MORPHOLOGICAL STUDIES ON POLYGALA SENEGA.*[,1]

BY PAUL D. CARPENTER.

Senega, the root of a small North American plant, Polygala Senega L., enjoyed a very early reputation as one of the new remedies produced by America. It has been official in every edition of the United States Pharmacopœia since 1820. The root is of considerable interest pharmacognostically because of the wide variation evidenced in its secondary development. It has been the object of this work to make a detailed study of this variation histologically, correlating with it certain evident macroscopical irregularities consistently observed in the root.

THE DRUG.

(A) DESCRIPTION OF THE FRESH ROOT.

The fresh roots of Senega are light yellow in color. They may occasionally attain a length of 20 cm. but for the most part they average about 10 to 15 cm. The main root just below the crown soon breaks up into two or more large roots which in turn freely branch producing many fine ramifications.

The main root near the crown is about 1 cm. in diameter in plants of 5 years or more and correspondingly smaller in younger plants.

The roots rarely grow straight but are more or less curved and "knotty."

There are two characteristic markings or formations in the fresh root. First, the larger roots are knurled or marked with cross lines. The lines are formed because of regions of lesser and greater diameter in the root which give rise to a series of bulges. They vary in size (longitudinal) from about 1 mm. to 10 mm. They are very pronounced on one side of the root and gradually diminish until on the opposite side they are completely lost, which gives rise to the second characteristic marking, a narrow slightly raised and smooth ridge running through the length of the larger roots. This ridge may be quite straight, or it may form in a long spiral around the root. It is at this area a prominent keel forms upon drying.

Transverse sections of the fresh root are usually widely ovate in shape. The keel subsequently appears at the narrow end.

(B) MACROSCOPICAL DESCRIPTION OF DRUG.

The drug occurs nearly entire with some of the larger roots as long as 15 cm. The average length is between 7 and 10 cm. and 2.0 to 10 mm. in diameter. They are branched and slenderly conical. Most of the smaller roots are broken off either in collecting or in drying and handling. The rather large knotty crowns (2–3 cm. in diameter) are included with the roots. The crown is composed largely of stem bases and numerous rose-tinted or purplish buds which give to the crown, as a whole, a decided rose or purplish color.

The larger roots are often curved and twisted. The twist is long, perhaps 180° or so in a root 10 cm. long. On the larger roots a characteristic raised ridge, running longitudinally and more or less spiral, following the twisting of the root, is frequently developed. It may be more pronounced on some roots than on others although there is some indication of it on all of the larger roots (3 mm. or more in diameter) and occasionally on some of the smaller ones. This ridge is called the keel. The keel forms upon drying and develops along that part of the root free from cross wrinkles (knurls) (Figs. 5, 6).

The knurls are less pronounced after drying than before but are nevertheless readily dis-

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] Part of a Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in the Graduate School in the University of Illinois.

Fig. 1.—*Polygala Senega.*—Transverse section of the root showing concentrate rings in the phloem.

Fig. 2.—*Polygala Senega.*—Photomicrograph of cortex. Note indication of cell division throughout this region.

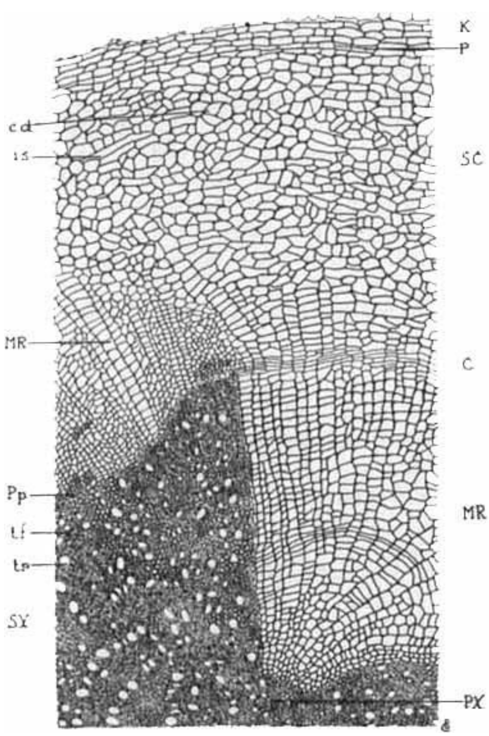


Fig. 3.—*Polygala Senega.*—Transverse section of the root.

K.—cork
P.—phellogen
c.d.—cells indicative of cell division
i.s.—intercellular space
S.C.—secondary cortex
C.—cambium
M.R.—medullary rays
M.R.—medullary ray (board medullary ray of the xylem)
P.p.—"phloem patch," sieve tissue
t.f.—fibre tracheids
tr.—tracheal tubes
S.X.—secondary xylem
P.X.—primary xylem

Fig. 4.—Photomicrograph of radial longitudinal section through the juncture of the parenchyma wedge and lignified portion of the xylem.

tinguishable in the drug. Most of the roots develop narrow and shallow longitudinal furrows after drying.

The color is brownish yellow, much darker than in the fresh root. The fracture is short. The drug has a peculiar and penetrating odor and a sweetish taste, afterward becoming acrid. In recently dried roots, or in those packed in tightly closed containers, there is a distinct odor of methyl salicylate which, however, disappears upon short exposure to the air.

