

# The morphology and anatomy of the flowers of *Mitragyna inermis* (Willd.) O.Kuntze

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The morphology and anatomy of the flowers of *Mitragyna inermis* have been described and a comparison made with the flowers of *Mitragyna ciliata* and *Mitragyna stipulosa*.

The alkaloidal contents of the leaves of the three species of *Mitragyna* growing in Ghana have now been fully reported (Beckett, Shellard & Tackie, 1963a, b; Shellard & Sarpong, 1969). The morphology and anatomy of the leaves of the three species have also been described (Shellard & Shadan, 1963; Pillay, 1964). Leaves of *Mitragyna stipulosa* and *M. ciliata* are almost identical and these species may be differentiated only by an investigation of the alkaloids present (Beckett & others, 1963a, b), or by an examination of the flowers (Shellard & Wade, 1967).

Although the alkaloidal patterns of *M. stipulosa* and *M. ciliata* differ, those of *M. ciliata* and *M. inermis* shows few differences. Major differences obtain between the leaves of the two species; nevertheless, for Rubiaceae and particularly the sub-family Naucleoideae, a study of the flowers is indispensable for determining the genus and species (Aubreville, 1959). The present work reports a detailed study of the flowers of *M. inermis*.

*Materials.* The flowering tops were obtained from trees growing in the coastal plains of the Sogakofe district of the Volta region, Ghana. The identity of the species was confirmed by Mr. A. Enti, Government Silviculturist and by comparison with specimens in the Herbarium at the Royal Botanic Gardens, Kew.

## Macroscopical features

The *inflorescence* consists of a number of globose flower heads, each growing to about 20 mm in diameter, arranged in a dichasial cyme (Fig. 1A). The 100 to 120 individual florets of each flowerhead are closely packed on a spherical receptacle, each floret in the mature flower head being surrounded by 10 to 15 closely packed overlapping paleaceous bracteoles (Fig. 1B). In the flower bud stage the bracteoles completely cover and protect the developing florets which, as they develop to maturity, gradually push their way through the centre of the group of bracteoles. The first part of the floret to become visible during this development is the corolla, the tube of which is closed by the infolding of the petal lobes so that the margins of each lobe are adjacent in a valvate arrangement. When the floret is mature, the combined length of the calyx and ovary is much shorter than the length of the bracteoles so that the calyx is not readily visible on the fully grown flower head. The long corollas with their recurving lobes also tend to mask the bracteoles (Fig. 1C).

In the mature flower the calyx with ovary is 1.08-1.70-2.03 mm long and the bracteoles 2.47-3.22-4.0 mm long, the ratio of the average dimensions being 0.53.

