SCIENTIFIC SECTION

STRUCTURAL VARIATIONS IN ERYTHROXYLON LEAVES.

BY C. W. BALLARD.

(Continued from p. 453, June JOUR. A. PH. A.)

Erythroxylon brevipes, *DC.*—(Plate XVII.) This leaf averages 210 microns in thickness. The upper epidermis is very thick and consists of a single layer of nearly square cells with convex inner walls and extremely thick cutinized outer walls. The lower epidermis is comparatively thin, the free surfaces of the cells being convex; this formation is perhaps the forerunner of papillae. The convexity

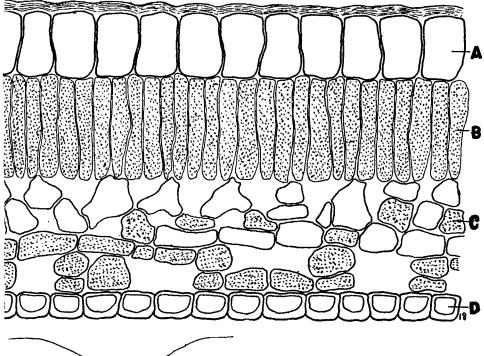


Plate XVIII.—Erythroxylon Urbanii, O. E. Schulz.

Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

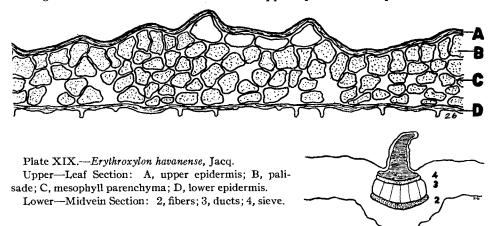
Lower—Midvein Section: 2, fibers; 3, ducts; 4, sieve.

shows a slight increase in wall thickness over the other portions of the cell wall and this strengthens the view that these free surfaces represent rudimentary papillae. The palisade cells are dark brown in color and in several instances are divided transversely so that this region may be in one or two layers in thickness. These cells vary in size to meet the convex inner walls of the epidermal cells. The number of cells abutting on an epidermal cell is three or four. Mesophyll paren-530 chyma consists of five to seven layers of isodiametric and flattened cells containing brown coloring material or chloroplasts. Prismatic calcium oxalate occurs abundantly within the cells of this region. The lower layers of parenchyma often show branching form, appearing in the section as circular cells.

Primary veins are deeply embedded in the mesophyll parenchyma and the amount of fibrous tissue in connection with them is so small as to cause but little displacement of the surrounding tissues.

Instead of a ridge on the upper leaf surface over the midvein, this specimen shows a slight depression or notch in this region. In general the tissues of the midvein are much reduced, with collenchyma practically absent. The lower surface of the vein consists of one or two layers of fibrous tissue within which occurs a narrow sieve zone. The sieve in turn is separated from the ducts by another layer of fibers, and a third layer of fibers is located on the upper surface of the ducts. This arrangement of layers of fibrous tissue alternating with sieve and duct zones is met with only in this specimen.

Erythroxylon Urbanii, O. E. Schulz.—(Plate XVIII.) Leaves of this specimen average 295 microns in thickness. The upper epidermis comprises about one-



fourth the total thickness of the leaf and consists of a single layer of radially elongated rectangular cells, the outer walls of which are heavily cutinized. This cuticle is thicker than in any of the other species examined and these cells are but slightly longer in a radial plane than broad. These epidermal cells are very regular in form and at least one of the leaves showed a white band between the inner wall of the epidermal layer and the palisade tissue, suggesting the possibility of a heavy cutinization of the inner as well as the outer walls. The side walls of these cells are thin and slightly wavy. The lower epidermis is devoid of papillae, the cells being rather thick-walled, especially on the sides and free surface. The palisade cells are brown, regular in form and five to six times longer than broad. Two or three cells abut on each epidermal cell. The mesophyll parenchyma consists of four to six layers of irregular and flattened cells, the latter often arranged in bands extending for considerable distance in the mesophyll. The collecting cells underlying the palisade are especially prominent. Many of the mesophyll cells are filled with a brown content. Large aerating chambers occur in this region. The cells of this zone may assume branched form and prismatic calcium oxalate is present.

The primary veins are provided with very thick layers of fibrous tissue on both sides and this tissue entirely displaces the palisade at the points of occurrence. These fibrous tissues do not extend to the lower epidermis, being separated from the latter by at least two layers of parenchyma.

No ridge occurs on the upper surface of the leaf in the midvein region and there is no apparent thickening of the lamina at this point. However, the upper surface of the leaf is slightly depressed over the midvein and the lower surface shows a corresponding elevation. Pericyclic fibers encircle the vascular elements and are thickest on the upper surface just below the midpoint of the depressed area. Collenchyma does not occur in this region.

Erythroxylon havanense, Jacq.—(Plate XIX.) Sections of this leaf are not at all uniform in thickness as both upper and lower surfaces show large elevations or ridges. Thus different parts of the same section range in thickness from 150 to 285 microns. Accurate details of the upper and lower epidermal layers are almost impossible of determination, as these parts appear as a rather thick cuticle with but little signs of cellular structure. The lower epidermis shows peglike projections somewhat similar, but more frequent and more pronounced than those occurring in a specimen marked *E. obovatum*, but subsequently identified as *E. areolatum* (Plate XXIII). The palisade is extremely irregular both in size and disposition, containing scattered chloroplasts and is at some points subjacent to what are apparently epidermal cells, especially in the projecting ridges of the upper leaf surface. Mesophyll consists of from three to seven layers of isodiametric and irregular cells surrounding large aerating chambers. Although prismatic calcium oxalate is present, the amount is very small.

