FOLIAR VASCULARIZATION AND HISTOLOGY IN THE CANELLACEÆ

BY N. PARAMESWARAN

(Department of Botany, Presidency College, Madras-5)

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DURING the course of a comparative morphological study of the Canellaceæ, the author had the occasion to investigate the petiolar vasculature and foliar venation in the taxa of the family. As an understanding of these features is essential for future discussions on the relationships of the family, the results are presented here. The early literature concerning the anatomy of leaves are those of Vesque (1882-83), De Bary (1884), Blenk (1884), Warburg (1895) and Rochebrune (1897). These reports, however, are largely concerned with general or only casual aspects. Van Tieghem (1899) has given a somewhat detailed account of the histology of the leaves, young stem, etc. Perhaps Solereder's "Systematische Anatomie der Dicotyledonen" (1899) contains the first detailed account on the anatomy of various organs in the family. Occhioni's (1948) work on Cinnamodendron axillare is of a monographic nature and is confined only to this species. Recently, Metcalfe and Chalk (1950) have contributed some more data on the general anatomy. That a comparative study of the petiolar vasculature, foliar vascularization and foliar histology over wide representatives of taxa is also useful in assessing the systematic relationships of the family, taken in combination with other features, has been demonstrated by Bailey and Nast for the Winteraceæ (1944 a, b) and for Illicium, Schisandra and Kadsura (1948). The same is also true for Alangium as has been recently demonstrated (Govindarajalu, 1958). These studies have proved significant also in ascertaining the range of histological, anatomical and morphological variabilities within the family, a knowledge of which affords reliable clues or criteria for the choice of diagnostic features of the taxa.

MATERIALS AND METHODS

The materials used in this study come chiefly from herbarium specimens. A complete list of materials with herbarium vouchers has been incorporated in the doctoral thesis submitted by the author to and approved by the Madras
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University (Parameswaran, 1960). Should any one be interested in the sources of materials the author would be glad to supply the necessary information and data on request.

The study of anatomy and gross structure of lamina necessitated the following types of preparations: (1) Cleared and unstained lamina, (2) Cleared and stained lamina, (3) Epidermal peels, and (4) Serial sections of lamina.

Clearing.—The siccate leaf material was immersed in simmering water in order to expel air. The treated material was removed into glass vials containing 2.5% sodium hydroxide and placed in a thermostat maintained at 60°C. The time required for clearing varied in different species. It was found, in some cases, necessary to keep the material for two days in the thermostat. After optimum clearing was obtained the material was thoroughly washed in several changes of water, until no trace of alkali was left. This material was then dehydrated in higher grades of alcohol and mounted in canada balsam. These preparations were used for determining the nature of mesophyll tissue, vein endings and crystal types.

Staining of cleared tissues.—Staining of the cleared lamina was carried out as per schedule given by Foster (1934) using tannic acid and iron chloride. The result was so good with thin and tenuous leaves as to give a complete picture of the epidermis, mesophyll, venation pattern, etc. The leaves were mounted in canada balsam.

Epidermal peels.—Features of foliar epidermis were studied from peel preparations. Small pieces of lamina were boiled in water and then transferred to the maceration fluid, made up of a 5% solution of chromic acid-nitric acid in equal proportions, to isolate the epidermis. Leathery leaves required placement in a thermostat at 60°C. As soon as the tissue became whitish the leaf bits were removed and washed in water. With the aid of a needle and fine brush the peels were separated. Both adaxial and abaxial epidermis were studied. A good staining of these peels was obtained by the use of Delafield’s haematoxylin and safranin.

Serial sections.—In almost all cases the siccate specimens were first re-expanded by treatment in boiled water. However, certain leathery materials required the agency of an “accelerator”. This was provided by a 2.5% solution of sodium hydroxide, the use of which had two effects on the tissues—that of expanding and slight softening. Care was, however, taken to see that no complete bleaching of the material occurred. Optimum results were obtained by pretreatment of the boiled specimens in a 2.5% sodium
hydroxide solution for about 12 hours at room temperature. Several washings were given after this treatment in order to remove all traces of alkali. Subsequent procedure was the customary one followed in paraffin method.