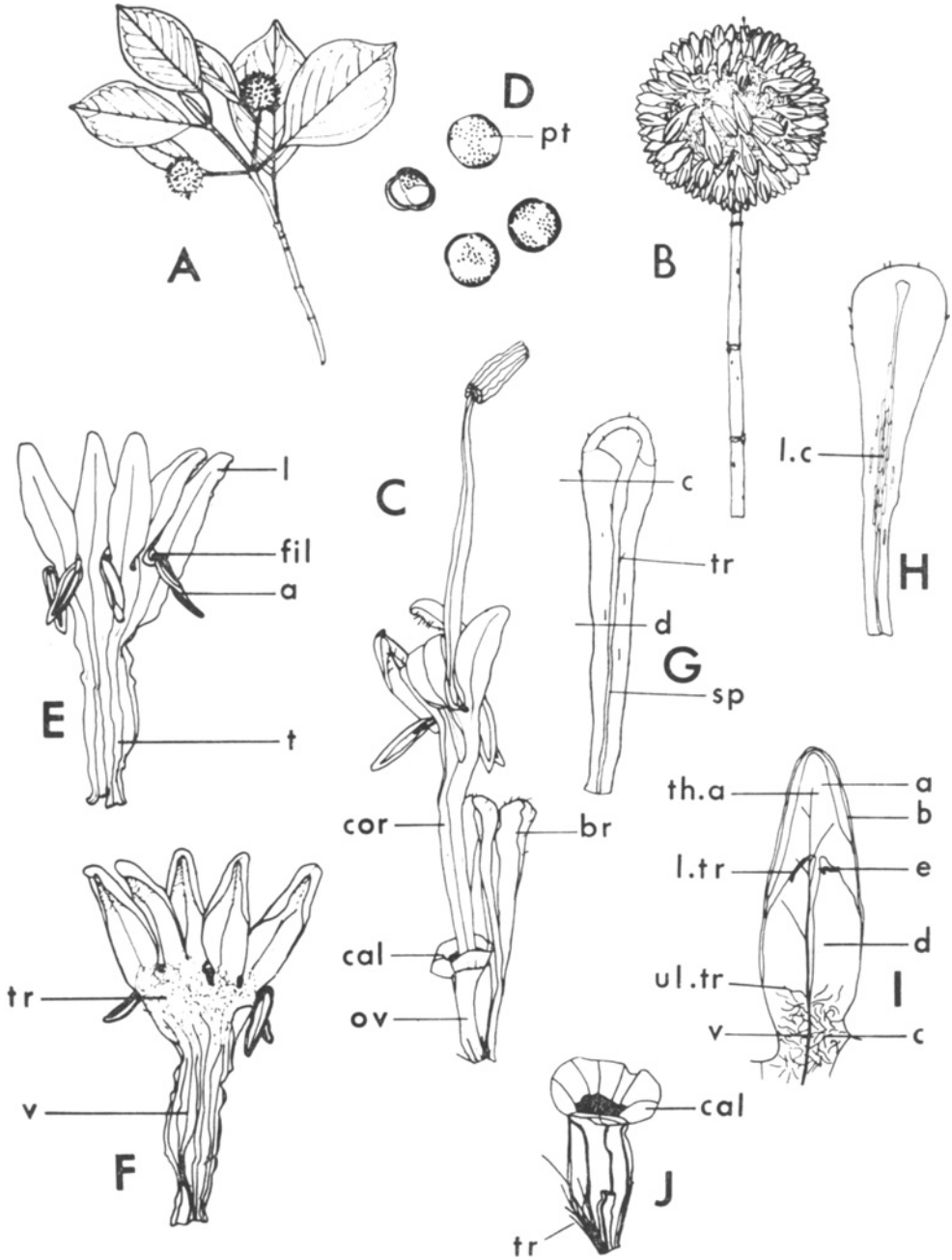


FIG. 1.

FIG. 1. *Mitragyna inermis*, A, inflorescence  $\times \frac{1}{4}$ ; B, flower head and peduncle  $\times 1\frac{1}{2}$ ; C, floret  $\times 9$ ; D, pollen  $\times 400$ ; E, corolla, outer epidermis and anthers  $\times 9$ ; F, corolla, inner epidermis  $\times 9$ ; G, bracteole, outer epidermis, surface view  $\times 15$ ; H, bracteole, inner epidermis, surface view  $\times 15$ ; I, corolla, inner epidermis of lobe, surface view  $\times 15$ ; J, calyx and ovary  $\times 15$ . a, anther; br, bracteole; cal, calyx; cor, corolla; fil, filament; l, lobe; l.c, lignified cell; l.tr, lignified trichome; ov, ovary; pt, pit; sp, spine; t, tube; th.a, thickened apex; tr, trichome; ul.tr, un-lignified trichome; v, vein.

