

## PHARMACOGNOSY OF INDIAN SUBSTITUTES OF MALE FERN

I. *DRYOPTERIS RAMOSA* (HOPE) C. CHR. AND  
*D. CHRYSOCOMA* (CHRIST) C. CHR.

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A taxonomic description, and the macroscopy and microscopy of the rhizome and leaf bases of *Dryopteris ramosa* (Hope) C. Chr. and *D. chrysocoma* (Christ) C. Chr. are given. Both the species compare very favourably in oleoresinous content and "crude filicin" with the official male fern. Because of their abundance in nature they could be commercially exploited. Like the official male fern, both the species possess schizogenous cavities in the ground tissue with internal glands. *D. ramosa* is devoid of sclerotic nests while a few of these are present in the other species both in the rhizome as well as in the leaf bases.

CONSIDERABLE quantities of the expensive male fern extract are imported into India for medicinal purposes. Search for an Indian substitute for this important drug has shown that there are substitutes that grow wild in the Himalayas, some abundantly enough to be exploited commercially (Mittal and Mehra, 1960). The present communication concerns the pharmacognostic investigation of two of these, namely, *Dryopteris ramosa* (Hope) C. Chr. and *D. chrysocoma* (Christ) C. Chr. Whereas the official B.P. requirements of the crude filicin is not less than 1.5 per cent, the two species contain a much higher percentage of oleoresin and crude "filicin." The amount of oleoresin in *D. ramosa* varies from 12-15 per cent and in *D. chrysocoma* 14-17 per cent. The crude "filicin" content of the former is approximately 3.8 per cent and in the latter 4.3 per cent as calculated by the B.P. (1953) method.

### MATERIAL AND METHODS

The material for *D. ramosa* was gathered from various localities at Pahlgam and Gulmarg, Kashmir (North-western Himalayas) while *D. chrysocoma* was sampled from Darjeeling (Eastern Himalayas).

The plants were uprooted so as not to dislodge the stele within the rhizome. These were cut into thin slices for microtomy, fixed in formalin, acetic acid and ethanol (5:5:90) and passed through the usual grades of ethanol and xylol, and embedded in paraffin. Because of the abundant starch and resin they were kept in each grade for 24-48 hr. and 2-4 days on the bath in paraffin. Sections were stained with safranin-fast green. A saturated solution of Sudan III in equal parts of glycerol and rectified spirit was used for detecting the presence of oleoresin in the intercellular cavities and glands. The latter are normally hyaline but with this reagent stain bright red and can be easily located. Schultze's and Jeffrey's fluids (Sauss, 1958) were used as macerating agents for the study of individual cells. For detailed study of glands a 5 per cent sodium hydroxide solution was found to be a better maceration fluid as with this reagent the glands

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do not undergo any distortion in shape. Whole mounts of scales were prepared in 50 per cent glycerol after clearing.

*Dryopteris ramosa* (Hope) C. Chr.

*General description.* This is one of the commonest species growing in Kashmir (North-western Himalayas) and is plentiful in Pahlgam and Gulmarg as an undergrowth in forests at an altitude of about 7,000 ft. The species is characterised by an ascending rhizome which is densely clothed, as are the bases of the fronds, with large, broad, pale brown ramenta each ending in a hair. The stipe is up to 45 cm. long, stout, pale brown or straw coloured, sometimes mottled. The fronds (Fig. 1A)

