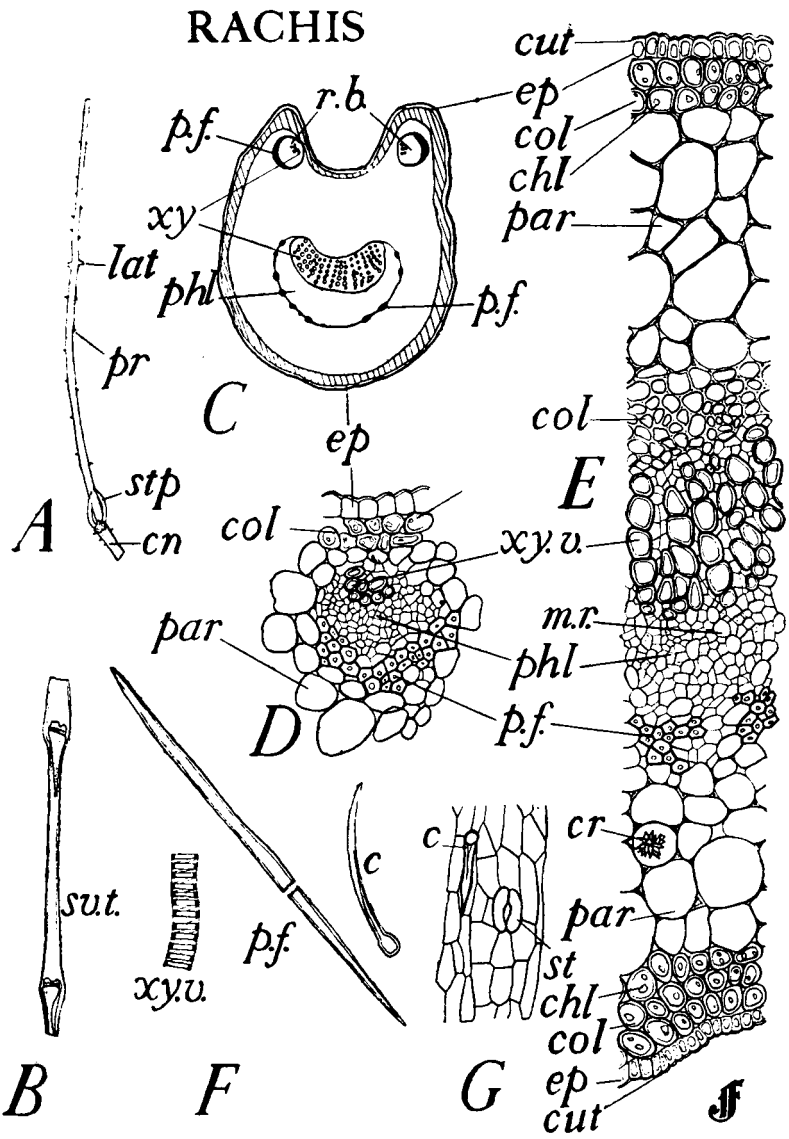

FIG. 3. Rachis of *Rubus idæus* L. *A*, rachis, denuded of leaflets. *B*, sieve-tube, from a cultivated species of *Rubus idæus*, and larger than, but otherwise similar to those found in the wild species. *C*, transverse section of rachis. *D*, transverse section of ridge bundle of rachis. *E*, transverse section through central region of rachis. *F*, isolated elements obtained by maceration. *A*, × $\frac{2}{3}$; *B*, × 250; *C*, × 40; *D*, *E* and *F*, × 200. *c*, covering trichome; *chl*, chloroplast; *cn*, cane; *col*, collenchyma *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *ep*, epidermis; *lat*, point of attachment of lateral leaflet; *m.r.*, medullary ray; *par*, parenchyma; *p.f.*, pericyclic fibre(s); *phl*, phloem; *pr*, prickle; *r.b.*, ridge bundle; *st*, stoma; *stp*, stipule; *s.v.t.*, sieve-tube; *xy*, xylem; *xy.v.*, xylem vessel.

An *endodermis* is not distinguishable, which agrees with Engard's statement[17] that it is absent in the genus *Rubus*.