(C) HISTOLOGICAL STUDIES.

1. *Lens View* (*T.S.*).—It would be impossible to describe all of the varied formations found in transverse sections of Senega root. Even to describe a "typical section" would be difficult, for there are no two alike and sections cut a few microns apart may show extreme differences (Fig. 7).

A description of a transverse section follows which in the main is often met with and may, with some flexibility, be called typical and at the same time comments will be made in general (Fig. 1).

The bark varies in thickness considerably, from 1 mm. at its narrowest point to 2.25 mm, at the widest. The xylem is eccentric and 2.3 mm. in diameter. The lignified tissue is broken by a wide medullary ray, where adjacent to the cambium it is 1.7 mm. wide and wedge shaped running to the primary xylem. The ray is only 0.8 mm. deep, thus the primary xylem is eccentric and more secondary growth is on that side opposite the wide medullary ray. In this specimen, it is 1.5 mm. thick.

The bark is easily differentiated into three regions. *First*, the cork which in unstained specimens appears as a narrow yellowish brown line, encircling the root. In stained specimens it usually takes up more of the stain than any of the other tissues and is then distinctly seen as a very dark line corresponding in color to the stain used.

Second, the cortex which is a narrow, light yellow region just inside the cork. It varies greatly in thickness; being thickest adjacent to the wide medullary ray. In this specimen it is 1 mm. It becomes gradually diminished in thickness until on the opposite side it may be only a few cells thick and hardly visible in this view. There is no apparent differentiation in the cells and the cortex appears homogeneous throughout.

Third, the phloem is well differentiated from the other bark regions. In the unstained specimen, it is of a somewhat darker yellow color than the cortex. In stained specimens, both regions stain equally. The phloem, however, is easily differentiated after slight magnification because of the regularity of its cells which give rise to two distinct groups of lines. *First*, medullary rays can be seen distinctly as fine lines running from the cambium out to the cortex. These rays, however, are not all straight, or radial, nor do they form all around the bark if a large medullary ray is present in the xylem as in the section being described. There are no rays opposite and adjacent to the cambium directly over the wide parenchyma wedge of the xylem (this ray does not usually continue into the bark as will be shown under the microscopic description). Near the tangential extremities of the wedge, that is away from the middle, short-curved medullary rays are plainly visible in the phloem. Farther away and approaching the opposite side of the root, the rays become very much curved and longer until directly opposite the wedge they begin to straighten out and are finally quite radial. At this region the phloem is very wide (2.25 mm.). It is here the keel forms.

The second group of lines seen in the phloem are concentric circles, especially distinct in the thicker regions, where in the specimen under discussion, there are some 12 to 14 lines. The lines closer to the cork are farther apart and they gradually become closer together until near the cambium they are only a fraction of a millimeter apart. These concentric circles of the phloem will be further discussed under the microscopic description.

THE XYLEM.

It is in the xylem region that extreme variations are seen in the lignified portion. As already mentioned the xylem is invariably eccentrically located in the

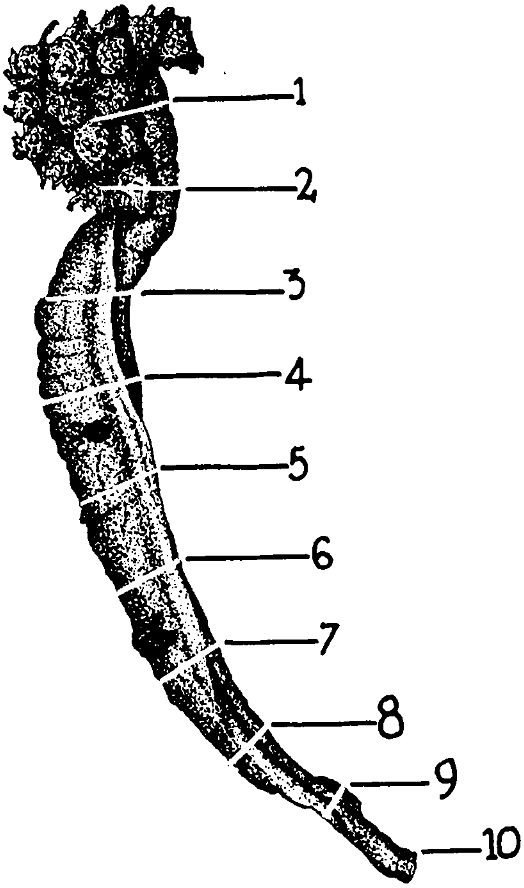


Fig. 5.—*Polygala Senega.*—Root from which transverse sections of Fig. 7 were cut at the various regions indicated by numbers 1 to 10.

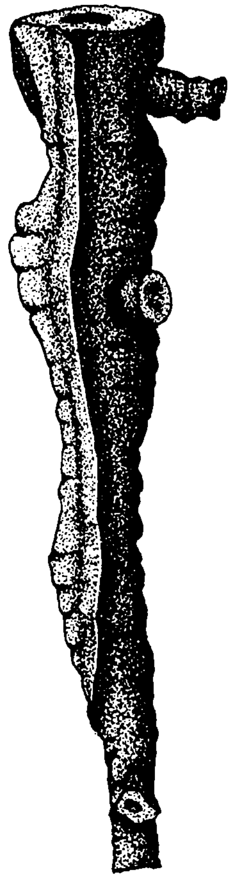


Fig. 6.—*Polygala Senega.*—Root from which model of xylem was made. The length of the root was 5 cm.