Fibrovascular tissues of the primary veins are much reduced and are centrally placed in the mesophyll parenchyma. The ridges on the upper leaf surface do not mark the locations of these veins and there is apparently no displacement of any tissue excepting the parenchyma.

There is a very prominent ridge on the upper surface of the leaf over the midvein. This projection is narrow, with crenate margin and the interior is of collenchymatic tissue. Ducts form the major portion of the midvein bundle. Sieve is a narrow and indistinct zone on the upper side of the ducts. Fibers are almost lacking, being reduced to a narrow band on the lower side of the duct region.

Erythroxylon minutifolium, Griseb.-(Plate XX.) This leaf is the thickest of all the species investigated in this work and averages 350 microns. Sections were cut with great difficulty, as they showed a tendency to disintegrate. The upper epidermis consists of nearly square cells the outer surfaces of which are covered by a thick cuticle, the inner surface showing a corresponding thickening but in lesser Thus the palisade is separated from the epidermis by a light colored layer. degree. The lower epidermal cells are about one-third the size of those of the upper epidermis, with thickened outer and side walls. Papillae are absent. Palisade consists of a single layer of extremely long and rather irregular cells of brown color. These cells are six to eight times longer than broad. Two or three palisade cells underlie each epidermal cell. Mesophyll parenchyma forms about one-half the total thickness of the section, the cells being of isodiametric and branched types and arranged in five to seven layers. Those cells adjoining the lower epidermis are apt to be flattened and show thickened walls. Brown contents are present in a great number of these cells and the aerating spaces are large. Branched fibrous elements ramify in this portion of the leaf and prismatic calcium oxalate is present.

Primary veins are located more toward the lower leaf surface and the fibrous zones occurring in connection with these veins do not penetrate the palisade laver.

Instead of a ridge or crest appearing over the midvein on the upper leaf surface there is a slight indentation at this point. The midvein is deeply embedded

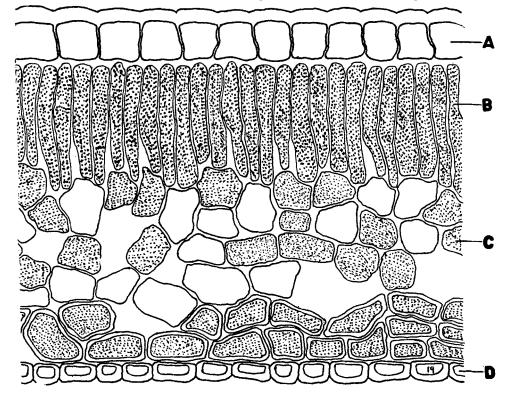
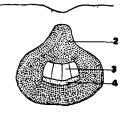


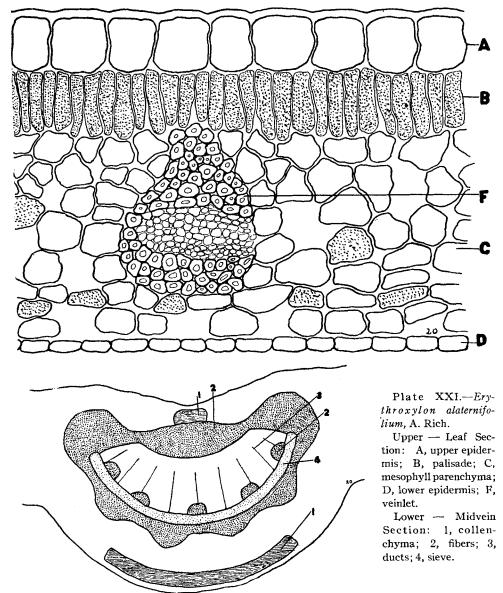
Plate XX.—Erythroxylon minutifolium, Griseb. Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower-Midvein Section: 2, fibers; 3, ducts; 4, sieve.



in the mesophyll and the vascular tissues are surrounded by a very thick layer of fibers. Ducts and sieve are much reduced and occur in the center of a mass of fibers.

Erythroxylon alaternifolium, A. Rich.—(Plate XXI.) Sections of this leaf average 315 microns in thickness. The upper epidermis consists of a single layer of large square cells, the outer walls being strongly cutinized. The lower epidermis is comparatively thin and devoid of papillae. Palisade is represented by a single layer of fairly regular cells as regards size and arrangement. These cells are filled with a brown content, are four to five times longer than broad and two to three are in contact with each epidermal cell. Mesophyll parenchyma is six to eight layers



of cells in thickness, isodiametric, irregular and flattened forms being present. The lower layers of parenchyma are apt to be of branched type and of brownish color. Branched sclerenchymatic elements ramify among the cells and prismatic calcium oxalate occurs freely. Large aerating chambers are present through the entire parenchymatic tissue.

The primary vein bundles are very large and in some instances the fibrous tissue extends toward the upper surface so as to partially displace the palisade, while in others the fibers are restricted to the mesophyll. In both instances a few layers of parenchyma intervene between the bundle and the lower epidermis.