The MERISTELE is crescent-shaped and well-defined.

The PHLOEM consists of strands of sieve-tissue and small-celled parenchyma, alternating with medullary rays. The *sieve-tubes* are small, individual segments being about 70 μ long and about 2 to 6 μ in diameter with transversely or slightly obliquely arranged sieve-plates, which are uniformly pitted; they resemble the sieve-tubes of the rachis (Fig. 3, B). The medullary rays are clearly marked and are usually one or two cells wide (Fig. 2, B).

CAMBIFORM TISSUE is rarely discernible in the midrib, but, where evident, consists of small, thin-walled rectangular cells.

The XYLEM consists of a number of rows of radiately arranged *vessels* about 4 to 22 μ in diameter; the rows are traversed by the medullary rays, which are about one or two cells wide. The vessels have lignified, spirally and occasionally annularly thickened walls (Figs. 2, A and B; 4, F).

In longitudinal sections, files of micro-clusters and occasional small prisms of *calcium oxalate* about 2 to 8 μ in diameter are frequently seen in the small-celled parenchyma within the meristele (Fig. 4, F).

The *lateral veins* exhibit similar anatomy to that of the midrib, all features becoming progressively smaller towards the margin.

Sections of fresh material mounted in ferric chloride solution exhibit a dark greenish-black colouration in the phloem and medullary rays of the meristele; the parenchyma below the meristele shows a weak reaction. The mesophyll of the lamina reacts strongly, but there is no reaction in the epidermis.

(*b*) RACHIS

The rachis is about 5 to 15 cm. long and 1 to 3 mm. wide and is deeply grooved on its upper surface so that, in transverse section, it presents a somewhat oval outline, with a deep groove on the upper side and a central, crescent-shaped meristele, with two small bundles, one in each ridge. Prickles are usually present throughout the length of the lateral and abaxial surfaces (Fig. 3, A and C).

The EPIDERMIS consists of cells similar in structure to those of the epidermis of the midrib. They are fairly heavily cuticularised, elongated longitudinally, and measure about H 8 to 14 μ, Lev B 5 to 14 μ, and Lev L 25 to 80 μ (Fig. 3, G); *stomata* of the anomocytic (ranunculaceous) type are present; they are elliptical in shape and measure about 20 to 28 μ in length, and 18 to 24 μ in width. *Covering trichomes*, similar in structure and nature to those on the upper surface of the lamina, occur frequently, measuring about 45 to 450 μ long and 7 to 20 μ wide at their bases. The *prickles* are rather larger than, but have similar structures to, those on the lower surface of the midrib.

The CORTEX, like that of the midrib, consists of two layers of tissue—an outer hypodermal layer of *collenchyma*, several cells wide, similar in character to the corresponding layer of the midrib; the cells measure about

L 10 to 140 μ, R and T 10 to 38 μ (Fig. 3, C, D and E). This collenchymatous layer forms a complete cylinder of tissue but is particularly well-developed in the two ridges. The inner cortex is *parenchymatous*, consisting of cells measuring about R and T 18 to 70 μ and L 38 to 190 μ; occasional idioblasts are present, containing cluster crystals of *calcium oxalate*, about 10 to 60 μ in diameter.

An *endodermis* is not distinguishable.

PERICYCLIC FIBRES occur below the arc of the stele; they measure about 350 to 1,300 μ in length, and 4 to 12 μ in diameter, and exhibit a bluntly pointed apex, lignified, smooth walls and a narrow lumen (Fig. 3, C, D, E and F).

The vascular tissue of the central MERISTELE is arranged in a crescent, and the structure of the vascular elements approximates closely to those of the midrib, except that those of the rachis are all somewhat larger. The PHLOEM consists of groups of *sieve-tubes* about 80 μ long and 4 to 6 μ wide, frequently accompanied by small rectangular parenchymatous cells, some of which contain micro-clusters of *calcium oxalate*. Medullary Rays traverse the phloem and are usually one or two cells wide. The XYLEM consists of rows of *vessels* about 4 to 25 μ in diameter, spirally thickened and lignified. There is but little xylem parenchyma; the medullary rays are clearly seen alternating with the rows of vessels (Fig. 3, B, C, E and F).