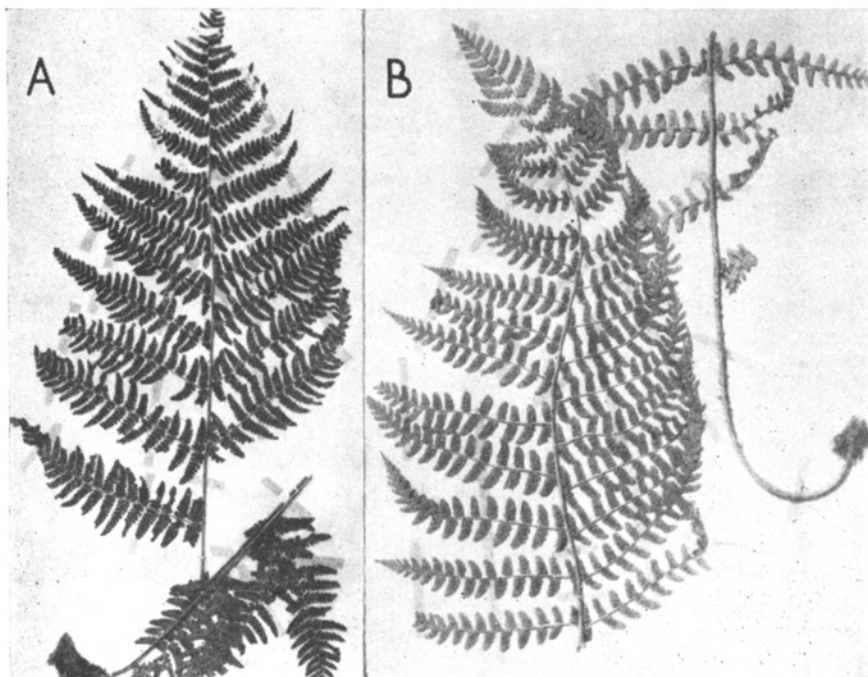


FIG. 1. A, *Dryopteris ramosa*, frond  $\times 1/5$ ; B, *D. chrysocoma* frond  $\times 1/5$ .

are 30–60 cm. long, 20–35 cm. broad, oblong or ovate-lanceolate, tripinnatisect. The rachises are straw coloured, sparsely covered with pale coloured, linear ramenta and fibrils, but sometimes glabrous; the pinnae are in 16–30 pairs besides the deeply pinnatifid apex, the middle pinnae being generally the largest, the upper and lower ones are gradually shortened. Pinnae near the base of fronds are distantly placed; the lowest pinnae are 13–25 cm. in length and 5–13 cm. broad, with 12–20 pairs of pinnules. Pinnules in the lower pinnae are 5 cm. or more long and 1 cm. broad at base, lanceolate, gradually acuminate from a deltoid base, pinnatisect to a narrow wing along the costule, the ultimate segments possess sharply acuminate deltoid teeth. The texture is herbaceous,

the colour pale green but on drying sometimes pale brown. The veins are pinnate in segments and forked in large lobes. The lower third of the frond is sterile; sori are uniseriate along the costule of the pinnules; the indusium is thick, reniform, moderate sized, persistent brown, and glabrous.

This species is very much like *D. marginata* (Wall.) Christ but differs in rather slender habit and smaller size of all parts excepting the rhizome. The pinnules in the lower pinnae of *D. marginata* are generally much longer and 2-3 cm. broad, oblong-ovate, short acuminate or blunt, and the sori are larger, not so close to the costules of pinnules or the

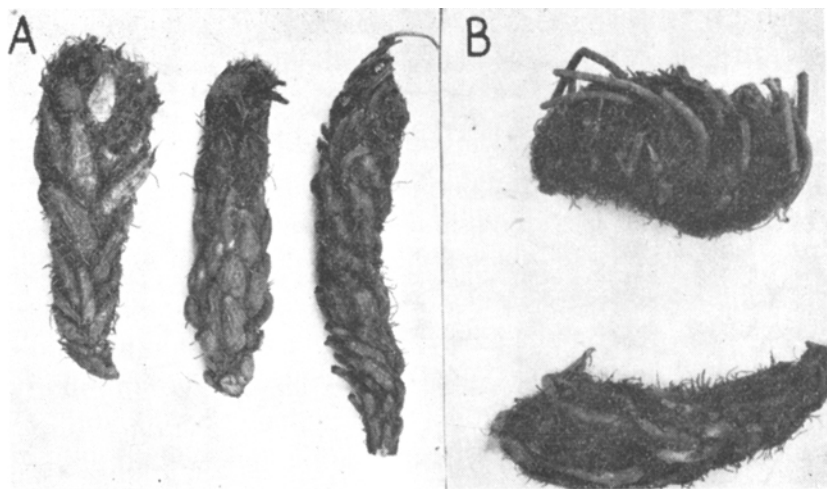


FIG. 2. A, *Dryopteris ramosa* rhizome  $\times 1/3$ ; B, *D. chrysocoma* rhizome  $\times 1/3$ .

midrib of segments in the lower pinnae. *D. ramosa* also resembles *D. blanfordii* (Hope) C. Chr. which may be distinguished by the dark ramenta and short frond base.