The upper leaf surface above the midrib shows a slight indentation. Beneath the central point of this indentation is a small patch of collenchyma and a zone of this material occurs on the lower surface between the epidermis and the fibrous tissue. Pericyclic fibers completely surround the vascular elements, the fibers toward the lower side being in irregular groups and the amount of fibrous tissue being greatest on the upper surface. A secondary zone of fibers occurs between the ducts and sieve.

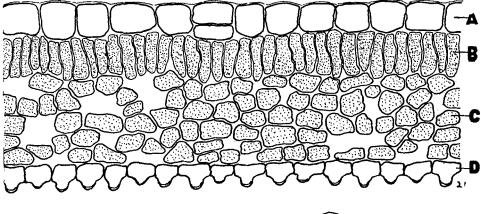
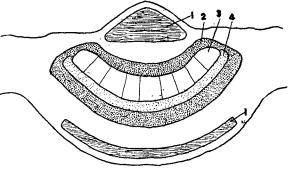


Plate XXII.—Erythroxylon obovatum, Macfad.

Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower—Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve.

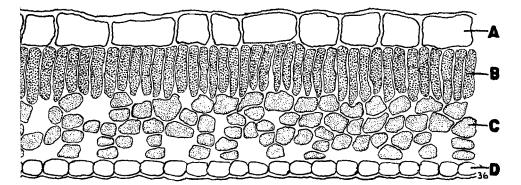


Erythroxylon obovatum, *Macfad.*—(Plate XXII.) Examination of four leaves from two herbarium specimens showed thicknesses ranging between 135 and 170 microns. The upper epidermis consists of rectangular cells which in some instances show subdivision into two. The tendency toward division of upper epidermal cells is more marked in this specimen than in the others examined. The outer cuticle is very thin. Lower epidermal cells bear prominent papillae. Palisade cells are rather irregular, about four times longer than broad and filled with chlorophyll. Two to four palisade cells adjoin each epidermal cell. Mesophyll parenchyma occurs in four to six layers of cells containing a brownish content or filled with chloroplasts. The lower layers are apt to show branched form and prismatic calcium oxalate occurs in moderate amounts. Fibrous elements in this region show a slight tendency toward branched form.

Primary veins are sufficiently large to partly displace the palisade cells but are separated from the lower epidermis by a few layers of parenchyma.

There is a slight ridge on the upper leaf surface over the midvein. Collenchyma occurs within this ridge and also as a narrow band on the lower side beneath the fiber zone. Pericyclic fibers surround the vascular elements, being more abundant on the upper surface of the bundle.

Erythroxylon Pelletierianum, Spreng.—(Plate XXIV.) Sections of this leaf average 145 microns in thickness. The upper surface is uneven and the epidermal cells are variable as regards size and form, ranging from tangentially elongated to



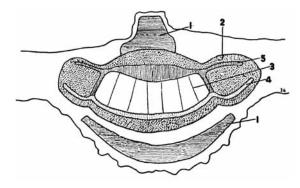


Plate XXIV.—Erythoxylon Pelletierinum, A. St. Hil.

Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower—Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve; 5, conducting parenchyma.

almost square types. The cuticle on the free surface is thick. The lower epidermis consists of rather small flattened cells entirely devoid of papillae and showing a moderately thick cuticle on the free surface. Palisade cells are filled with brownish content, are of varying sizes, and the ratio of height to width averages five to one. The number of palisade cells in contact with each epidermal cell depends upon the size of the latter and varies from two to six. Mesophyll parenchyma consists of three to six layers of isodiametric cells, many of which are filled with a brown content. Prismatic calcium oxalate occurs mainly in connection with the fibrous elements of this region and, while many of the latter are of branched type, little evidence of thickening is apparent. Aerating chambers are rather small and in consequence the parenchyma zone appears fairly compact. Primary branch veins are very large and the fibrous tissue sheath completely displaces the palisade on the one side and the mesophyll parenchyma on the other; thus the vein extends from upper to lower epidermis. The irregular upper surface mentioned above is due, at least in part, to the large amounts of fibrous tissue causing a slight elevation of the upper epidermal layer over the veins.

The ridge on the ventral leaf surface above the midvein is flattened and similar to that occurring in *E. carthagenense* but is not as much raised above the general leaf surface. Collenchyma occurs both in the interior of the ridge and as a band between the bundle and lower epidermis. The vascular tissues occur as a large central group with a smaller group on each side, these side groups being separated from the central portion by a thin layer of conducting parenchyma. The entire vascular tissue, central as well as side groups, is surrounded by pericyclic fibers. The sieve zone is continuous on the lower side of the ducts and a mass of conducting parenchyma occurs on the upper side. This division of vascular tissues into a central and two subsidiary groups is peculiar to this species.