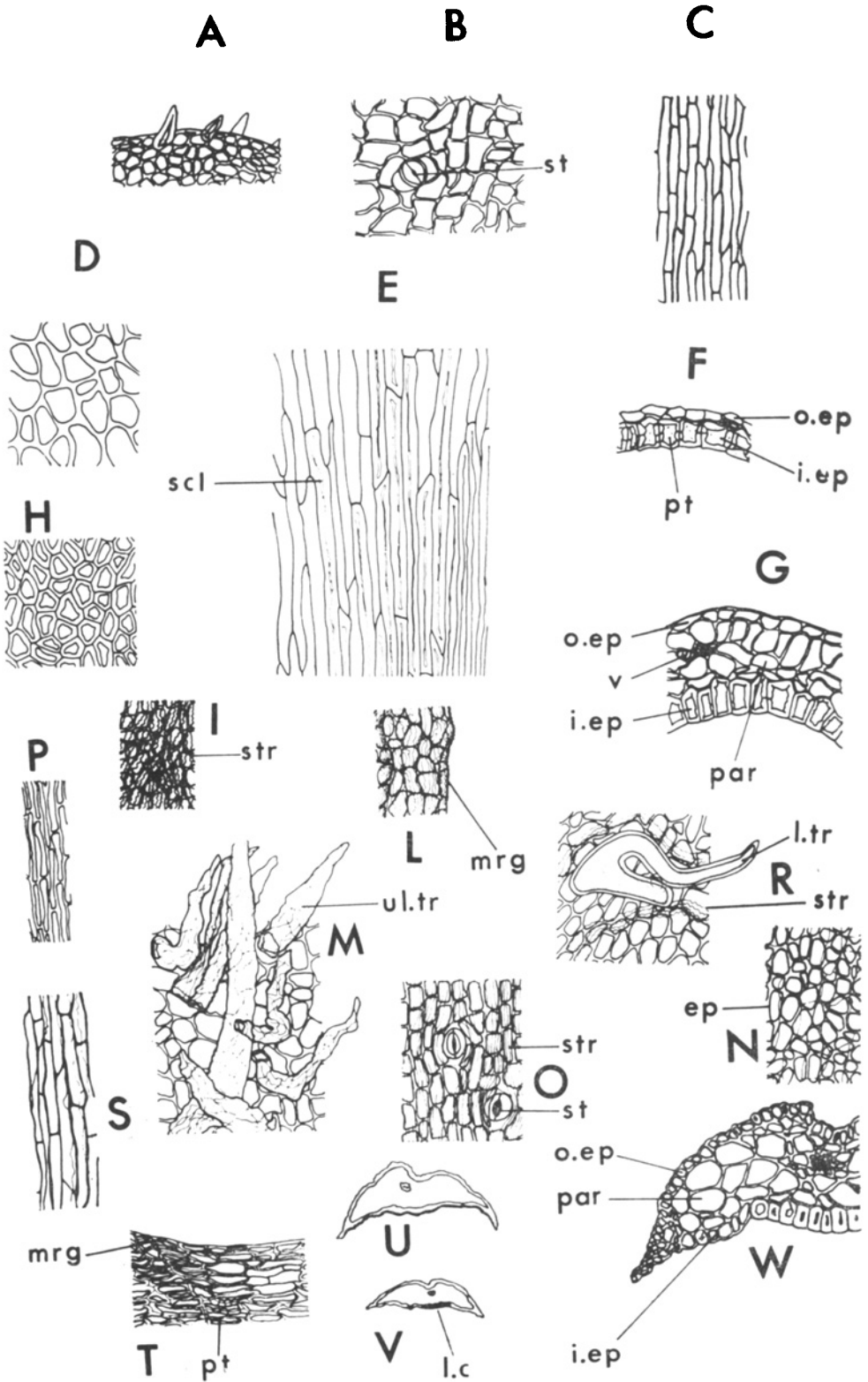


FIG. 2.

Each floret is pentamerous, complete, perfect, actinomorphic and epigynous. The peduncle is 20 to 65 mm long, up to 1.5 mm in diameter, and woody with a thin brown cork; it bears longitudinal ridges and alternate pairs of leaf scars. The receptacle is spherical and is about 4 to 5 mm in diameter, the individual florets being attached over its entire surface. It is brown and covered with long stiff trichomes, the surface being rugose because the base of each ovary is attached to a raised portion of the receptacle. The transversely cut surface is white and exhibits numerous vascular strands.

The bracteoles are clavate to spatulate and are 2.4 to 4.0 mm long. They are thickened along a central spine which, on the outer surface, extends over most of the apical region, so that they have a planoconvex transverse section near the apex (Fig. 1G and H).

The calyx is gamosepalous (Fig 1J) consisting of five sepals united into a truncate cone about 300  $\mu\text{m}$  deep and 0.9–1.05 mm in diameter at the open end. The veins on the outer surface are inconspicuous. The calyx is thicker near the base where it is fused to the ovary wall.

The corolla is gamopetalous consisting of five petals which are joined for over half their length. It is from 4.0 to 5.5 mm long of which the lobes comprise 1.0 to 1.9 mm, the diameter at the open end being 0.9 to 1.3 mm (Fig. 1E and F).

The individual petals are linear, but widen slightly near the lobes, which taper to a blunt incurving point at the apex. Closure is valvate. The tips of the lobes are wrinkled but otherwise the outer surface of the corolla is matt and glabrous. The inner surface is wrinkled and the top half raised into a thickened pad. Occasional trichomes occur in the middle region of the lobe but there are many white tangled trichomes where the lobes unite to form the tube. Towards the base of the tube the inner surface is ridged (Fig. 1 I).

The androecium consists of five epipetalous stamens which alternate with the corolla lobes. Each anther is borne on a short filament attached to the corolla just below the lobes. The bilobed anthers are exserted and pendulous between the lobes of the corolla, dehiscence being through a longitudinal slit in each lobe. The spindle-shaped lobes of the anthers are joined for most of their length and are 1.0–1.4 mm long and 0.3 to 0.6 mm wide, the filaments being about 300  $\mu\text{m}$  long and 50  $\mu\text{m}$  wide. Pale yellow pollen is found on the stigma and entangled in the trichomes of the corolla lobes (Fig. 1D).

The gynaecium consists of a bicarpellary, syncarpous, inferior ovary and a reddish-brown cylindrical style surmounted by a lobed and grooved mitriform fleshy stigma. The ovary, which is 0.8 to 1.7 mm long and up to 1.0 mm in diameter at the top, is conical but flattened, with longitudinal ridges. It is inserted on a small mound on the receptacle and surrounded by the bracteoles. Long, stiff pale yellow trichomes

FIG. 2. *M. inermis*, A, bracteole, margin of head, after epidermis, surface view; B, bracteole, centre of head, outer epidermis, surface view; C, bracteole, shaft, outer epidermis, surface view; D, bracteole, head, inner epidermis, surface view; E, bracteole, shaft, inner epidermis, surface view; F, calyx, margin, transverse section; G, calyx, base, transverse section; H, calyx, base, inner epidermis, surface view; I, L, M and N, corolla, inner epidermis, surface view at a, b, c and d respectively on Fig. 1, I; O, corolla, lobe, outer epidermis, surface view; P, corolla, tube, outer epidermis, surface view; R, corolla, inner epidermis, surface view at e on Fig. 1, I; S, corolla, tube, inner epidermis, surface view; T, calyx, margin, inner epidermis, surface view; all  $\times 150$ . U and V, bracteole, transverse sections at c and d respectively on Fig. 1 G  $\times 35$ ; W, bracteole, shaft, transverse section  $\times 150$ . o.ep, outer epidermis; i.ep, inner epidermis; l.c, lignified cell; l.tr, lignified trichome; mrg, margin; par, parenchyma; pt, pit; scl, sclerenchyma; st, stoma; str, striation.