**Macroscopy of the rhizome.** The rhizome (Fig. 2A) is woody, stout, cylindrical and curved or nearly straight, tapering towards one end, 10-25 cm. long, 2-5 cm. thick (the thickness of the rhizome without leaf bases 1-3 cm.), externally reddish brown, covered over by hard, persistent, curved, flato-convex, reddish brown frond bases, the latter up to 1 cm. in thickness and somewhat wrinkled longitudinally or smooth showing transverse scars of fallen ramenta. The upper one-third to one-half of the rhizome and the frond bases are furnished with linear-lanceolate golden brown ramenta. The fracture is short. The transversely cut surface of the fresh rhizome is pale green, later turning pale brown, spongy, marked by whitish patches of 4-6 cauline meristeles and a few groups of 7-11 smaller vascular strands, each group is arranged in the form of closed "C" and constitutes a leaf trace.

The ramenta (Fig. 5A) are lanceolate to ovate, hair pointed, pale brown to golden brown, and measure 1-2 cm.  $\times$  2-6 mm. The texture is thin,

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the surface is more or less corrugated, and the margin wavy with a few inconspicuous marginal peg-like projections, each consisting of two parallel and contiguous cells (Fig. 5Bb).

*Histology of the rhizome.* The rhizome in transverse section presents the appearance shown in Fig. 3, and its cortical region on an enlarged scale is represented in Fig. 5E.

The epidermis is composed of a single layer of oblong to rectangular brownish cells which contain occasional tannin-resin masses. Many of the cells bear ramenta.

The hypodermis consists of a zone of 3-8 layers of thick walled lignified fibres which appear polygonal in transverse section. This region is compact and lacks intercellular spaces. In unstained preparations the

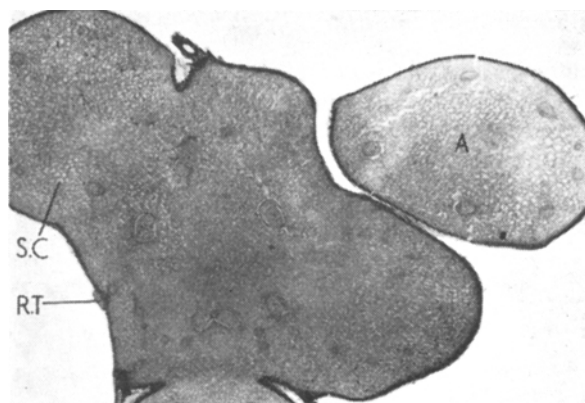


FIG. 3. *Dryopteris ramosa*, T. S. rhizome  $\times 3\frac{1}{2}$ ; A, T.S. stipe. R.T., root trace; S.C., schizogenous cavity.

walls of the fibres appear yellowish brown due to the deposition of a tannin like substance. In maceration they possess blunt ends and a few rounded pits on their walls.

The cortex consists of many layers of large parenchymatous cells with slightly thick cellulosic or suberised walls. They are often filled with starch grains which are roundish to oval in outline, 3-21  $\mu$  in diameter, and do not exhibit any marked striations. Irregular aggregates of tanoid masses are commonly present in the cells surrounding the meristemes and in the outermost layers of the cortex adjoining the hypodermis. In maceration and longitudinal sections the cells of the cortex appear predominantly rectangular in shape, each measuring 100-300  $\times$  35-65  $\mu$ . In transverse section they number about 500/mm.<sup>2</sup> Within the cortex as well as the pith are observed numerous small intercellular spaces and large schizogenous cavities (Fig. 5E). Into the latter project characteristic unicellular glandular hairs each of which possesses a short stalk and round head which under natural conditions is surrounded by orange yellow secretion. When stained with Sudan III these become very conspicuous (Fig. 6A). However, when the secretion is dissolved by suitable reagents

the glands appear hyaline (Fig. 6B). The schizogenous cavities are distributed all over the ground tissue but are more abundant around the meristemes and the outer zone of the cortex. The cavities number 50–70/mm.<sup>2</sup> in the area of maximum distribution. In longitudinal section they measure 500–1,400 × 50–112 μ, and each contains 3–10 unicellular glands. Often more than one gland is found attached to a single subtending cell. Each gland measures 50–80 μ in length, and consists of a distinct stalk, 20–30 μ long with a globular or rounded head, 25–55 μ in diameter. Since the glands do not arise with any regular orientation within the cavities they are commonly cut in various planes in transverse sections and consequently their true shape and structure is seldom revealed.

As a general feature the sclerotic nests are absent in the species, but very rarely isolated and imperfectly developed sclerosed cells may be observed (Fig. 5E).

Pith is well developed and is continuous with the cortex in the regions intervening the bundles. It is composed of parenchymatous tissue and resembles the cortex in all respects.