The bundle in each of the ridges exhibits a very simple structure, being partially surrounded by an arc of *pericyclic fibres* similar to those occurring below the meristele. The *phloem* is fairly extensive, but there are only a few spirally thickened *xylem vessels* (Fig. 3, C and D).

The reaction of sections of the rachis with ferric chloride solution is similar to that of the midrib, but the cortical collenchyma also gives a positive reaction.

(*c*) STIPULES

The paired stipules are adnate to either side of the base of the rachis. They are about 5 to 10 mm. long and 0·5 to 1 mm. wide, subulate and hairy.

EPIDERMAL CELLS of both upper and lower surfaces are small and elongated, measuring about H 7 to 12 μ, Lev B 6 to 12 μ and Lev L 12 to 70 μ. *Stomata* are present on both surfaces and commonly are raised above epidermal level; they are usually elliptical in outline and measure from about 23 to 28 μ in length and 20 to 24 μ in width (Fig. 4, B, D and E). Very numerous *covering trichomes* are present; at the apices and around the edges of the stipules they are large, measuring about 80 to 170 μ in length and 8 to 17 μ wide at their bases, spiral markings well defined (Fig. 4, A and B); towards the centre, they become shorter, similar to that depicted in Fig. 4, D, being about 15 to 70 μ long and 4 to 12 μ wide at their bases; in other respects, they have the general character of those of the upper epidermis of the leaflets. Occasional *glandular trichomes* occur, usually over the narrow midrib, and are very similar in structure to those found on the leaflets (Fig. 4, C).