arise from the receptacle and pack the space between the ovaries and bracteoles. The ovary is bilocular with axile placentation, numerous minute ovules arranged in an overlapping pattern filling each loculus. The ovules are roughly triangular in section and each is joined to the placenta by a short funicle. In the opened, mature flower the style is 8.0 to 11.0 mm in length, thus extending about 4 mm beyond the corolla (Fig. 1C).

#### *Microscopical features*

*Peduncle.* The general anatomy is similar to that of the young stem described by Pillay (1964). The outer layers of the peduncle consist of 1 to 2 rows of thick-walled, flattened cork cells with dark brown contents. The outer cortex varies in thickness from 1 to 3 rows of isodiametric thin-walled parenchymatous cells while the inner part of the cortex is collenchymatous and consists of up to 5 rows of cells with thickened walls, although the innermost cell walls are thinner. Occasional cells are slightly lignified and pitted like the pith cells.

Within the cortex is a ring of thickened unlignified phloem fibres. The zone is 1 to 3 cells deep; individual fibres are 1–5 mm long and 10 to 18.3 to 27  $\mu\text{m}$  in diameter. The phloem is made up of groups of 1 to 4 lignified and thick-walled phloem cells and parenchyma, sieve cells and some latex cells with yellow to brown contents. No medullary rays can be distinguished in the phloem at the mature flower stage.

There is a thin yellowish zone of tightly-packed cambial cells with thin walls, inside which is a rectangular zone of xylem. The xylem has vessels 12 to 30  $\mu\text{m}$  in diameter, with lignified walls bearing reticulated or spiral thickening. On two opposite sides of the rectangle the large vessels are outermost, but on the other two sides they are usually nearer the axis. In each case the remainder of the xylem consists of fibres with narrow lumens, occasional xylem parenchyma and prominent medullary rays. The rays are mainly uniseriate, but some are biseriate, and the cells often have dark brown contents.

The central pith consists of a narrow outer zone of small yellow pith cells and an innermost zone of large polygonal cells with lignified, pitted and thickened walls. No crystals of calcium oxalate are present.

*Receptacle.* The epidermis consists of small polygonal cells from which the bracteoles arise together with numerous straight, unicellular trichomes with thick lignified walls, and measuring 160 to 400 to 720  $\mu\text{m}$  in length and about 20  $\mu\text{m}$  in width.

The internal structure is somewhat similar to that of a young stem but with numerous groups of spirally thickened and lignified vessels, each running to the base of one of the 120 or so ovaries. In consequence, these vascular strands may appear in any one section to have been cut obliquely, transversely or longitudinally. There is usually a pale band of phloem cells on the outer side of the strand. The cortex consists of cells with thickened cellulosic walls. Nearer the centre there are several groups or a continuous ring of lignified vessels arranged radially round the centre of the receptacle. No scattered lignified fibres or calcium oxalate crystals are present.

#### *Bracteoles*

The *outer epidermis* of the unthickened margin of the apical region consists, in surface view, of polygonal cells with somewhat sinuous slightly thickened walls (Fig. 1G and 2B). They are 13 to 30.6 to 44  $\mu\text{m}$  long, 10 to 18.3 to 33.3  $\mu\text{m}$  wide and 8 to 22  $\mu\text{m}$  deep. A thin, slightly wrinkled cuticle is visible. There are several

unicellular conical trichomes along the margin (Fig. 2A) with thickened, lignified walls, and measuring from 30 to 50  $\mu\text{m}$  in length. On the thickened central spine there are also several trichomes, but these are about 80 to 120  $\mu\text{m}$  in length. Both types have thickened, pitted and lignified bases. There are numerous anisocytic and paracytic stomata, about 30  $\mu\text{m}$  in diameter, particularly on the thicker centre of the bracteole head.

Where the head begins to merge with the shaft the polygonal cells become narrower and more elongated. Along the shaft the cells have thickened cellulosic walls and are not lignified. Calcium oxalate crystals are absent and cuticle is not visible. The cells are 35 to 155  $\mu\text{m}$  long and 6 to 12  $\mu\text{m}$  wide. There is an occasional long trichome near the base of the shaft similar to those on the receptacle. Along the central thickened spine the cells have thin brown walls with dark brown contents.