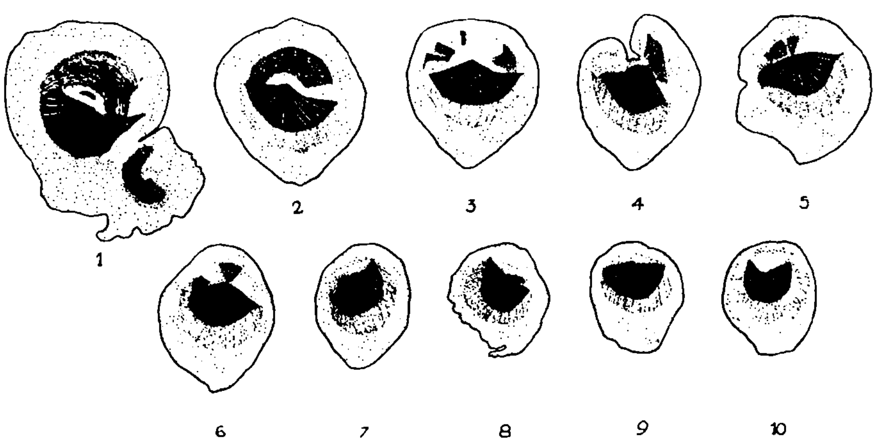
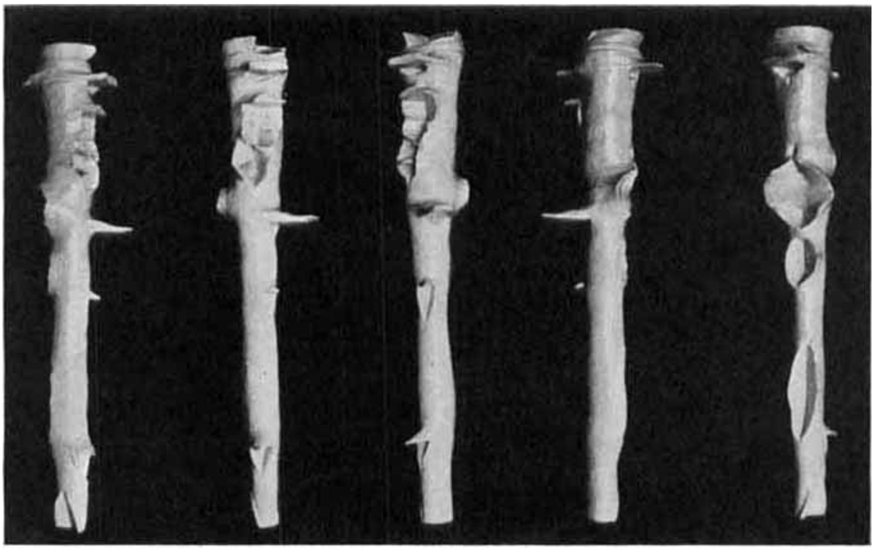


Fig. 7.—Transverse sections showing variations in the xylem of the root shown in Fig. 5, cut at the corresponding numbers.



0° 45° 90° 180° 270°

Fig. 8.—Photographs of model rotated through degrees indicated to show all sides.

root and it in itself is eccentrically formed, that is, the primary xylem is seldom in the geometric center. Even when it, as a whole, appears almost circular, it is found to have much more secondary development on one side than on the other. More growth is found on the same side as the greatest development in the phloem, or if a wedge of parenchyma is present on the opposite side to this. The lignified portion may vary from what appears to be a normal xylem (circular in transverse section) to a very small and irregular V-shaped xylem (Fig. 7). It is pointed out here that in practically all roots studied, the xylem as a whole is circular in transverse view. By definition the xylem of a root is all tissue "produced by the cambium and found inside of, and surrounded by the cambium." This then takes in the wide areas of parenchyma already referred to as wide medullary rays or parenchyma wedges. In most of the works on Senega, the term xylem has been loosely used to mean only the lignified portion of the entire xylem. Even in sections showing a V-shaped lignified area, the cambium can often be seen to form a circle in transverse section.

The cells of the wide parenchyma wedges of the xylem are in radial rows. By location, by origin, by arrangement of cells, by shape, these wedges of parenchyma are medullary rays definitely belonging to the xylem. The marked variation in the xylem, then, is not in it as a whole, but in the lignified portion of it, or which amounts to the same thing, wide variations in the medullary rays of this region. It is, however, convenient to use the term xylem to mean the lignified portion only and it will be used in this way except in specific instances where reference will be made to its more exact usage.

In an unstained specimen the lignified portion of the xylem is of a very pale yellow color, almost white in comparison to the bark. The various shapes may readily be observed in even the small roots by cutting transversely and examining the cut surface with a hand lens, or in the larger roots with the unaided eye.

Annual rings may be seen in sections either stained, or unstained, at a magnification of about 5 ×, showing much better at this mangification than under the higher power of the microscope. They appear as faint concentric lines especially well, if oblique light falls on the section. In the particular specimen used in the discussion so far, there were five such rings apparent, indicating the root is six years old. Very fine but distinctly seen radial lines, some straight, some slightly curved, are seen as narrow medullary rays in the xylem. There were about twenty-four in this specimen (they are more prominent toward the outer half of the xylem). The radial arrangement of the cells in the wide medullary ray can be plainly seen, especially in contrast to the cortical cells farther out.