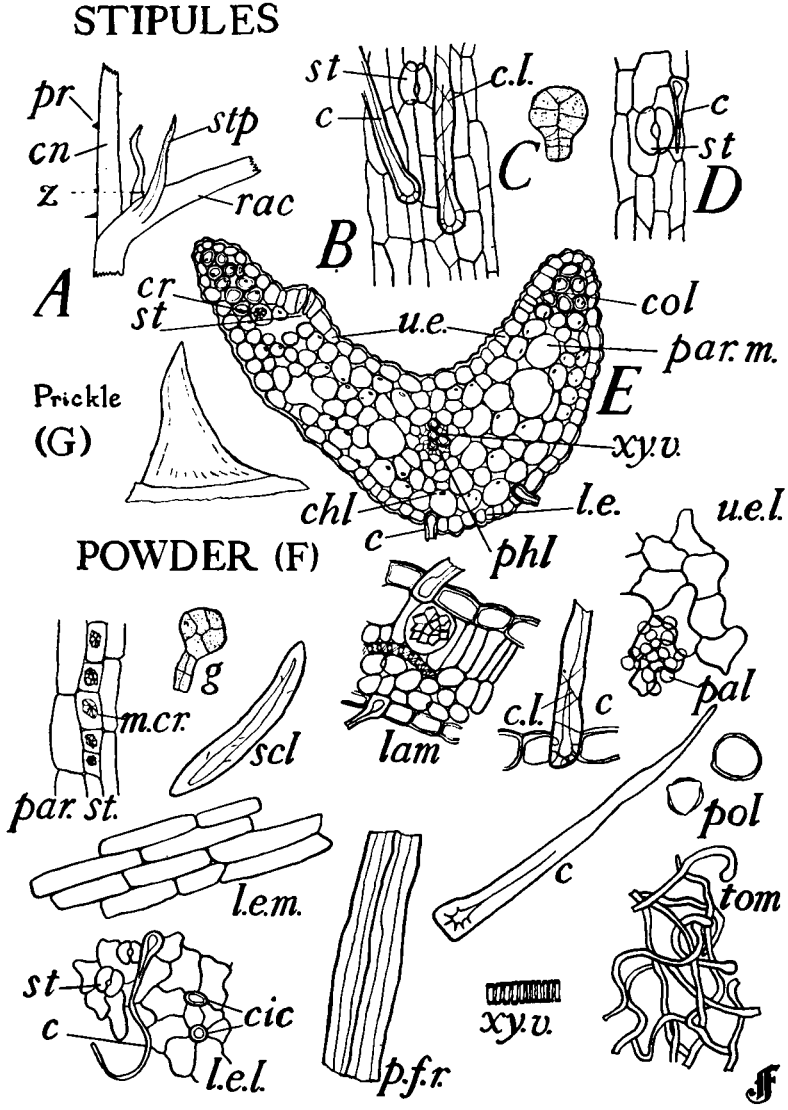

FIG. 4. Stipules, prickle and powder of *Rubus idæus* L. *A*, paired stipules at base of rachis. *B*, lower epidermis of stipule. *C*, glandular trichome. *D*, upper epidermis of stipule. *E*, transverse section of stipule at the position *z*, Fig. *A*. *F*, powder. *G*, prickle. *A*, × 2½; *B*, *C*, *D* and *F*, × 200; *E*, × 120; *G*, × 35. *c*, covering trichome; *chl*, chloroplast; *cic*, cicatrix; *c.l.*, crossed-line effect; *cn*, cane; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *g*, glandular trichome; *lam*, fragment of lamina in transverse section, showing upper and lower epidermises with hair bases, idioblast with cluster calcium oxalate crystal, vascular strand, palisade and spongy mesophyll; *l.e.*, lower epidermis; *l.e.l.*, lower epidermis of lamina; *l.e.m.*, lower epidermis of midrib; *m.cr.*, micro-cluster of calcium oxalate; *pal*, palisade; *par.m.*, parenchymatous mesophyll; *par.st.*, parenchyma from stele; *p.f.r.*, fragments of pericyclic fibres from rachis; *phl*, phloem; *pol*, pollen; *pr*, prickle; *rac*, rachis; *scl*, sclereid of prickle; *st*, stoma; *stp*, stipule; *u.e.*, upper epidermis; *u.e.l.*, upper epidermis of lamina; *xy.v.*, xylem vessel.

The MESOPHYLL has very simple structure, is undifferentiated and consists of rounded or somewhat elongated cells, measuring about H 8 to 17 μ, Lev B 10 to 22 μ and Lev L 8 to 25 μ, and containing chloroplasts about 1 to 4 μ in diameter. Occasional idioblasts occur, containing cluster crystals of *calcium oxalate* about 15 μ in diameter. Towards the base of the stipule, the hypodermal tissue near the margin is strongly collenchymatous.

The MIDRIB is the only vein present and is very simple in structure, consisting of a few xylem vessels about 6 μ in diameter and but little phloem. Fibres are absent.

POWDER

A No. 85 powder varies in colour from light-green to greyish-green; it has a slight and pleasantly aromatic odour and an astringent, slightly bitter taste. A No. 10 powder is similar, but has a light texture, being more felted since the trichomes are less fragmented in the coarser powder. When some of the powder is mixed with solution of ferric chloride in a watch-glass, a deep greenish-black colour is observed.

To examine the powder for structural features, it should be mounted in one of the following: 50 per cent. v/v glycerol solution, solution of chloral hydrate, or phloroglucin and hydrochloric acid.

The diagnostic characters of the powder (Fig. 4, F) are:—

Very numerous curved or curled, unlignified fragments of *covering trichomes* from the lower surface of the lamina, up to about 16 μ wide, also fragments of larger, lignified, acutely pointed, unicellular trichomes from the upper surface, up to 30 μ wide, apical fragments being solid and basal fragments having linear pits; numerous *fragments of the lamina*, showing a transverse sectional view, about 70 μ thick, with a single or occasionally double layer of palisade in which there are rounded idioblasts, each containing a cluster crystal of *calcium oxalate* about 8 to 30 μ in diameter; particles showing in surface view the very slightly curved walls of the cells of the *upper interneural epidermis* of the lamina and usually, immediately beneath it, the palisade with its idioblasts; fragments showing the *lower interneural epidermis* consisting of wavy-walled cells with scattered anomocytic (ranunculaceous) stomata and cicatrices of trichomes surrounded by radiating groups of about 8 epidermal cells; fragments of the *veins* with small spiral vessels accompanied by files of small-celled parenchyma, each cell containing a micro-cluster, or more rarely a small prism of *calcium oxalate*; fragments of the *prickles* consisting of *lignified sclereids* with oblique, linear pits; infrequent entire and broken *glandular trichomes* with a multiseriate stalk and yellowish-brown, multicellular, subspherical, glandular head; lignified fragments of fibres from the rachis; *pollen grains* of *Rubus idæus* L., tricolpate and about 25 to 30 μ in diameter (Fig. 4, F).