The *inner epidermis* (Fig. 1H, Fig. 2D) has cells similar to those of the outer surface except that on the head of the bracteole the walls are somewhat thicker. On the shaft the cells become elongated and some groups of cells near the centre of the shaft have lignified walls and narrow lumens. The non-lignified cells alongside, similar in size and shape but longer than the outer epidermal cells, measure 130 to 320  $\mu\text{m}$  in length and 6 to 18  $\mu\text{m}$  in width (Fig. 2E). Lignified cells are absent from the base of the shaft and there are no stomata or trichomes present (Fig. 2W).

The *mesophyll* is composed of parenchymatous cells and there is a single vascular strand running up the centre of the shaft (Fig. 2U, V and W). Cluster crystals of calcium oxalate occur but are uncommon.

### *Calyx*

The cells of the *outer epidermis* are polygonal with fairly straight, thin walls which become a little thicker towards the base of the calyx. Occasional isolated cells have cellulosic thickening and are 15 to 35  $\mu\text{m}$  long, 6 to 22  $\mu\text{m}$  wide, and 8 to 14  $\mu\text{m}$  deep.

The *inner epidermis* shows two zones. In the marginal region the cells are elongated transversely and have thickened lignified walls apart from a few small patches of similar unligified cells. They are 15 to 45  $\mu\text{m}$  long, 5 to 15  $\mu\text{m}$  wide and 20 to 30  $\mu\text{m}$  deep. Small pits are visible on the walls of some groups of lignified cells and the middle lamella is very prominent (Fig. 2T). This marginal region extends for 10 to 12 cells towards the base, i.e.  $\frac{1}{4}$  to  $\frac{1}{3}$  of the length of the calyx.

In the basal region the cell walls become more heavily thickened and lignified, the cells are wider, and pits are absent. These cells are 18 to 45  $\mu\text{m}$  long, 11 to 24  $\mu\text{m}$  wide and 20 to 40  $\mu\text{m}$  deep, the longest axis being generally aligned towards the ovary. This zone is about 12 to 14 cells deep (Fig. 2H).

The mesophyll consists of thin-walled parenchyma increasing in thickness from one row of flattened cells near the margin, to 4 rows of larger cells near the ovary (Fig. 2F and G). Stomata, trichomes, cuticle and calcium oxalate are absent from the calyx.

### *Corolla*

On the lobes the cells of the *outer epidermis* are generally rectangular with straight walls. Near the tube, the cells are narrower and more elongated. The cells, which are 16 to 25.5 to 42  $\mu\text{m}$  long and 6 to 13.6 to 22.0  $\mu\text{m}$  wide, are covered by a thick cuticle bearing parallel slightly sinuous striations. Near the edge of the lobe the striations are very sinuous. There are numerous paracytic stomata measuring about

30  $\mu\text{m}$  in diameter which are raised above the level of the surrounding cells. There are no trichomes on the outer surface (Fig. 2 O).

On the corolla tube, the cells of the outer epidermis are narrower and more elongated along the corolla axis than on the lobes; they are 43 to 58.2 to 70  $\mu\text{m}$  long and 5 to 7.1 to 12  $\mu\text{m}$  wide. The surface is ridged over the vascular bundles and has a faint, thin, slightly striated cuticle (Fig. 2S).

The *inner epidermis* of the corolla lobes consists of polygonal to rectangular cells with thin, straight walls measuring 11 to 21.7 to 40  $\mu\text{m}$  long, 6 to 14.2 to 27  $\mu\text{m}$  wide, and 3.3 to 7.1 to 12  $\mu\text{m}$  deep. The surface of the fleshy pad which covers the top half of the lobes is heavily ridged and covered by a thick cuticle with prominent sinuous striations. These striations become more patchy and less prominent towards the middle of the lobe. There are a few lignified trichomes near the middle of the lobes. They are generally bent over near their lignified bases and appear to lie between the ridges on the surface. They measure 200 to 300  $\mu\text{m}$  in length and about 25  $\mu\text{m}$  at the base. There are occasional lignified cells on or just below the epidermis near these trichomes (Fig. 2I, L and R). The lower half of the lobes is very thin. In the region where the lobes join to form the tube, in the throat of the corolla, the surface is covered with thin-walled sinuous twisted trichomes measuring 100 to 175 to 400  $\mu\text{m}$  long and up to 50  $\mu\text{m}$  in diameter at the base (Fig. 2M).

The inner surface is heavily indented with ridges which follow the main vascular bundles (Fig. 3E). The cells are rectangular and elongated; they measure 70 to 100 to 120  $\mu\text{m}$  long, 8 to 11 to 15  $\mu\text{m}$  wide, and 5 to 9.7 to 17  $\mu\text{m}$  deep, and are covered by a thin cuticle with faint longitudinal striations (Fig. 2S).