Erythroxylon nitidum, Spreng.—(Plate XXV.) This Erythroxylon possesses very thin leaves, sections averaging less than 90 microns in thickness. Despite

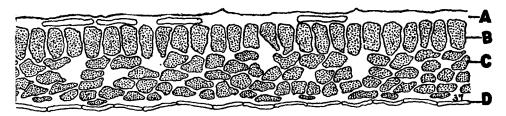


Plate XXV.—Erythroxylon nitidum, Spreng. Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

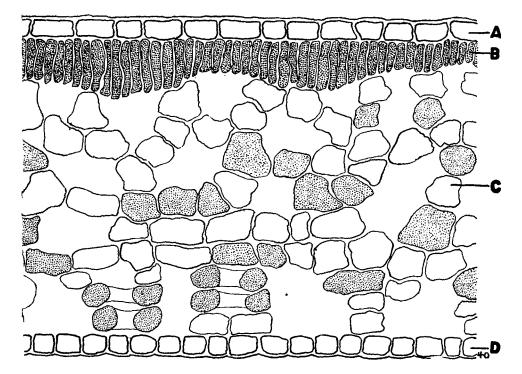
Lower—Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve; 5, conducting parenchyma.



repeated attempts it was impossible to prepare sections showing distinct cellular structure in the upper epidermis. The upper surface appears to be covered by a thick cuticle in which every here and there a few cells of an elongated epidermal type are visible. This upper surface also shows many ridges and projections appearing as local thickenings of the cuticle. Lower epidermis was in a better state of preservation and consists of an extremely thin layer of tangentially elongated cells devoid of papillae. Palisade cells are rather broad and irregular in form, being about onehalf as broad as they are high. A brown content completely fills these cells. Mesophyll parenchyma is in three to six layers of isodiametric to irregular cells filled with brown content and surrounding aerating chambers of fair size. Although prismatic calcium oxalate is apparent, the amount is very small.

Fibrovascular tissue of the primary vein branches fills the entire region between the upper cuticle and the lower epidermis.

The upper surface of the leaf is depressed in the midvein region and a small crest or ridge, directly over the midvein, is present at about the central point of this depression. This ridge is flattened as in E. carthagenense and E. Pelletierianum and its upper or flattened surface is about level with the general leaf surface. The interior of this projection is filled with collenchyma containing a brownish content.



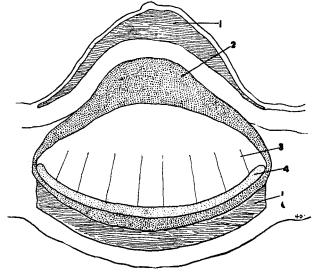


Plate XXVI.—Erythroxylon Columbianum, Mart.

Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower—Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve.

A very broad band of collenchyma is located between the bundle and the lower epidermis, extending into the leaf blade for some distance on each side. In proportion to its thickness, this leaf contains more collenchymatic tissue in the midvein region than any of the species examined and the amount of fibrous tissue is correspondingly reduced. There are no clear indications of fibers in the midvein bundle excepting two small groups on the upper side of the ducts and conducting parenchyma. The sieve is much reduced but conducting parenchyma is increased in amount and projects into the duct zone.

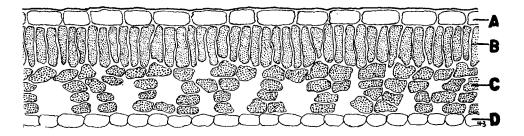
Erythroxylon Columbianum, Mart .-- (Plate XXVI.) Sections of this leaf range from 290 to 320 microns in thickness. The upper epidermis is comparatively thin and consists of tangentially elongated cells varying slightly in length and bearing a moderately thick cuticle on the free surface. The lower epidermis is slightly thinner but consists of the same type of cells, bearing a cuticle and without papillae. Palisade in this specimen shows extreme variability as regards height of the cells, but the variations are so graded as to give an undulated appearance to the lower surface of this tissue. These cells are filled with a brown content and three to five abut on each epidermal cell. The ratio of height to width ranges from two to one up to six to one. The mesophyll parenchyma zone is very extensive, consisting of up to twelve layers of cells and with a large part of this region occupied by the aerating chambers. Collecting or funnel cells appear in the upper part of this region and in general the whole tissue is made up of chains of cells connecting with these. Branched parenchymatic cells are present toward the lower epidermis and these appear in sectional view as two circular cells joined by a slightly constricted band Prismatic calcium oxalate occurs in small amounts both in the mesoor isthmus. phyll parenchyma cells and also in the lower epidermal cells.

Fibrous tissue of the primary branch veins is extensive and displaces a part of the palisade but does not extend to the lower epidermis, two or three layers of parenchyma intervening.

The upper leaf surface shows a large and wide elevation over the midvein. This is not the typical ridge of *E. Coca* and differs from the latter in its great width. Collenchyma occurs within this elevation and as a narrow band between the lower epidermis and the fiber zone. The amount of collenchyma in the midvein region is rather reduced as, because of the great amount of fibrous tissue present, this element is not required for adequate support. A band of cells with brownish contents intervenes between the upper collenchyma and the fiber zone. This brown band appears to be a continuation of the palisade across the midvein region but the cells are isodiametric with slightly thickened walls. Pericyclic fibers completely surround the vascular elements, being well developed at all points but greatest in number toward the upper surface of the leaf. The sieve zone is also well marked and the cells show a brownish content. Ducts occur in radiating rows alternating with rays of parenchymatic tissue and a large mass of the latter tissue occurs between the ducts and fibers toward the upper leaf surface.