Cortex and pith are separated from each other by a ring of 4–6 cauline meristemes. Numerous smaller vascular strands are present in the cortex

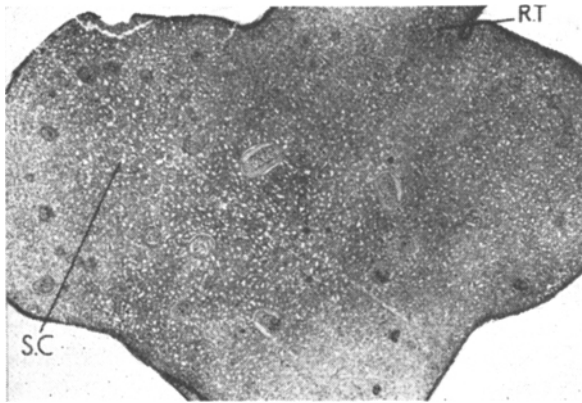


FIG. 4. *Dryopteris chrysocoma*, T.S. rhizome × 4 R.T., root trace; S.C., schizogenous cavity.

in the region of leaf gaps constituting the leaf traces. Each leaf trace is in the form of a semicircle comprising of 7–12 small vascular bundles (Fig. 3). Occasionally a root trace is abstracted from the outer limits of the cauline meristeme and is observed passing out through the cortex.

*Structure of a meristeme.* The structure of a meristeme is similar to that generally found in ferns.

*Histology of the stipe.* Fig. 3 at A shows transverse section of the stipe. It is oval in outline. The epidermis has dark brown outer walls. Attached to it are manyramenta, cut variously. The hypodermis is composed of 6–10 layers of thick walled, lignified sclerenchymatous

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fibres. The fibrous zone extends around the petiole except on the two lateral sides where the cells are broader and loosely arranged, forming a ventilating system for the petiole.

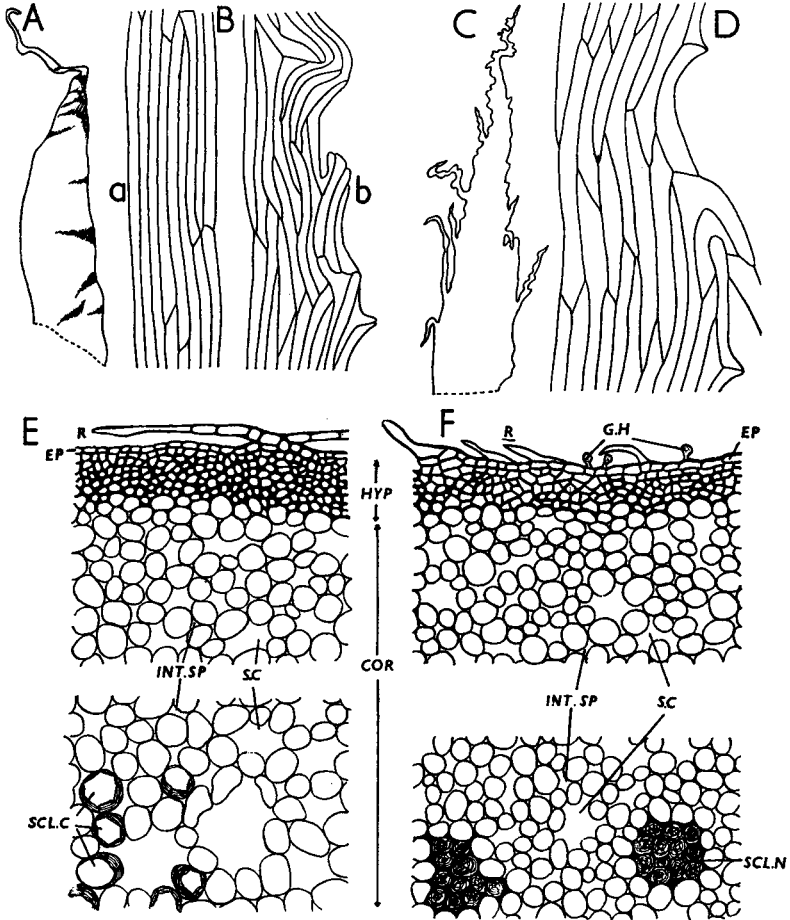


FIG. 5. A, *Dryopteris ramosa*, ramentum from rhizome  $\times 5$ . B, centre and margin of ramentum  $\times 80$ . C, *D. chrysocoma*, ramentum from rhizome  $\times 5$ . D, Margin of ramentum  $\times 80$ . E, *D. ramosa*, T.S. rhizome, a section magnified  $\times 80$ . F, *D. chrysocoma*, T.S. rhizome, a section magnified  $\times 80$ . COR, cortex; EP, epidermis; G.H, glandular hair; HYP, hypodermis; INT.SP, intercellular space; R, ramentum; S.C, schizogenous cavity; SCL.C, sclerosed cell; SCL.N, sclerotic nest.