The *mesophyll* consists of two main tissues. In the lobes there is a variable region of cellulosic collenchymatous cells immediately below each epidermis. The main part of the mesophyll is composed of large, yellow, thin-walled parenchymatous cells (Fig. 3A and F). Lower down in the ridged corolla tube the cells are mainly collenchymatous with thickened cellulosic walls, but there are also very large parenchymatous thin-walled cells, measuring 20 to 50  $\mu\text{m}$  in diameter in transverse section, which appear to be lacunae in the mesophyll, (Fig. 3E and G). Cluster crystals of calcium oxalate occur mainly in the lobes and are 6 to 10 to 17  $\mu\text{m}$  in diameter. The vascular tissue consists of lignified spiral vessels with associated phloem cells.

### *Androecium*

The characteristic tissue of the anther lobe is the "fibrous layer," the cells of which are isodiametric, with a contorted shape in surface view. The cell walls bear spiral thickening which appears to be linked with that of adjacent cells in an intricate pattern. The thickening is only slightly lignified. The cells are 6 to 13 to 19  $\mu\text{m}$  in diameter and 11 to 20.5 to 37  $\mu\text{m}$  deep. Epidermal cells were not visible in the material examined (Fig. 3H).

The connective consists of small yellowish cells, some containing cluster crystals of calcium oxalate. Crystals are, however, more common in the larger paler cells next to the fibrous layer and measure 5 to 11.1 to 17  $\mu\text{m}$  in diameter. There is a vascular bundle consisting of small lignified vessels (Fig. 3H).

The *pollen* grains are subspherical and measure 14 to 16.7 to 18  $\mu\text{m}$  in diameter. The exine is covered with minute pits and there are three germinal furrows and pores (Fig. 1D). There are occasional immature pollen grains that appear more triangular in polar view.

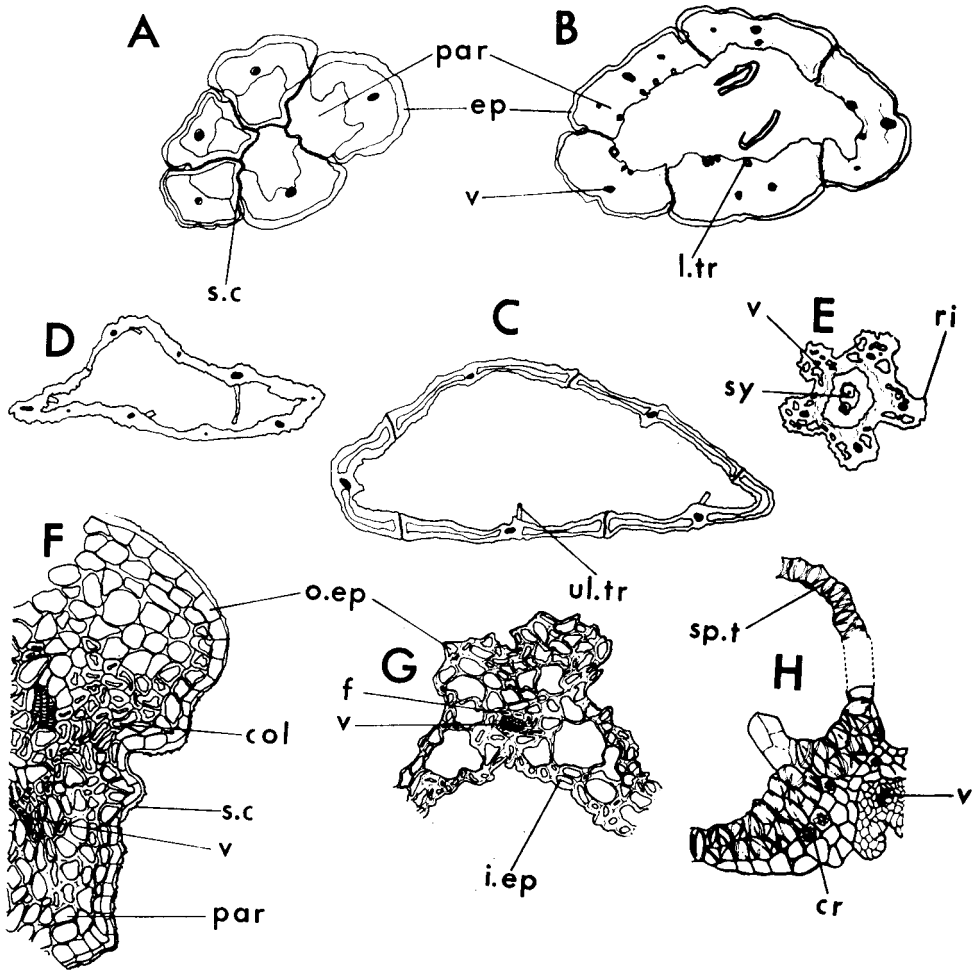


FIG. 3.