2. *Microscopical Description. The Bark—the Cork.*—The cork layer of Senega root is relatively thin, seldom over 4 or 5 cells thick except in an occasional area of wound cork where it is thicker (10–20 cells) and in a few instances it has been noted that these cells are lignified.

In transverse section the cork cells appear oval to rectangular for the most part, but as this region approaches that portion of the root opposite the wide medullary ray, the cells tend to become more and more isodiametric and often appear almost round in this view.

The average size of the cork cells in transverse section is about 60 microns (tangential diameter) by 20 microns (radial diameter) although there is considerable variation from these sizes, especially in the tangential diameter. The cell wall is quite thin (1–2 microns) with only slight variations in thickness. In longitudinal radial view the cells appear more square and average about 20 x 30 microns with the radial diameter shorter than the tangential. In longitudinal tangential (surface) view, the average size is 20 x 60 microns, the cells being elongated tangentially. The middle lamella is not apparent in any of the sections.

The cork cells of Senega root do not form in the characteristic radial rows usually observed in most dicotyl corks. The cork appears rather as a layer of suberized parenchyma. Very often the outermost layer of cells (whole cells) shows protoplasts, again suggesting a resemblance to parenchyma. The cell walls are a light yellow color, the outer wall of the outermost cells slightly darker. There are partially disintegrated or decayed cells similar to the cork cells adhering as uneven broken fragments on the outer surface. Possibly these are old primary cells but because of their close resemblance to the cork cells just described, they are probably of the same tissue.

Phellogen.—Evidence of a phellogen is only occasionally seen even in very young roots. Many sections show none at all. Now and then, however, a single cell just below the cork appears to be a meristematic cell. Occasionally 3–6 such cells have been observed in a tangential row. The absence of a well-developed phellogen is not surprising, however, since there are (even in a 5-year old root) only 3–5 rows of cork cells. The phellogen cells as seen now and then are in transverse section about 8 microns (radial diameter) by 20 to 40 microns (tangential diameter).

Secondary Cortex.—The secondary cortex is made up entirely of living parenchyma cells which are filled with a substance globular in shape, varying in size and being oil-like in appearance.

These cells immediately below the cork are narrow and elongated tangentially. In transverse view they are about 15 x 60 microns. This type of cell comprises the first 4–6 rows of the outer cells. Sometimes they gradually widen until they become nearly isodiametric or only slightly tangentially elongated or they may remain about the same size and a sudden change in size and shape is then seen in the parenchyma lying adjacent to this narrow zone of cells. The cell walls at this juncture are very often much thicker than at any other place in the cortex. The author has seen them as thick as 12 microns, especially at the corners. The average wall thickness of the cortical parenchyma is 2–4 microns.

The cells making up the rest of the cortex are more or less isodiametric in transverse section, although they are as a whole slightly elongated tangentially. The cells in this region show no evidence of radial rows which one might expect if their origin was entirely from a secondary meristem. In the reporter's opinion this is because cell division takes place throughout the entire cortex during the life of the plant. With properly stained specimens many cells distinctly show evidence of recent cell division (Fig. 2). Often after staining, a cell that first appeared as a single cell will show 2 nuclei and a very thin protoplasmic wall, separating the 2 new cells. Others show a cellulose wall extremely thin, while still others show walls of varying thickness up to the maximum for this tissue. Mitotic figures have been seen in occasional cells.

PHLOEM.

The phloem is for the most part made up of parenchyma in radial rows. There is considerable variation in the size and shape of the cells, sieve is confined almost entirely to the inner half adjacent to the cambium and found in small isolated patches (Fig. 3). As described under the lens view, the region almost always shows more development on one side of the root than the other. The various differences are described in the cellular structure of the phloem working through the thicker portion from the cortex to the cambium and using the same specimen as in the lens view as a type. The phloem at its widest point runs to within 6 or 10 cells of the cork. It is not difficult to definitely see where the phloem and cortex join because of the regular radial arrangement of phloem cells in contrast to the irregularity of the cortical cells. This juncture is not even, however, some medullary rays running farther in the cortex, some less.

There are two groups of cells which form nearly concentric bands in the phloem alternating with each other and gradually becoming closer together until when rather near the cambium, they are only a few cells apart.

The parenchyma cells of the phloem in transverse section vary from 5 to 50 microns in diameter. The larger ones are closer to the cortex and gradually diminish in size until near the cambium they are extremely small. It is in this portion of the phloem that sieve tissue is found in small isolated patches.

The parenchyma varies in shape from isodiametric to rectangular cells. Invariably the elongation is in the tangential diameter. The isodiametric cells form bands in the phloem. The bands are from 2 to 8 cells wide and alternate with similar bands of the tangentially elongated cells. These give rise to the concentric lines seen in the lens view. The isodiametric cells average, in the outer portions of the phloem, about 35 microns. The cell walls are about 4 microns thick, showing only slight variations in thickness and composed of cellulose. Occasional simple pores are seen on all walls. The middle lamella often stands out very distinctly, especially in stained specimens in which case it takes up more of the stain than the thickened portions. The phloem parenchyma shows the same cell contents as the cortical cells.