The cortex is composed of many layers of parenchymatous cells similar to those of the rhizome excepting that they are much broader and shorter. They number only about 200/mm.<sup>2</sup> in contrast to 500 in the rhizome. The marked decrease in this number in the frond base is due to the cells being predominantly of larger diameter and the oleoresin cavities being much broader than in the rhizome.

The pith is similar to the cortex. Sometimes the free surface of the cells of the cortex and pith lining the schizogenous cavities are pressed inwardly and are transformed into crescent shaped cells. In between the cortex and the pith is a ring of 7-12 vascular strands. The number of true bundles in the petiole varies from 7-10 and the rest are smaller accessory strands connecting the bundles in their longitudinal course.

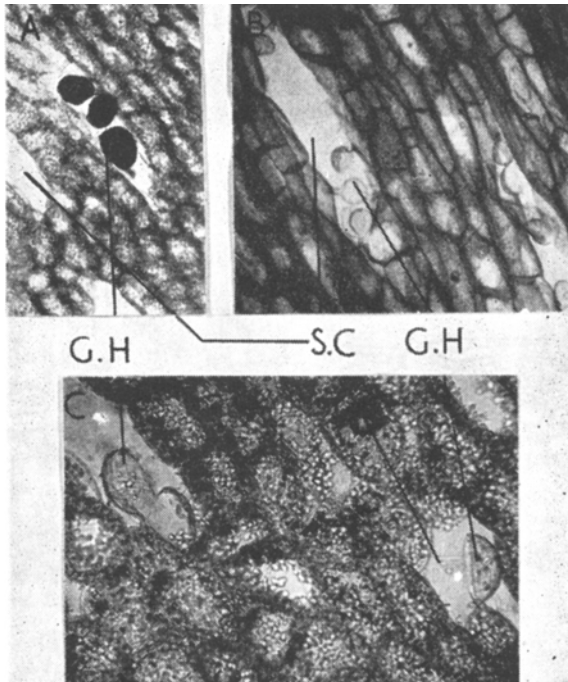


FIG. 6. A, *Dryopteris ramosa*, L.S. rhizome showing schizogenous cavities containing glands enveloped in oleoresin secretion, stained with sudan III  $\times 40$ . B, Unstained glands in the cavities  $\times 100$ . C, *D. chrysocoma*, unstained glands  $\times 166$ .

The two adaxial bundles are larger and conspicuous because of the inward notching of the xylem which forms prominent hooks. The structure of the individual bundle is on the same pattern as in the rhizome.

*D. chrysocoma* (Christ) C. Chr.

The species occurs in abundance around Darjeeling between the altitudes of 5,000-8,000 ft. On the way to Sandakphu from Tonglu (Dist. Darjeeling), the hillocks are almost covered with the species as if cultivated. It is also common along the Mussoorie-Tehri Road, between 6,000-8,000 ft.

The species is characterised by a short, ascending, thick and tufted rhizome. The stipe is up to 20 cm. long, stramineous, and densely scaly or muricated due to the persistent bases of fallen ramenta. The

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laminae are lanceolate, less pointed, bipinnate, acute, 30–60 cm. long and 8–20 cm. broad at the middle, the lower pinnae gradually shorten (Fig. 1B). The pinnae are alternate or sub-opposite in the lower part of the frond, they are sessile, lanceolate to ovate, sub-arcuate and the middle pinnae are up to 13 cm. long, while the basal ones are up to 6 cm. long. The ultimate pinnules are up to 20 in number, 2 cm. long and 5 mm. broad, sessile, with a broad base opposite or sub-opposite, oblong, crenate or lobato-incised. The venation is pinnate, veinlets bifurcating into the teeth, the sori are in two rows, one on either side of the midrib of the pinnae, dorsal on veinlets, distinctly kidney shaped, in 2–6 pairs. The indusium is large and light brown, crustaceous and convex.