FIG. 3. *M. inermis*, A, B and C, corolla, transverse sections at a, e and c respectively on Fig. 1, I; D, corolla, just below lobes, transverse section; E, corolla, near base, transverse section; all  $\times 35$ . F, corolla, apex of lobes, transverse section; G, corolla, tube, transverse section; H, anther lobe and connective, transverse section; all  $\times 150$ . cr, cluster crystal of calcium oxalate; col, collenchyma; ep, epidermis; f, fibre; i.ep, inner epidermis; l.tr, lignified trichome; o.ep, outer epidermis; par, parenchyma; ri, ridge; s.c, striated cuticle; sp.t, spiral thickening; sy, style; ul.tr, unlignified trichome; v, vein.

### Gynaecium

**Stigma.** The epidermal cells of the stigma are elongated radially and have thin suberized anticlinal walls. Small globules of fixed oil are present in these cells. The cortex has an outer parenchymatous region of larger thin-walled cells and an inner layer of thick-walled collenchymatous cells. The centre of the stigma consists of parenchyma and some of the cells contain cluster crystals of calcium oxalate. There are also two major groups of lignified spiral vessels orientated in various directions (Fig. 4E, F and H).

**Style.** The epidermal cells of the style are small and polygonal with thick cellulose walls and a thin striated cuticle. The cortex is composed of larger collenchymatous



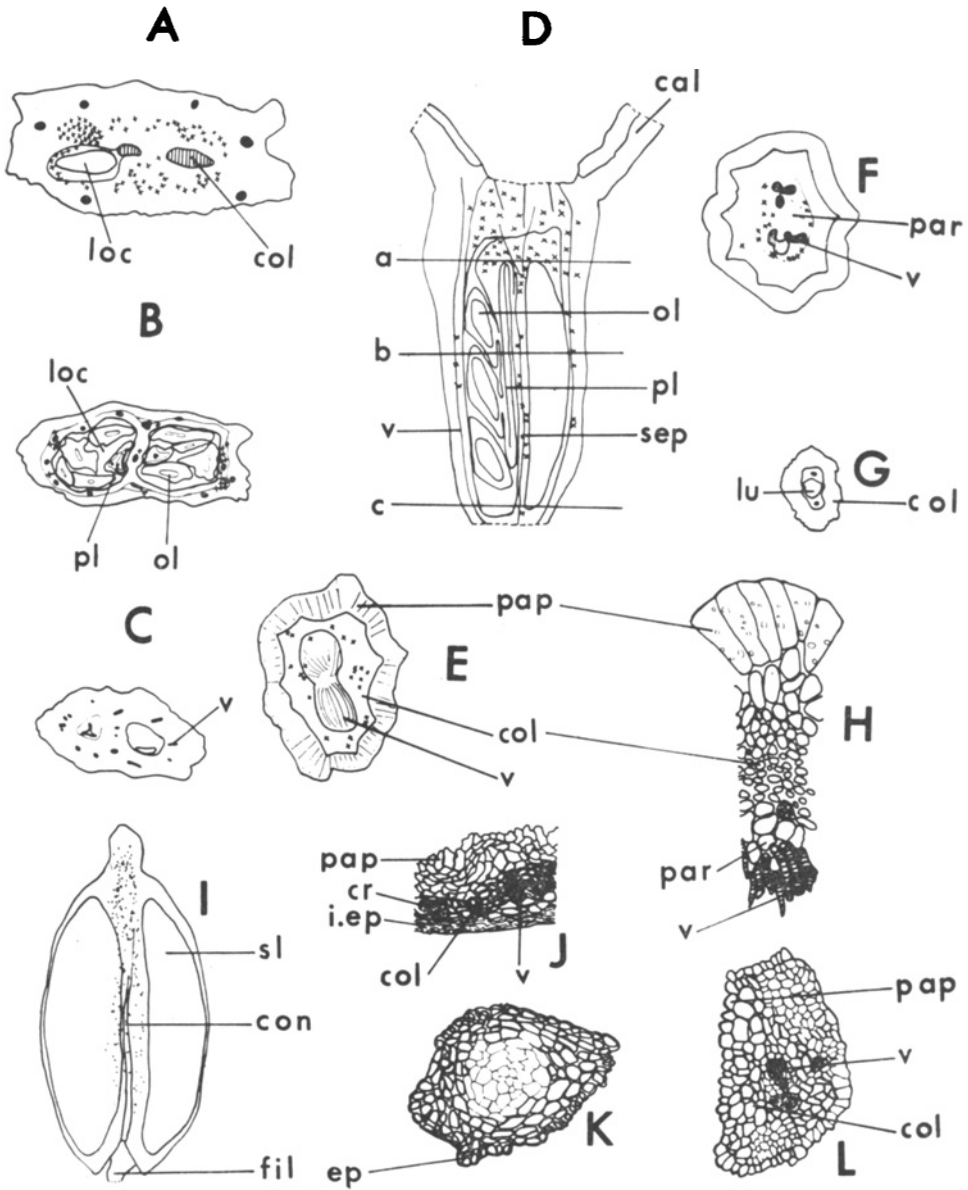


FIG. 4.