Erythroxylon orinocense, H. B. & K.—(Plate XXVII.) Sections of this leaf average 105 microns in thickness. The upper epidermal cells are nearly alike in size and form, being of the usual elongated type. A moderately thick cuticle is developed on the free surface. Lower epidermal cells are about half the length of those of the upper layer and devoid of papillae. Palisade constitutes about onehalf the tissues between the upper and lower epidermal layers and the cells are variable in form. The chlorophyll throughout this leaf has apparently undergone partial oxidation and is of a brownish green color. The height of the palisade cells ranges from five times to twice their width, three to four cells abutting on each epidermal cell. Mesophyll parenchyma consists of three to four layers of isodiametric to irregular cells containing brownish chlorophyll and enclosing large aerating chambers. Prismatic calcium oxalate occurs especially in connection with the fibers and these crystals appear in sacs. The lower layers of mesophyll appear as branching cells. Astrosclereids extending into the palisade appear in some sections and not in others.

The primary branch veins are provided with a thick fibrous sheath which not only partially displaces the palisade but also causes an elevation of the upper epidermis at the points of occurrence. On the lower side of the veinlet the fibrous sheath extends to the lower epidermis.



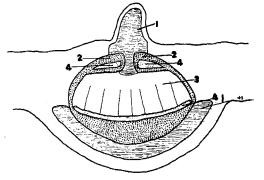


Plate XXVII.—Erythroxylon orinocense, H. B. & K.

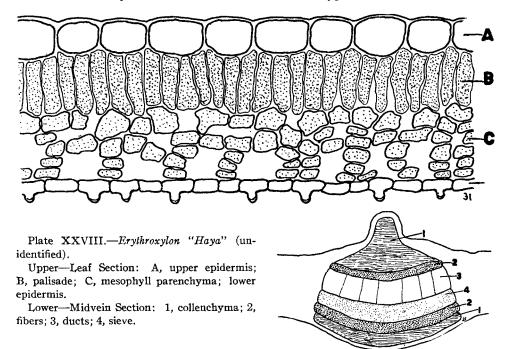
Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower-Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve.

A midvein ridge is fairly prominent on the upper leaf surface and is of a type similar to that in *E. Coca.* Collenchyma occurs both within the ridge and as a broad band between the lower epidermis and the fibrous layer. The cell walls of this tissue appear greenish and as if an infiltration of chlorophyll had taken place. The fibrous tissue completely encircles the vascular elements excepting a small area immediately beneath the ridge which is filled with collenchyma. Sieve is well developed and occurs in normal position, but two narrow strips of a similar tissue occur on the upper side of the duct zone. These strips are enclosed by fibrous tissues in which thick-walled ducts also appear. The ducts occur in radiating rows with layers of parenchyma intervening and a mass of the latter material is placed on the upper side of the ducts.

Erythroxylon, "*Haya*."—(Plate XXVIII.) Sections of this leaf average 170 microns in thickness. The upper epidermis consists of large oblong to nearly square cells, the free surface bearing a moderately thick cuticle and the inner wall being

decidedly convex. The lower epidermis is thin and many of the cells bear papillae. Palisade cells vary slightly in length owing to the convexity of the lower side of the epidermal cells and are slightly irregular in form. The proportion of height to width is about four to one and the chloroplasts appear to have collected at the upper ends of the cells. Mesophyll parenchyma is loosely arranged in four to seven layers and the lower part of this region contains large aerating chambers separated from each other by one or two vertical rows of cells. Each of the cells of the uppermost layer of mesophyll parenchyma acts as a collecting cell for several adjoining palisade cells, conforming to the irregularities of the latter. The lowermost cells of this region may be of branched character and appear as ring-like forms in sectional view. Prismatic calcium oxalate occurs mainly in connection with the fibrous elements and many of the latter are of the branched type.



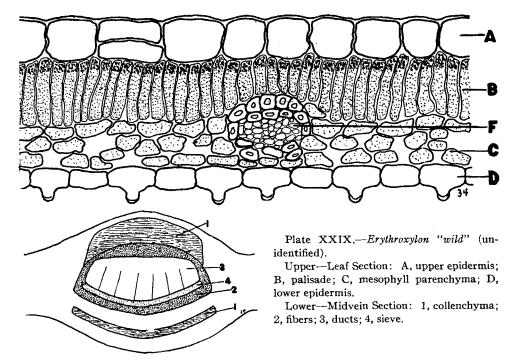
Primary veins are very large and displace both palisade and mesophyll parenchyma, thus extending from the upper to the lower epidermal layer.

The ridge on the upper leaf surface over the midvein is fairly prominent. The interior of the projection contains partially collenchymatized cells which extend inward to the fibers of the midvein bundle. A collenchyma zone also occurs between the lower epidermis and the midvein bundle. Fibrous tissues occur on both sides of the vascular tissues but do not form a complete ring around the latter and at best the amount of fibrous tissue is smaller than in the majority of species examined. Thus the various tissues of the midvein bundle are arranged in definite layers in the following order: fibers, sieve, ducts and fibers.

Erythroxylon--"Wild."—(Plate XXIX.) This leaf averages 170 microns in thickness. The cells of the upper epidermis are very large and for the most part

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nearly square, with the inner walls slightly convex. Occasionally these cells have undergone division, the two cells occupying but little more space than the undivided cell. A thick cuticle is present on the free surface of this upper epidermis. Lower epidermal cells are also large and frequently bear papillae of fair size with tips much thickened. Palisade appears as a uniform layer as regards size, form and arrangement, except in the vicinity of the branch veins. These cells are filled with greenish contents, but the outer ends show a brownish deposit which under low magnification appears as a characteristic brown zone immediately beneath the epidermis. Mesophyll parenchyma consists of three to five layers of flattened and isodiametric cells, most of which are filled with brownish material. Prismatic calcium oxalate occurs mainly in connection with the fibrous elements and crystal sacs within the fiber have been observed. Some of the fibers appear in branched



form and with rather thin white walls. The lower layers of mesophyll parenchyma may be of branched type, appearing in surface preparations as a net work in which each cell communicates with several others. Owing to the brown coloration this network is very plainly apparent through the epidermis in surface preparations.