The bands of tangentially elongated cells are not as wide as those composed of the parenchyma just described. Usually there are fewer cells in the ring and the radial diameter is considerably less. This type of cell averages in transverse section 35 by 10 microns. The cells do not break up the radial rows because their tangential diameter is the same as that of larger cells. The walls are somewhat thicker (5 to 6 microns). The bands closer to the cambium consist of smaller cells, although in radial number they remain about the same.

It will be seen from the foregoing description that the wide portion of the phloem is made up of two distinct types of parenchyma cells giving rise to alternating bands of tissue having different physical characteristics. This arrangement is referred to again under the discussion of the keel.

The sieve tissue in transverse section is seen in small patches close to the cambium and sometimes out to about the middle of the phloem; seldom farther. Each patch is made up of about 15 to 20 cells. The average sieve cell is 15 microns in diameter and isodiametric in transverse section. Associated with the sieve cells are smaller cells (companion cells) about 7 microns in diameter. The sieve patches appear to be made up of cells with rather thick cell walls. This, however, is only relative and due to the small size of the lumen. The cell walls are about 3 microns thick which is often slightly thicker than the walls of the surrounding parenchyma. This frequently causes the sieve patch to be more refractive and stands out from the other tissues more pronouncedly.

Adjacent to the parenchyma wedge of the xylem the phloem may be entirely wanting or consist of a few cells in radial rows forming a continuation of the medullary ray through the cambium and into the bark for perhaps 5 to 10 cells. Opposite the middle portion of the ray there is less likely to be phloem tissue present while near the juncture of the ray and lignified portion of the xylem, the phloem is definitely present (Fig. 3).

The author has never seen sieve tissue in the phloem directly adjacent to the wide medullary ray of the xylem. If the lignified portion of the xylem is circular in transverse section and especially if the primary xylem is nearly centric, the phloem is quite normal in its development and forms completely around the xylem.

If the primary xylem is markedly eccentric, the phloem is also eccentric and forms about the same way as when the large parenchyma wedge is present.

Longitudinal Section of the Cortex and Phloem—Cortex.—The cells in the cortex, in either radial longitudinal section or tangential longitudinal section, show little difference in appearance from the transverse section already described. The cells are for the most part isodiametric. The cells near the cork which are tangentially elongated in transverse section are almost circular in the radial longitudinal section and elongated in tangential longitudinal section. They are, therefore, similar in arrangement and somewhat in size and shape to the cork cells. There is one noticeable difference in the size of the cortical cells which it is believed is significant and accounts for the peculiar rings or knurls seen in the fresh root and to some extent in the dried root. The cells at the narrowest diameter of the root, that is between or separating the bulged portions, are appreciably smaller than those found in the region of greater diameter. They are often more radially elongated also. Assuming that the cells in a given longitudinal plane all originate at about the same time, it can readily be seen that if a certain portion of the cells increase greatly in size and another portion only slightly, the surface area will be increased unequally and this in turn will cause a bulging where the greater increase in the size of the individual cells has taken place. As stated under the description of the root, the knurls do not run all the way around the root. There are none where the keel forms. The microscopic examination shows little or no cortex at this region and the cells are about equal in their development. The knurls in size (thickness) follow well the thickness of the cortex; as the cortex narrows down so do the knurls correspondingly decrease.

Often oblique bands of elongated cells are seen in the longitudinal sections which appear to be part of the cortex but are probably phloem cells of a lateral root. It is believed that in many cases the lateral roots leave the main root in a long curve rather than making a sharp turn. This would account for the fact that after selecting a small portion of root free from lateral roots on the surface, there still appears strong evidence of their presence in the various microscopic sections.

Phloem—Longitudinal Section—Radial.—All of the cells of the phloem are very much elongated. They average about 150 microns. Some will occasionally attain a length of 250 microns. The end walls of a radial row of cells are usually in the same plane and if a cut is made as nearly radial as possible, these end walls form almost a straight line across the field of view. If the cut is not radial or varies only slightly from it, the lines are lost because the end walls are not found in the same plane in tangential rows of cells.

The sieve cells are not easily differentiated in the longitudinal view. Being only 5 to 10 microns in diameter, they appear as long lines of cell wall material and can hardly be distinguished from the narrow parenchyma surrounding them. Occasional cells nearer the cambium are free from the characteristic oil-like cell contents and these are undoubtedly the sieve cells. The smaller companion cells seen in the transverse section are extremely difficult to observe in the longitudinal view.

The same bands of cells seen in the transverse section are also plainly visible in the radial longitudinal section. Those with the thicker walls are narrower and more highly refractive to light. In many of these cells the end walls are slightly thicker than the lateral walls.

In the bands made up of the larger cells, the only noticeable difference besides their size, is that in many cases, especially those nearer to the cortex, the end walls are more oblique or rounded, often giving the individual cells a cigar-like shape. Small intercellular spaces are seen at the juncture of such cells.