FIG. 4. *M. inermis*, A, B and C, ovary, transverse sections at a, b and c respectively on Fig. 4, D; all  $\times 45$ ; D, ovary and calyx, medium vertical section  $\times 35$ ; E, stigma, upper part, transverse section; F, stigma, middle part, transverse section  $\times 35$ ; G, style, transverse section; all  $\times 35$ . H, stigma, upper part, transverse section  $\times 150$ ; I, anther, adaxial surface,  $\times 35$ ; J, ovary, wall, transverse section; K, ovule, transverse section; L, placenta, transverse section; all  $\times 150$ . col, collenchyma; con, connective; cr, cluster crystal of calcium oxalate; ep, epidermis; fil, filament; i.ep, inner epidermis; loc, loculus; lu, lumen; o.ep, outer epidermis; ol, ovule; pap, papillose epidermis; par, parenchyma; pl, placenta; sep, septum; sl, slit; v, vein.

cells. There is an inner region of thick-walled cells, each with a narrow reddish lumen, surrounding two distinct groups of lignified spiral vessels. In the lower half of the style there is a central lumen but this becomes filled with parenchyma in the upper part of the style (Fig. 4G).

*Ovary.* The *outer epidermis* is of small subrectangular papillose cells with thin walls. The cells are 13 to 23·8 to 43  $\mu\text{m}$  long and 6 to 11·1 to 17  $\mu\text{m}$  wide. There are occasional conical lignified trichomes near the top of the ovary, similar to those growing on the receptacle. They are 180 to 350  $\mu\text{m}$  long and about 15  $\mu\text{m}$  wide.

The *inner epidermis* is not readily visible in surface view, but in section is seen to consist of 4 to 5 rows of similar radially flattened thin-walled cells (Fig. 4B and J).

The *mesophyll* consists of a layer of parenchymatous cells beneath each epidermis. Below the inner epidermis some of these cells contain cluster crystals of calcium oxalate, particularly towards the top of the ovary. The crystals measure 3·5 to 9·4 to 19  $\mu\text{m}$  in diameter. In each half of the ovary there are 4 to 6 groups of spirally thickened and lignified vessels, each group being surrounded by small dark brown cells. The septum dividing the two loculi has a structure similar to that of the inner region of the ovary wall (Fig. 4A, B and C), with a central vascular bundle. The placenta is only attached to the septum at the top and bottom of the ovary (Fig. 4B and D). It has an epidermis of clear cells, those facing towards the septum being papillose. There is a yellow layer of large thin-walled cells with yellow contents below the papillose cells and a central vascular bundle of lignified spirally thickened vessels. The remainder of the tissue is composed of small parenchymatous cells, some of which contain cluster crystals of calcium oxalate (Fig. 4L).

When immature the ovules consist of an outer epidermis of thin-walled yellowish cells and an inner region of small undifferentiated cells. In transverse section these cells appear to radiate from the centre of the ovule, with the cell walls decreasing in thickness towards the centre (Fig. 4K).

#### DISCUSSION

Although at first sight the globose flower-heads of *M. inermis*, *M. ciliata* and *M. stipulosa* look similar, the flowers of *M. inermis* differ markedly from those of the other two species. Neither the calyx of *M. inermis* nor of *M. ciliata* are visible above the surface of the globular receptacle, as is the case with *M. stipulosa*, but dissection of the flower-heads shows that the calyx of *M. inermis* is small with an entire, glabrous margin while that of *M. ciliata* is large with a lobed, ciliate margin. The ratio of the average length of the calyx plus ovary to the average length of the bracteole in mature flowers is—*M. inermis*, 0·53; *M. ciliata*, 0·95; and *M. stipulosa*, 1·42. This clearly distinguishes each of the three species.

Other macroscopical differences are that the corolla of *M. inermis* does not have a hood in the corolla lobe, the top half being fleshy, and further, there are no trichomes on the outer epidermis. With *M. ciliata* and *M. stipulosa*, on the other hand, there are many golden trichomes present. The anthers of *M. inermis* are exerted and pendulous on a short filament, while those of *M. ciliata* and *M. stipulosa* are sessile with introrse dehiscence.

*M. inermis* also differs by having an ovary in which the placentas hang freely from the septum and a style with a distinct central lumen.

Microscopically, the three species differ in many details, though only a few are of major diagnostic significance, for example, the absence of crystals of calcium

oxalate from the peduncle, receptacle and calyx of *M. inermis*. This species also differs in having lignified cells only down the centre of the shaft of the bract, whereas in the other two species almost the entire shaft is lignified. Further, stomata are absent from the calyx of *M. inermis* but are present in the other species, while they are numerous on the lobes of the petals of *M. inermis* and absent or rare on the lobes of the petals of *M. ciliata* and *M. stipulosa*.

The differences described above should be sufficient to distinguish between the leaves and flowering tops of *M. inermis* and *M. ciliata* in the coarsely powdered condition.

#### *Acknowledgements*

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