The primary branch veins are located centrally in the leaf and while the fibrous sheath surrounding them encroaches but slightly upon the palisade it usually extends to the lower epidermis.

The upper leaf surface over the midvein shows an elevation which can hardly be compared with the distinct ridge occurring in several other species and the thickening of leaf blade in this region is no greater than occurs in leaves generally. A broad strip of collenchyma occurs on the upper side of the bundle and a narrow strip on the lower side. Pericyclic fibers completely surround the vascular elements and occur in greatest amount on the upper side. Sieve and ducts occupy normal positions and show no abnormal characters.

"Wild" Coca.—(Plate XXX.) This leaf averages 210 microns in thickness. The cells of the upper epidermis appear in large rectangular forms, occasionally occurring in two layers, and with the free surface strongly cutinized. Papillae occur freely on the cells of the lower epidermis and the thickest portion of the papilla wall is around the apex. The palisade occurs both as single and double layers, being irregular both as to size and form. Three to five cells abut on each epidermal cell. The mesophyll parenchyma consists of six to eight layers of cells surrounding

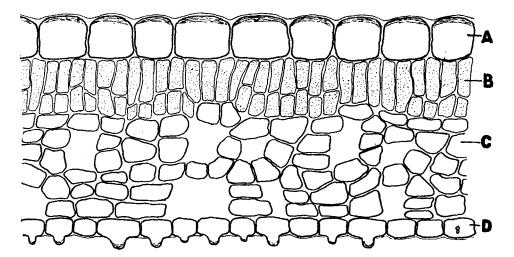
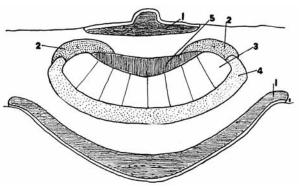


Plate XXX.—*Erythroxylon* (wild unidentified).

Upper—Leaf Section: A, upper epidermis; B, palisade; C, mesophyll parenchyma; D, lower epidermis.

Lower—Midvein Section: 1, collenchyma; 2, fibers; 3, ducts; 4, sieve; 5, conducting parenchyma.



large aeration cavities. Some of these parenchyma cells are of branched form. Calcium oxalate in the usual prismatic form is rather scarce, being more frequent in the fibers. Fibrous tissue in the mesophyll zone shows a slight tendency toward branching form.

The fibrous tissue of the primary veins displaces the palisade layer over these structures and the veins are so large as to fill the space between upper and lower epidermal layers to the exclusion of all other tissues.

The ventral ridge over the midvein is reduced to a mere projection consisting of collenchyma and the fibrous supporting elements of the midvein are largely replaced by collenchyma. The fibers appear in two groups toward the upper side of the bundle and extend for a short distance around the ends of the duct and sieve zones. Collenchyma is also well developed on the lower surface of the leaf in the midvein region. The sieve zone is well marked and scattered bast fibers occur sparingly in this tissue but the fibrous layer is incomplete on the lower side of the sieve. A mass of partially collenchymatized parenchyma is placed on the upper side of the duct zone.

SUMMARY.

The salient histological characters of the various specimens examined are presented in tabular form as Table 2, page 55.

Perhaps the outstanding feature in this investigation is the difference in histological structure between the leaves of different species. Coupled with this is the fact that these structural differences have been found to be constant in specimens from different sources. Therefore may we not assume that the hypothesis of constancy of histological structure for a given species rests upon a reasonable foundation?

The character of the epidermal layers, both upper and lower, the form of palisade and more especially the arrangement of the midvein tissues would appear to be of value in diagnostic work. The midvein structure has been brought forward by both Hartwich (3) and Greenish (1) but the former based his opinions upon the general form of the midvein fibrovascular bundle and Greenish has shown that the form varies in different parts of the leaf. In verifying the statement of Greenish by sectioning leaves of Erythroxylon Coca at different points I have found that the general arrangement of the midvein tissues is constant to within 10 mm. of the base and about 5 mm. of the apex. I am therefore satisfied that these midvein structures are of undoubted value in diagnostic work. The question of the crest or ridge on the upper surface of the midvein is often difficult to decide. To my mind the mere size or height of an elevation at this point should not be the deciding factor. Many of the illustrations will show an elevation which gradually rises well above the general level of the leaf and it is doubtful if such an elevation constitutes a midvein ridge or crest, for a majority of leaves in other genera show a local thickening at this point. In view of these facts I am inclined to limit the term "crest" or "midvein ridge" to structures in which the elevation above the general leaf surface is at least equal to the base of the ridge.

Even with due allowance for variation in histological structure we may make use of the following characters in identification work:

- 1. Presence or absence of papillae in connection with the lower epidermis.
- 2. Presence or absence of a ridge or crest on the upper surface of the midvein.