SUMMARY

1. Raspberry leaves were collected from plants growing wild to obtain material typical of the species *Rubus idæus*. Polyethylene glycols were

used for embedding the fixed material for sectioning and a method of preparing permanently mounted sections by carbon dioxide freezing and subsequent double-staining with crystal violet and Bismarck brown is described.

2. Both *epidermises* of the leaflets are characteristic. The *upper epidermis* consists of cells with slightly wavy anticlinal walls and bears scattered unicellular, lignified, *covering trichomes* with acute and almost solid apices and thickened, pitted bases. The *lower epidermis* consists of cells with wavy anticlinal walls and bears very numerous unicellular, curly, non-lignified felted trichomes. *Glandular trichomes* are present on the stipules and on the upper epidermis of the leaflets; each has a multiseriate stalk and multicellular, subspherical head. *Stomata*, of the anomocytic (ranunculaceous) type occur in the lower epidermis only; they are circular to oval in outline.

3. The *lamina* of the leaflet is thin and dorsiventral, with, usually, a single row of palisade in which are rounded or ovoid idioblasts each containing a cluster crystal of *calcium oxalate*.

4. The *midrib* of the leaflet contains a meristele consisting of spirally thickened xylem vessels, a phloem of simple sieve-tubes with transverse or oblique sieve-plates, and rows of parenchymatous cells in longitudinal files, each cell containing a micro-cluster or small prism of *calcium oxalate*.

5. *Prickles* of midrib and rachis are composed of lignified, elongated sclereids.

6. Lignified *pericyclic fibres* are abundant in the rachis and provide a diagnostic feature for detecting the presence of rachis in the drug.

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REFERENCES

1. Sowerby's *English Botany*, edited by J. T. B. Syme, Vol. III, Hardwicke, 1864, p. 160.
2. Bentham and Hooker, *Handbook of The British Flora*, Reeve & Co., Ashford, 1930, p. 138.
3. Clapham, Tutin and Warburg, *Flora of the British Isles*, Cambridge University Press, 1952, p. 462.
4. Wallis, *Textbook of Pharmacognosy*, 3rd Ed., Churchill, London, 1955, p. 143.
5. Thoms, *Handbuch der Pharmazie*, Vol. V, Urban & Schwarzenberg, 1931, p. 1021.
6. Moeller-Griebel, *Mikroskopie der Nahrungs- und Genussmittel aus dem Pflanzenreiche*, 3rd Ed., Springer, Berlin, 1928, p. 365.
7. Béguin, *Pharm. Acta Helvet.*, 1945, **20**, 41.
8. British Pharmaceutical Codex, 1949, p. 772.
9. Burn and Withell, *Lancet*, 1941, **241**, 1.
10. Whitehouse, *Brit. med. J.*, 1941, **2**, 370.
11. Beckett, Belthle, Fell and Lockett, *J. Pharm. Pharmacol.*, 1954, **6**, 785.
12. Johansen, *Plant Microtechnique*, McGraw-Hill, 1940, 126.
13. Fell and Rowson, *J. R. micr. Soc.*, 1955, **75**, 111.
14. Van Horne and Zopf, *J. Amer. pharm. Ass.*, 1951, **40**, 31.
15. Miles and Linder, *J.R. micr. Soc.*, 1952, **72**, 199.
16. Moll and Janssonius, *Botanical Pen Portraits*, Nijhoff, The Hague, 1923, p. 22.
17. Engard, *Organogenesis in Rubus, University of Hawaii Research Publication*, No. 21, Honolulu, 1944, p. 220.