Longitudinal Tangential Section.—Since the tangential diameter of the phloem cells varies only slightly except that the diameter gradually becomes less toward the cambium, there is no marked difference in the appearance of the cells in a single section. In comparison to the radial view, however, there are besides the uniformity of the cells two noticeable differences. *First,* the end walls do not fall in the same plane and the tissue appears as a network of elongated cells. *Second,* the end walls are not straight across, that is, at right angles to the long axis of the cells but are oblique and often rounded. The end walls of the parenchyma of the phloem are therefore wedge shaped. Sections near the xylem show cells very difficult to differentiate because of their narrow diameter and relatively thick walls and appear more as a mass of lines, the end walls being indistinct.

3. Cambium.—The cambium forms a complete circle around the xylem. It is much more distinct adjacent to the wide parenchyma wedges of the xylem where it often forms a rather wide zone (Fig. 3, C.). In specimens that have been dried and subsequently softened, it is seldom seen except at this region. In fresh material, especially if collected during the growing season, it may be seen adjacent to the lignified portion but differs from that over the ray in that it seldom forms a "zone" but is only one cell wide for the most part. The cambium cells correspond in tangential diameter to the tissues lying adjacent to it (Fig. 3). The radial width is from 3 to 6 microns. The nucleus and the rest of the protoplast are very prominent. Iodine stain often brings out the cambium ring more pronouncedly because of the dense protoplasmic content.

4. *Xylem (Transverse, Tangential, Radial).*—The xylem may conveniently be divided into two parts, the lignified portion and the non-lignified portion. The former consists of tracheal tubes, wood fibres (fibre tracheids) and the narrow lignified medullary rays. The latter consists entirely of parenchyma and has been referred to several times before as the parenchyma wedge or wide medullary ray.

Transverse Section.—The elements of the lignified portion are first described in the order named.

Tracheal Tubes.—The tracheal tubes are oval to circular, a few showing more or less straight sides and are therefore somewhat angular. Most of them, however, are only slightly oval, the greater diameter being radial. They vary in diameter from 20 to 50 microns. Most of them average 40 microns, the larger and smaller being less frequent. Those of the primary xylem, which are plainly visible in all roots, are 5 to 15 microns in diameter.

The common cell wall, between either two tracheal tubes or between a tracheal tube and fibre, show slight variations; the average thickness being from 5 to 7 microns. Pores of a very peculiar formation are seen in the wall of the treacheal tubes. They appear as a chain of elongated small intercellular spaces about 5 microns long and 1 to 2 microns wide.

The tracheal tubes seldom occur in groups of more than two or three and are for the most part separated from each other by a few fibres or medullary ray cells. In a low power view the tracheal tubes can be seen to form in rather obscure tangential rows, more pronounced near rings of growth which are for the most part made up of fibres.

Wood Fibres or Fibre Tracheids.—The fibres are quite angular in this view varying from almost square to triangular to several sided. They vary in size from 5 to 18 microns. The thickness of the common cell wall between two fibres is about the same as that between the tracheal tubes and fibres, 5 to 7 microns. It is possible that as a whole the thickness is very slightly less between the fibres. The same type of pores is found in the walls of the fibres as those described in the tracheal tubes.

The fibres generally are elongated somewhat in their tangential diameter. This is especially noticeable when they occur in radial rows of 4 or 5 cells as they do in the annual rings. Generally there are only 1 to 3 fibres separating the tracheal tubes from each other.

The middle lamella stands out rather distinctly in unstained specimens, being more refractive than the secondary wall thickening. In stained specimens it takes up more of the stain.

The Medullary Rays of the Lignified Portion of the Xylem.—It is very difficult to distinguish between the fibres and medullary ray cells in the transverse view. The walls of both are lignified and are the same thickness and the cells are about the same size and shape. However, close observations under the high powers of the microscope will differentiate them. In general the medullary ray cells are radially elongated. It must be borne in mind that occasionally the fibres are also radially elongated or isodiametric. Also, sometimes a few medullary ray cells will be tangentially elongated or isodiametric.

In fresh material the medullary ray cells often show the characteristic cell contents of the bark parenchyma. This is not so easily seen in material that has been dried and later softened with water, nor is it seen in material which has been kept for some time in strong alcoholic solutions. The most constant and perhaps surest way to distinguish these cells from the fibres is by the type of pores found in their walls. The pores of the medullary ray cells are simple and in transverse view show the typical picture of this common type of pore, the middle lamella being free from secondary thickening for a short distance. They are about 1 to 3 microns in diameter and occur less frequently than do the pores of either the fibres or tracheal tubes.

The lignified medullary rays are from 1 to 4 cells wide and run in many cases almost to the primary xylem but never up to it. At the start of secondary growth in the xylem the cambium invariably produces several layers of fibres almost always free from tracheal tubes and in all the observations made, entirely free from medullary rays (Fig. 3, P.X.).

The Wide Parenchyma Wedge or Medullary Ray of the Xylem.—This type of ray is of course not always present in the transverse section of the root. About five of the sections cut (which totaled some 2000) were free from such non-lignified rays. Two or more may be seen in some sections. The individual cells of the unlignified xylem rays resemble very closely the parenchyma cells of the phloem rays, perhaps somewhat larger especially near the cambium. The cells form in remarkably straight radial rows (Fig. 3). They show considerable variation in size. Those nearer the center of the root are much smaller than those nearer the cambium. They gradually increase in size from about 8 microns to 40 microns. Those cells adjacent to the lignified cells show much less increase in size and remain throughout the length of the ray about the same size as the bordering lignified cells. Sometimes they also exhibit partially lignified walls (Fig. 4).