3. Presence or absence of sclerenchymatized elements in the mesophyll and palisade regions.

- 4. The relative thickness of the upper epidermis.
- 5. The thickness of the cuticular layer in connection with the upper epidermis.
- 6. The constancy in form of the palisade cells.

The schematic arrangement or analytical key, page 56, is based upon these characters:

A distinct crest or midvein is indicated by +. Absence of a distinct crest is indicated by - and figures in connection with this sign represent the general elevation of the upper surface over the mid- rib. Absence of both crest and general elevation over the midvein is indicated by D and in these leaves a depression over the midvein was apparent.	Leaves of E. Coca (wild) E. Coca (wild) E. Coca (wild) E. Coca (cult.) E. Subracemosum E. pauciflorum E. Bangii E. Popayenense E. areolatum E. areolatum E. areolatum E. areolatum E. areolatum E. areolatum E. areolatum E. Truxillense E. Truxillense E. Truxillense E. Truxillense E. Trux. (Ceylon) E. carthagenense E. Trux. (Ceylon) E. carthagenense E. Novagranatense E. Novagranatense E. Novagranatense E. Novagranatense E. Novagranatense E. Wovagranatense E. Wov
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	++++++++++++++++++++++++++++++++++++++
	+ + + + + + + + + +
	++ ++ + + ++ ++ + Branched scleren- chyma in mesophyll.

July 1926

ANALYTICAL KEY	BASED ON HISTOLOGICAL CHARACTERS.			
Papillae present				
Distinct ridge or crest on upper	r surface of midvein			
Sclerenchymatized elements present in mesophyll				
Upper epidermis of large ce				
E. Coca (wild growing)-	-midvein fibers completely encircling vascular tissue; aerating			
	chambers small			
"Haya" Coca	-midvein fibers not completely encircling vascular tissues;			
	aerating chambers large			
Upper epidermis of small o				
E. Coca (Cultivated)	-lower collenchyma separated from fiber zone of midvein;			
E. Popayenense	aerating chambers large —lower collenchyma adjoining fiber zone of midvein; aerating			
E. Popayenense	chambers small			
Sclerenchymatized elements				
Sclerenchymatized elements absent in mesophyll Upper epidermis of large cells or with thick cuticle				
E. havanense	-upper surface roughened; midvein ridge bent			
E. Truxillense	-midvein fibers partly encircling vascular tissues; midvein			
	ridge rounded			
E. carthagenense	-midvein fibers completely encircling vascular tissues; mid-			
-	vein ridge flattened			
E. Novagranatense	-midvein fibers grouped at sides of vascular tissues; midvein			
	ridge rounded			
Upper epidermis of narrow	cells or with thin cuticle			
	-midvein fibers much reduced or lacking			
	-midvein fibers completely encircling vascular tissues			
Ridge or crest absent on upper s				
Sclerenchymatized elements	• • •			
Upper epidermis of large ce				
?Wild Coca	-massive collenchyma zone on upper surface midvein bundle			
Upper epidermis of narrow cells				
E. areolatum (Type B)	-midvein fibers completely encircling vascular tissues but			
T. C.	interrupted by collenchyma on lower side			
E. Spruceanum	-midvein fiber zone incomplete on upper surface and enclosing			
Sclerenchymatized elements	two small sieve groups on upper side of ducts			
-	etely encircling vascular tissues			
E. subracemosum	lower collenchyma zone adjoining fibers and thicker than			
13. subracemosum	upper; conducting parenchyma prominent on upper side			
	ducts			
E. cumanense	-lower collenchyma zone as thick as upper and both adjacent			
	to fibers; conducting parenchyma reduced in amount			
E. obovatum	-lower collenchyma zone thinner than upper and both sepa-			
	rated from fibers; conducting parenchyma absent			
Midvein fiber zone not con	npletely encircling vascular tissues			
?E. Coca (wild)	-fibers at sides of vascular elements; lower collenchyma zone			
	extensive			
Papillae absent				
Ridge or crest apparent on upp				
Sclerenchymatized elements				
E. pauciflorum	-lower collenchyma not in contact with fibers of midvein			
E. Bangii	-lower collenchyma in contact with fibers of midvein; fibers			
The anima second	completely encircling vascular elements of midvein			
E. orinocense	-lower collenchyma in contact with fibers of midvein; fibers			
	not completely encircling vascular elements of midvein;			
	two subsidiary sieve groups on upper side of ducts			

Sclerenchymatized elements	absent in mesophyll			
Upper epidermis of large	cells			
E. anguifugum	fibers not completely encircling vascular elements of mid-			
	vein; upper collenchyma zone extending to the ducts			
E. Pelletierianum	-fibers completely encircling midvein vascular elements;			
	lower collenchyma zone separated from fiber layers			
Upper epidermis of small cells				
E. Columbianum	fibers completely encircling vascular elements of midvein			
E. nitidum	-fibers in two small groups on upper side of midvein bundle;			
	supporting tissue largely collenchyma			
Ridge or crest absent on upper	surface of midrib; leaf surface depressed at this point			
Sclerenchymatized elements	present in mesophyll			
Upper epidermis of large cells, heavily cutinized				
E. minutifolium	collenchyma absent in midvein region; fibers strongly de-			
	veloped and completely encircling vascular elements			
E. alaternifolium	-traces of collenchyma in midvein region; subsidiary fiber			
	groups on lower side ducts			
Sclerenchymatized elements absent in mesophyll				
Upper epidermis of large cells, heavily cutinized				
E. brevipes	collenchyma absent in midvein region; fibrous elements in			
	three layers alternating with vascular elements			
E. Urbanii	-collenchyma absent in midvein region; fibers completely			
	encircling vascular elements of midvein			

CONCLUSION.