*Macroscopy of the rhizome.* The rhizome (Fig. 2B) is cylindrical to conical, up to 15 cm. long and 2–5 cm. broad (without leaf bases only 1–3 cm. in diameter), densely covered with shining reddish brown chaffy ramenta which form a felt like covering at the apex. The stipe bases are hard, persistent, curved, spirally arranged; dark coloured wiry roots emerging in between the bases. The petiole is stramineous, 2–5 mm. thick, furnished at the base with cushion like mass of reddish ramenta and showing transverse scars of fallen ramenta on the upper part. The fracture is short. The transversely cut surface is yellowish green or greenish brown even on drying, and is marked by 5–8 cauline meristemes. In the peripheral region are present 3–4 leaf traces each comprising of 6–10 relatively smaller bundles arranged in the form of a closed "C." Dark spots which represent the position of sclerotic nests are observed in the rhizome (Fig. 4) as well as in the petiole. The taste at first sweetish, later becoming acrid and nauseous.

The ramenta (Fig. 5C) are lanceolate to ovate-lanceolate with a hair pointed apex, pale brown to chestnut brown, up to 3 cm. long and 6 mm. broad. The margin of the mature scale is markedly wavy bearing a few laciniae, and with twin celled peg-like projections common along the margin (Fig. 5D). Unicellular glands may occasionally be observed on the surface of the ramenta. Young ramenta at the apex of the rhizome are characterised by fimbriated margin.

*Histology of the rhizome.* A transverse section of the rhizome is represented in Fig. 4. The histological pattern is the same as described in the previous species except that a few dark patches of sclerenchyma or sclerotic nests can be observed in the ground tissue. The salient features are as follows.

The epidermis is formed of rectangular to oblong, brownish cells, and bears numerous cut ramenta and single celled epidermal glands (Fig. 5F). The hypodermis consists of 3–7 layers of sclerenchymatous fibres whose position is variable. This zone may be next to the epidermis, or it may be separated from the epidermis by 1–3 layers of cellulosic fibres. The cortex consists of many layers of isodiametric parenchymatous cells. The cells of the outermost region of the cortex and a few layers around the meristemes possess aggregate granules of tannoid material, otherwise the cells are packed with starch grains. The latter are oval to



oblong in outline and measure 2–16  $\mu$ . A few cells may occasionally contain globules of fat. In maceration the cortical cells appear round to rectangular, each measuring 60–140  $\times$  35–70  $\mu$ . In transverse section such cells number about 500/mm.<sup>2</sup>

Numerous intercellular spaces and schizogenous cavities are present all over the cortex and pith. The frequency of the latter varies from 40–60/mm.<sup>2</sup> in transverse section. In longitudinal section the cavities measure 200–480  $\times$  48–96  $\mu$ , and into each project 2–4 unicellular glandular hairs (Fig. 6C). The diameter of the head is up to 40  $\mu$ . Pith is similar to cortex.

As stated above a few sclerotic nests are scattered in the ground tissue. Each nest is composed of a few sclereids. The individual sclereid is polygonal in transverse section and on maceration appears as an elongated cell with pointed or blunt ends measuring 200–450  $\times$  30–50  $\mu$ . Its wall is markedly pitted and the lumen is almost occluded due to the thickening on the walls. The latter are impregnated with a colouring matter because of which the nests appear dark in colour.

There is a diffused circle of 5–8 cauline meristemes. About 6–10 smaller strands, arranged in the form of closed "C," constitutes a leaf trace and there are observed 3–4 such leaf traces in a transverse section of the rhizome (Fig. 4). The structure of the meristeme is the same as the ferns generally. Occasionally root traces are observed passing out of the cortex.

*Histology of the stipe.* The structure is similar to the previous species except for the presence of a few, dark coloured sclerotic nests in the ground tissue, and a number of unicellular glands on the epidermis. The number of vascular strands varies from 6–10. The cortical cells are relatively broader than the corresponding ones in the rhizome, and in transverse section number about 350/mm.<sup>2</sup>

Apart from the presence or otherwise of sclerotic nests, the general pattern of the internal structure in both the species is essentially the same.

The epidermis bears two types of appendages, single celled glandular hairs andramenta. Below is the hypodermis composed of fibrous cells which lack intercellular spaces. The cortex is parenchymatous. The important feature is the occurrence of varying number of schizogenously developed cavities in the cortex and the pith in both the species. Into each cavity project numerous, unicellular, glandular hairs which are enveloped in oleoresinous secretion. In *D. ramosa* scattered sclerosed cells occur in the ground tissue while in *D. chrysocoma* sclerotic nests are present. The stele is dissected and composed of 4–6 cauline meristemes in the former and 5–8 meristemes in the latter.

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