Occasionally a band of tangentially elongated parenchyma is seen to run through the ray. In the author's opinion this is formed as an annual ring because it is usually continuous with such a ring in the lignified portion of the xylem. The formation of rings in this region is, however, by no means constant (Fig. 3, M.R.).

The same cell content as found in the bark is also found in the parenchyma of the ray.

Longitudinal Tangential Section—Tracheal Tubes.—The tracheal tubes are made up of segments, or units, of about 160 microns long, although there is considerable variation from this. Many are much shorter. Occasionally one is seen that is no longer than it is wide. The end walls are only partially dissolved out so that the diameter of the opening between segments is less than the diameter of the tracheal tubes away from the ends. The end walls, or rather the portion remaining, is readily seen in radial view, and it is often impossible in the view to tell that only a portion of it has dissolved out. They are oblique, forming rather sharp angles with the side walls, and one segment dovetails with the section directly above or below.

The pores of the tracheal tubes in this view are long and slit-like, running very nearly at right angles to the long axis of the cell. The pores vary in size and give rise to two distinct types of markings, those with very narrow openings, 1 micron or less, and those with wider pores, 2 to 3 microns, which because of their length (5 to 10 microns) and closeness to each other, give rise to the typical reticulate form of tube. The pores are 3 to 6 microns apart and about equally distributed throughout the walls. It is because of their closeness to each other that they appear in the transverse section as a string of small openings in the walls. Although the sections may be very thin, they are usually greater than the longitudinal diameter of the pore.

Longitudinal Radial Section—Fibres.—The wood fibres are elongated, tapering cells, whose end walls dovetail together. They average about 225 microns long. The pores of the fibres are also slit-like but much shorter than those of the tracheal tubes. They differ also in that they are oblique and a shadow forming the so-called border is more distinctly seen around the pores of this tissue than those of the tracheal tubes. Often only a single row of pores is seen along one surface of the fibre. Their maximum length is about 3 microns and their width 2 microns or less. They occur in the walls about as frequently as those of the tracheal tubes (Fig. 4).

Longitudinal Tangential Section—the Lignified Medullary Rays of the Xylem.—It is in this view that the lignified medullary rays are most easily recognized. The cells are longitudinally elongated and resemble in general very short wood fibres. Most of the cells have tapering end walls, but frequently the side walls show some tapering and the end walls form at right angles to these. The average length of the cells is about 60 microns, but here again much variation is often seen. An isodiametric cell is not uncommon. In length they may approach the fibres.

The one factor that remains constant is the less frequent occurrence of pores in the walls. Often in this view an entire radial wall may not show a single pore. This is not due to the fact that there are actually a far less number of pores in the medullary ray cells, but due almost entirely to their very small size. The pores are only slightly elongated and are 2 microns, or less, in diameter, so although their centers may be about the same distance apart as the centers of the longer and larger pores of fibres and tracheal tubes, they are cut less frequently in sectioning, hence the apparent absence of pores on large portions of the walls.

On the tangential wall the pores appear as small bordered elongated dots. They are on the average about 6 microns apart.

The medullary rays vary in height from a few cells to many hundreds of cells, often being several millimeters long. Their width is seldom over 5 cells.

Longitudinal Tangential Section—the Wide Parenchyma Ray.—The parenchyma are essentially alike in this view. The cells are isodiametric and corresponding in size to their tangential diameter as seen in the transverse view. The cells of the sections cut closer to the cambium are larger than those farther in toward the center of the root. The cells do not occur in rows but rather form a network.

Longitudinal Radial Section.—The radial longitudinal section simulates the tangential longitudinal section in most respects. The following differences may be observed—medullary rays are rarely seen for their entire depth—visible portions of them show cells slightly radially elongated. The markings appear similar to those described in the longitudinal section tangential view.

In radial section the junctures of a large number of tracheal tubes appear obliquely circular. In contradistinction to this, these same junctures in tangential section appear as oblique lines. The end walls, therefore, before being dissolved out, lie for the most part obliquely in a tangential direction.

5. *Primary Root.*—The primary roots show a very simple and normal development. They are very small, almost hair-like, and the average primary root is 0.3 to 0.5 mm. in diameter.

The epidermis consists of oval-shaped cells in transverse section, the longer diameter being tangential. The average tangential diameter is about 18 microns and the radial 10 to 12 microns. The cell walls are about 2 microns thick, except the outer, which is thicker because of a cuticle 1 micron or so thick. Only occasional root hairs are seen.

The primary cortex is relatively large and is made up of rather large parenchyma. The entire cortex, however, is only 4 to 5 cells wide. The outer half consists of one or two rows of cells about 35 by 30 microns tangentially elongated. The next row of cells is usually somewhat larger and radially elongated, about 35 by 40 microns. Next to these, a circle of smaller cells 18 by 12 microns is easily recognized because of the sharp contrast in size with the cortical cells. This is the endodermis. The cell walls are hardly thicker than the cortical parenchyma.