**Observations**

*Petiolar vasculature.*—The cortex of the petiole in the family as a whole is homogeneously parenchymatous. Only in one collection of *Canella alba* (L.F. 2909; Text-Fig. 4) and in *Cinnamodendron axillare* (Text-Fig. 2) three to five subepidermal rows of cells assume collenchymatous thickenings in varying degrees. The genus *Cinnamosma* is distinctive by the possession of a subepidermal layer of cells recalling the morphological features of a foliar hypodermis (Text-Fig. 1). In one collection of *Canella alba* (Pret. 2711/43·48) isolated stone cells are encountered embedded in the cortex (Text-Fig. 5).

The histological structure of the petiole in *Canella alba* is subjected to further variability. It may be emphasized that the variability is seen in the different collections originating from the same locality. Thus, within different collections from Cuba, a crescent-shaped arc of cells possessing incipient collenchymatous thickening confront the adaxial and abaxial sides of the petiolar vasculature strand (Text-Fig. 4). Such a feature is absent in *Canella alba* (Pret. 2711/43·58; Text-Fig. 5). Again, in *Canella alba* (L.F. 2909) nests of sclerenchyma fibres occur outside but adjacent to the arc of collenchymatous caps (Text-Fig. 4), while the remaining collections from the same locality fail to show such a feature.

The presence of collenchymatous caps on the adaxial and abaxial sides of the foliar vascular strand is not noticed in the remaining taxa of the family. In these, the sclerenchymatous fibres confront the corresponding sides of the vascular strand (Text-Fig. 6). Generally the fibres are disposed in a relatively continuous band on the protoxylem side, although often the band is broken up by parenchymatous cells. As a rule the fibres on the protophloem side always exhibit a lesser degree of morphological expression (Text-Figs. 1, 6). It is only in *Capsicodendron pimenteira* (Text-Fig. 3) that the sclerenchymatous band of cells encircles the vascular strand in an unbroken manner.

*Foliar vascularization.*—The size, form, thickness and texture of the lamina fluctuate very markedly in different taxa of the family, as does the venation pattern of the leaves. The differences are only one of degree and the pattern as such shows an underlying homogeneity. It is remarkable how the different species, disjunctively distributed, could show identical features.
TEXT-FIGS. 1-7
In general, the gross pattern of laminar vascularization in all the genera and species presents rather highly stereotyped features, thereby rendering it impossible to distinguish even groups of genera on this basis. Subtle differences, however, are noticeable not only between genera but also between species of the same genus, and it is possible that such variations are encountered in the leaves of one and the same plant: for example, young and old leaves, sun and shade leaves. Therefore, such variations as these cannot be taken to represent true differences between taxa.

The general features of foliar vascularization in the Canellaceae may be outlined as follows: The secondary and tertiary vein systems comprise of relatively thin veins. The outline of the areoles is somewhat polygonal, the number of sides varying from four to six. The degree of branching of the veinlets is extensive and uniform, the ultimate branches being bifurcate or trifurcate. It may be incidentally mentioned here that the leaves of *Pleodendron macranthum* (Text-Fig. 21) may be readily distinguished from the other taxa of the family not on the basis of foliar vascularization but on account of the presence of numerous crystal-bearing cells in the mesophyll, a feature which apparently obscures the stereotyped pattern of vascularization.

With reference to the minute characters of veins and veinlets there is again an emphasis of uniformity. However, the relative thickness of the veins varies depending upon the form, number and density of the sheathing sclerenchymatous cells. Species of *Cinnamodendron* and *Capsicodendron* may be placed at one extreme of the gamut representing the thicker veins and *Canella* at the other extreme illustrating the thinner veins.

The presence of sclereids associated with vascular bundles is a common feature for a majority of the family. Although the morphological expression of the sheathing cells is fairly uniform, the minute characters of similar cells situated terminal to the vein endings significantly differ among taxa. The extent of diversity in the minute features, it may be remarked, is rather amazing as compared with the stereotyped pattern of gross vascularization.