Early in the course of this investigation it became apparent that, aside from the narrow confines of the subject of leaf variations, the genus Erythroxylon offered excellent opportunity for proof or disproof of the value of histological data in connection with the usual procedures in systematic botany. The number of species and varieties in the plant world is constantly increasing and while in many instances this increase is justified, there is the possibility that in others it is not. Slight variations are so common in plants that it becomes a matter of individual judgment whether or not a given plant represents a form sufficiently different from those already described to warrant a new title. It is granted that the easiest way out of the difficulty is to coin a new name but this procedure is not founded on sound scientific principles and must ultimately result in bringing systematic work into disrepute. Even with the natural increase in the number of species, due to botanical exploration work and to the evolution of new forms, our volumes on flora are becoming unwieldy. On the other hand we must not go to the equally objectionable extreme of trying to make a plant fit the written description if the latter is clearly at variance with the material under consideration. Can we accept histological data in connection with morphological characters as an aid toward the happy medium between too few and too many species? If the results of this research are indicative of conditions in general, and it is not unreasonable to conclude that they are, the answer must be in the affirmative. The work of Solereder (15) dealing with the anatomy of the dicotyledons points the way and the use of histological characters in distinguishing between species is merely carrying the plan one step further. Therefore the following broad propositions are offered as worthy of further investigation:

1. Histological characters of the different species in a given genus, while showing a certain degree of agreement also show differences characteristic of the individual species. 2. The histological characters of a given species are sufficiently constant to warrant the admission of this evidence in systematic work.

3. In instances of doubt regarding specific or variational rank, histological data should be given equal value with morphological characters in determination.

While a review of the literature presented in summary form in another part of this work includes the conclusions of a number of botanists and pharmacognosists, these conclusions are largely based upon morphological characters and the variability of opinions has already been commented upon. With the exception of the studies of Hartwich, Greenish and Holmes, dealing with but few species in each instance, no references on comparative histological structure are available. This research has been concerned with the histological structure of a greater number of species and perhaps a greater number of individuals of the same species, and as a result the following conclusions are formulated:

1. That the morphological characters of the leaves of *Erythroxylon Coca* are greatly influenced by environment.

2. That the histological characters of the leaves are not as greatly affected by changes in environment as are the morphological.

3. The modifications in leaf characters as a result of escape from cultivation noted by Rusby (14) are substantiated.

4. Erythroxylon Novagranatense appears to be distinct from both E. Coca and E. Truxillense.

5. The series of specimens (*E. areolatum*) representing Jamaica plants are distinct from *E. Novagranatense*, contrary to the view of Trimen (9).

6. Leaves of a Ceylon grown plant are substantially identical in structure with E. Truxillense.

7. Papillae occur in several other species than those noted by Hartwich (3).

8. A crest or ridge may be present on the upper side of the midvein in *E. Truxillense* and in *E. Novagranatense*.

9. "Haya" coca as received, is a form of E. Coca.

10. Erythroxylon Truxillense, E. carthagenense and E. Novagranatense are all closely related.

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CHEMICAL EXAMINATION OF OVARIAN RESIDUE.¹

II. ALCOHOL INSOLUBLE, WATER SOLUBLE NITROGENOUS EXTRACTIVES.

BY FREDERICK W. HEYL AND BRYANT FULLERTON.

We have conducted the systematic examination of the extractives of ovarian residue, using the methods of Kutscher,² with the exception that preliminary percolations of the tissue for the purpose of exhausting the lipoids and alcohol soluble extractives have been carried out. The constituents of the alcoholic extract which may for example contain the betaines, and also the constituents of the filtrate of non-basic materials from the phosphotungstic acid precipitation will be reported upon later.

The phosphotung states precipitated from the aqueous extract from 4.53 kilograms of the desiccated tissue yielded 27.8 Gms. of organic material containing 4.466 Gms. nitrogen. This material yields 1.11 Gms. creatinine = 0.413 Gm. creatinine N.

The purine nitrogen amounts to 0.508 Gm. But a small portion of this was accounted for by the adenine and xanthine isomer isolated. In fact the usual procedure for the analysis of the purines accounts for less than 0.4 Gm. of purine or perhaps 30% of the purine nitrogen.

The so-called Silver II fraction was not examined quantitatively, but nothing was definitely isolated.

The Silver III or arginine fraction contained 1.06 Gms. nitrogen; yielded 1.2 Gms. arginine equivalent to 0.386 Gm. nitrogen, so that 36% of the nitrogen of this fraction is accounted for.

The lysine fraction containing approximately 1.0 Gm. of nitrogen is almost entirely of unknown composition, 0.71 Gm. of lysine picrate containing but 0.05 Gm. of lysine nitrogen being isolated (0.275 Gm. lysine).

Since it has been customary in this kind of work to report the products isolated, without designating the quantitative considerations it will be noted that the

¹ From the Chemical Research Laboratory of The Upjohn Company. Received for publication April 17, 1926.

² "Handbuch Der Biochem. Arbeits. Abderhalden," II, p. 1044.