In *Canella alba*, *Cinnamosma macrocarpa*, *Cinnamosma madagascariensis*, *Warburgia stuhlmannii* and *Warburgia ugandensis* the pattern of distribution and structure of the sheathing sclerenchyma are similar. In these species the sclereids sheathing the veins are elongate, unbranched cells with uniformly thick walls. The side walls of these cells contain simple elongate pits (Text-Figs. 11, 12). Associated with this type of cells are the sclereids that confine themselves to the terminal part of the veinlets. They are either solitary or in clusters. The wall pitting in these cells differs from the above-mentioned
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Text-Figs. 8-17
in being either elongated and extending right across the cell, or small, alternate and few (Text-Figs. 11, 12). In *Cinnamosma fragrans* and *C. madagascariensis* var. *namoronensis* the vertically sheathing sclerenchyma cells show simple circular pits with thick walls, while the terminal sclereids are relatively large with circular pits (Text-Fig. 8). In *Cinnamosma madagascariensis* the sheathing sclereids are more nearly fibre-like with simple circular pits while the terminal sclereids are elongated to a lesser degree with broader lumen, however, retaining the same type of pitting. In *C. madagascariensis* var. *namoronensis* certain sclerenchyma cells about the veinlets are unpitted and possess extremely thick walls (Text-Fig. 8). In *Cinnamodendron* and *Capsicodendron* the elongate sclerenchyma cells flanking the veinlets possess simple pits of circular outline. Terminal sclereids are absent in these genera (Text-Fig. 10). In *Pleodendron macranthum* the sclerenchyma flanking the veins are elongate structures with more or less scalariform pitting on their side walls. The terminal sclereids are unpitted (Text-Fig. 9).

A résumé of the sheathing sclerenchyma of the veins and the terminal sclereids may be summarized as follows:

(a) Pits on the sheathing sclerenchyma transversely elongate—*Canella, Cinnamosma macrocarpa, C. madagascariensis, Warburgia stuhlmannii, W. ugandensis*.

Pits on the sheathing sclerenchyma circular—*Cinnamodendron*.

Pits on the sheathing sclerenchyma scalariform—*Pleodendron*.

(b) Pits on the terminal sclereids transversely elongate or/and oval, opposite—*Canella, Cinnamosma macrocarpa, C. madagascariensis, Warburgia stuhlmannii, W. ugandensis*.

Pits on the terminal sclereids circular—*Cinnamosma fragrans, C. madagascariensis* var. *namoronensis*.

Terminal sclereids unpitted—*Pleodendron*.

Terminal sclereids absent—*Cinnamodendron, Capsicodendron*.

**Foliar histology.**—In the Canellaceae the cuticular thickening is generally pronounced on the adaxial than on the opposite side. In diverse species, the thickness of the cuticle varies within the same genus from 4 μ to 8 μ. In both the species of *Capsicodendron* and in *Cinnamodendron sampaoanum* the thickness of the cuticle is very pronounced (12 μ). It may be incidentally noted that the thickness of the cuticle does not correspond to that of the lamina. Obviously it is a feature that appears to be variable depending upon
external environment. The thickness of the lamina in comparable samples is highly variable even within genera and species. In *Cinnamosma fragrans*, *C. madagascariensis*, *Warburgia stuhlmannii*, *Cinnamodendron sampaoanum* and *Pleodendron macranthum* the maximum thickness is below 200 μ, ranging from 156 μ to 198 μ; in the remaining taxa the laminal thickness is above 200 μ, the range being 285 μ to 412 μ. The thinner extreme is represented by *Cinnamosma madagascariensis* (148 μ) and the thicker extreme by *Cinnamosma madagascariensis* var. namoronensis (412 μ).

The adaxial epidermis is uniseriate in all the species except in that of *Cinnamosma*, where there is always a subtending layer of hypodermis (Text-Fig. 19). The epidermal cell wall on the adaxial side is straight and polygonal in all the taxa except in *Pleodendron macranthum* where it is slightly wavy in contour (Pl. XII, Fig. 5). An abaxial epidermis, the side walls of which are straight and polygonal in outline, is shared by the majority of species except *Cinnamodendron sampaoanum*, *Cinnamosma fragrans*, *C. madagascariensis* and *Pleodendron macranthum* where the cells show a slight undulate contour. The epidermal cells are squarish in transection, their outer tangential walls showing a curved outline (Text-Figs. 13, 14, 15).

The bifacial leaves of the Canellaceae possess a well differentiated mesophyll tissue. The demarcation of the mesophyll into a typical adaxial palisade and abaxial spongy parenchyma is distinct in all the species (Pl. XII, Figs. 18, 28) except in *Pleodendron macranthum* and *Canella alba* (Pl. XII, Fig. 3) where a conventional type of palisade layer is absent; instead, the cells homologous with this layer are more or less polygonal in outline. In the leaves of species wherein a conventional type of palisade is present, it occurs in more than one layer on the adaxial side. The number of layers fluctuates even within different collections of the same species, the more common being three. There is a certain amount of variation in regard to the predominance of the spongy parenchyma as well. This tissue is loosely arranged in all the species with abundant intercellular lacunae. In the two species of *Capsicodendron* the cells are closely packed, thus minimizing the intercellular areas. The spongy tissue is the most predominant in species of *Cinnamosma* (Pl. XII, Fig. 2) occupying about three-fourths of the entire thickness of the lamina. Oil cells occur amidst the palisade and spongy tissues of the mesophyll.

The vascular system of the lamina conforms to the “embedded” type of Solereder (1899), a condition where the veins are surrounded on all sides by assimilatory tissue (Text-Fig. 7). As cautioned by Solereder himself, care has been taken to select veins of the same order, inasmuch as the
principal vein and all the larger lateral veins show varying "appearance of fibrovascular system in a transverse section and the differentiation of the mechanical tissue accompanying the vascular bundles", which differs as the vein is examined at its base or in its further course. In the Canellaceae the veins possess a jacket of chlorenchymatous cells. The lateral veins are flanked on the adaxial and abaxial sides with bands of sclerenchyma cells forming cap-like areas. The penultimate veins are enclosed by scattered sclerenchyma cells on the adaxial and abaxial sides. In this connection it is perhaps interesting to note that the main costal vein also shows varying amounts of sclerenchyma cells both on the adaxial and abaxial sides.

Stomata.—There are two stomatal forms in the family, the rubiaceous or paracytic (Text-Fig. 17) and the ranunculaceous or anomocytic (Text-Fig. 16).

**Taxa with rubiaceous stomata**
- Canella alba
- Cinnamosma madagascariensis var. namoronensis
- Cinnamodendron axillare
- Cinnamodendron sampaioanum
- Capsicodendron dinisii
- Capsicodendron pimenteira
- Pleodendron macranthum

**Taxa with ranunculaceous stomata**
- Cinnamosma fragrans
- Cinnamosma madagascariensis
- Cinnamosma macrocarpa
- Warburgia stuhlmannii
- Warburgia ugandensis

Thus, the genera Canella, Cinnamodendron, Capsicodendron and Pleodendron possess the rubiaceous type. Genera Warburgia and Cinnamosma (excepting C. madagascariensis var. namoronensis which possesses a rubiaceous stoma, Pl. XII, Fig. 6) possess the ranunculaceous type. On the whole, it may be noted that the ranunculaceous stomata are exclusively associated with the African and Madagascan taxa, while the rubiaceous is likewise associated with the taxa of the New World.

The stomata occur in level with the abaxial epidermis and generally their form conforms to an ellipse, the longer dimension coinciding with the vertical axis of the stoma (Text-Figs. 16, 17). The cuticular thickening around the stoma arises in the form of a vestibule (Text-Figs. 14, 15). This feature attains an exaggerated development in Capsicodendron. The distribution of stomata per unit area is a variable feature in the diverse species, and on account of the fact that ecological conditions determine, to a large extent, the frequency of distribution, no attempt has been made towards correlations.
However, in the african species of *Warburgia* and of *Capsicodendron* the distribution appears to be relatively denser than in other genera.

**Crystals.**—The presence of crystals in the lamina of the Canellaceae is neither so universal nor so extravagant as the oil cells. The most commonly occurring category of crystals is the druse. Less commonly single rhomboidal crystals are also encountered, but these are found only in the lamina of one collection of *Canella alba* (L.F. 2909; Pl. XII, Fig. 7) and in the secondary xylem of the same collection. In the lamina of *Warburgia* and *Cinnamodendron* the druses are distributed along the veins and veinlets. The bountiful presence of this type of crystal even obscures the venation pattern in *Pleodendron macranthum* (Pl.XII, Figs. 4, 8). The various types of crystals reported in the lamina of *Cinnamodendron axillare* by Occhioni (1948) are not met with in the materials available to the present author. It is likely that such gross and monstrous crystal types are only artifacts produced by reaction with potassium or sodium hydroxide used for clearing the laminal tissues.

In the cortex of the petiole of *Canella alba*, *Cinnamosma fragrans*, *C. macrocarpa*, *C. madagascariensis*, *C. madagascariensis* var. *namoronensis*, *Cinnamodendron sampaioanum* and *Pleodendron macranthum* druses are present in a rather meagre quantity.

**CONCLUSIONS**

The nodal anatomy of the family Canellaceae is remarkably stereotyped and constant. Three foliar traces are related to a corresponding number of lacunae in the vascular cylinder of the stem. Thus, all the taxa of the family show typical trilacunar nodal structure. Soon after the traces enter the petiole, they undergo a lateral concrescence so as to form a broad shallow arc of vasculature as seen in transection. At the subcostal levels the strands again appear to split up into individual components to form the median and lateral veins of the lamina. Prominent secondaries are given out from the median vein throughout its length, which, in turn, anastomose with the rather poorly developed lateral veins. The intercostal venation is derived from the branch systems of the secondaries.

Foliar vascularization is so uniformly stereotyped throughout the family that no obvious trends of evolutionary modifications are recognized. As has been emphasized before, the only variable feature concerns the relative thicknesses of the categories of veins. It has also been observed that the ranges of variability overlap extensively not only between genera and species, but also between individuals of one and the same species, and reasonably within the same tree. Just as the vessel-ray and vessel-parenchyma pitting
constitute an exception to the stereotyped condition (author's own observation) of the remaining structural features of the secondary xylem, the minute morphological features of the sheathing and terminal sclerenchyma cells of the foliar vein system are subject to a high degree of diversity. Unfortunately these diverse features do not show correlation with any exomorphic, endomorphic or geographical factors. However, their value in checking up the diagnosis of certain taxa cannot be ignored.

In the majority of genera of the family, there occurs on the adaxial part of the mesophyll two or three layers of a conventional type of palisade tissue. Only in the Central American genera, Canella and Pleodendron, such a distinctive tissue is absent. The Madagascarean genus Cinnamosma is also perspicuous by the possession of a well-defined hypodermis in the costa and petiole.

Both rubiaceous and ranunculaceous types of stomata occur in diverse genera of the family. Broadly speaking the African and Madagascarean genera (with the exception of Cinnamosma madagascariensis var. namoronensis) possess the ranunculaceous type, while the Central American and New World genera possess the rubiaceous type.

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EXPLANATION OF TEXT-FIGURES

Text-Figs. 1-7. Figs. 1-6. Anatomy of the petiole. Fig. 1. Cinnamosma macrocarpa. Fig. 2. Cinnamodendron axillare. Fig. 3. Capsicodendron pimenteira. Fig. 4. Canella alba (L.F. 2909). Fig. 5. Canella alba (Prot. 2711/43-58). Fig. 6. Capsicodendron dinisii. (Symbols used to indicate tissues are given in square blocks.) All, × 16. Fig. 7. Warburgia ugan- densis. A single vein bundle enlarged to show the details, × 305.

Text-Figs. 8-17. Figs. 8-12. Histology of the vein endings. Fig. 8. Cinnamosma madagascariensis var. namoronensis. Fig. 9. Pleodendron macranthum. Fig. 10. Capsicodendron dinisii. Fig. 11. Canella alba. Fig. 12. Warburgia stahlmannii, All, × 300. Figs. 13-15. Transections of stoma. Fig. 13. Warburgia ugardensis. Fig. 14. Cinnamosma fragrans. Fig. 15. Canella alba (Cienfuegos), All, × 516. Fig. 16. Warburgia ugardensis: Surface view of stoma, × 300. Fig. 17. Canella alba: Surface view of stoma, × 300.

EXPLANATION OF PLATE XII

Figs. 1-8.

Fig. 1. Warburgia ugardensis: Transection of lamina, × 240.

Fig. 2. Cinnamosma fragrans: Transection of lamina showing the palisade tissue and a veinlet with abaxial and adaxial sclerenchyma caps, × 220.

Fig. 3. Canella alba: Transection of lamina. Note the absence of a typical palisade tissue, × 100.

Fig. 4. Pleodendron macranthum: Surface of cleared lamina showing the abundance of crystals, × 15.

Fig. 5. Pleodendron macranthum: Surface view of adaxial epidermis, × 15.

Fig. 6. Cinnamosma madagascariensis var. namoronensis: The abaxial epidermis with rubia- ceous stomata, × 450.

Fig. 7. Canella alba: Surface of cleared lamina showing rhomboidal crystals, × 220.

Fig. 8. Pleodendron macranthum: Surface of cleared lamina showing druses, × 100.