The radial fibrovascular bundle is a diarch. The xylem is made up entirely of tracheæ, of which there are about 15. They are 5 to 15 microns in diameter. The two phloem patches on either side of the row of tracheæ consist of a few very small sieve tubes (15 to 20 cells). Occasionally larger parenchyma cells are also found in this region. The average sieve cell is about 6 microns in diameter in transverse section. These roots very soon become secondary and give rise to roots which at first appear to be normal, but very soon the eccentric xylem becomes apparent, which is always the result of secondary growth in Senega root.

(D) DISCUSSION OF SOME OF THE ABNORMAL DEVELOPMENTS EXHIBITED BY SENEGA ROOT.

In order to study the abnormal development in the xylem a model of the lignified portion was built. A typical root 5 cm. long was cut into 1135 consecutive sections, each mounted in glycerin jelly, and each in proper order and orientation with respect to the original root. With the aid of a camera lucida the lignified portion of the xylem of each section was drawn at a magnification of about 15 × onto cardboard. Each drawing was then cut out and numbered. The cardboards thus represented, to a fair degree, magnified sections of the xylem. They were then glued together in proper order one after another. To insure proper alignment in building up the model, a straight longitudinal cut was made through the bark of the original root before the sections were cut. Then, in drawing the outlines of the xylem, a ruler was placed parallel with this cut and a line drawn across the xylem.

With careful attention these lines were made to fall directly over each other in gluing the sections together. After all the sections were thus built up, a thin layer of plastic paint was spread over the surface to smooth out the slight ridges unavoidably formed by the layers of cardboard. The whole was then painted pale yellow, the natural color of the xylem (Fig. 8).

Several interesting observations may be made from the model. Although it is invariably stated that the keel forms opposite the wide medullary rays only, it will readily be seen by comparison with the original root (Fig. 6) this is not always true, for if it were true, in the particular specimen the keel should be markedly spiral at some parts of the root and entirely absent at others. It is on the contrary quite linear and uniform in size and shape.

At the outer portion of the medullary ray wedge, the lignified xylem exhibits a distinct flare, the edges of which often extend beyond the normal limit of the xylem cylinder.

It is also interesting to note that a rather wide but short medullary ray is formed immediately below each root branch. In cutting many other specimens, this condition has been found to be quite constant in Senega root.

It can also be readily understood why sections of the same root, cut rather close to each other, present such marked and widely different formations in the xylem. It is not unusual to cut transverse sections 1 mm., or less, apart and find in one section a normal circular xylem and in others a xylem, semicircular, or even V-shaped.

Perhaps the most interesting abnormal development in Senega root is the development of the keel upon drying. The literature I have been able to review has not adequately explained nor offered a satisfactory theory as to how the keel is formed. The following is offered as a physical explanation of their formation.

If one were to imagine a small piece of apparatus built from strips of metal and a block of wood in a manner to be described, it would be possible to observe a similarity in the physical make-up of it and the phloem of Senega root. If several strips of metal of varying lengths were securely fastened to a block of wood in such a way as to cause the strips to form in wide ellipses as indicated in Fig. 9, one could imagine these to represent the bands of radially narrow and thicker walled cells described under the miscroscopic description of the phloem. Now if a tenacious and adhesive material, which would shrink upon drying, were filled in between these bands, it would not be difficult to see that forces would result which in turn would cause a sharper bow to take place in the strips of metal (which resist any change in length) at their greatest diameter (Fig. 9a, b and c). This would appear as a ridge when viewed from the side. The adhesive material corresponds to the bands of larger and thinner walled cells of the phloem.

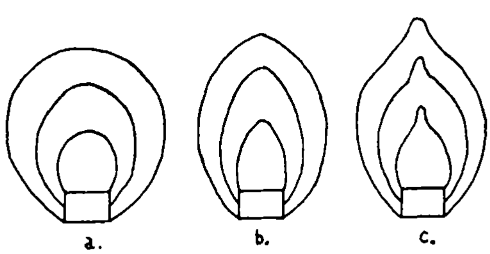


Fig. 9.—Diagram of apparatus to illustrate formation of keel.

While the bands of cells do not by any means simulate the actual physical properties of the metal and adhesive material, their behavior upon drying, due to their arrangement and cell characteristics, must correspond somewhat to the imaginary piece of apparatus.

In other words, there are in the phloem two sets of alternating bands, one composed of larger and thinner walled cells, which will shrink more upon drying than the second set, composed of narrow tangentially elongated and thicker walled cells which will resist shrinking and will, therefore, be subjected to the pull of the cells of the first set and as with the metal, a ridge will result.

The bands are more pronounced in some roots than in others, some showing practically no trace of them. Likewise, some roots form prominent keels, others do not.

In concluding, the author wishes to acknowledge the many valuable suggestions given by Professor E. H. Wirth, of the Department of Pharmacognosy, during the progress of this work.

THE HISTOLOGY OF CRACCA VIRGINIANA LINNÉ ROOT.*,[1]

BY B. V. CHRISTENSEN[2] AND ELBERT VOSS.

Cracca virginiana, Linné, root (Leguminosæ—devil's shoestring, goat's rue, catgut, etc.) has recently come into special prominence following the discovery of its insecticidal properties by Little (1), and the isolation of rotenone and tephrosin from the root by Clark (2). The fact that commercial samples of rotenone are at present limited to foreign sources, being obtained only from derris and cube roots, makes it highly desirable to develop a native source yielding an abundance of this insecticidal material. The United States Department of Agriculture, Bureau of Plant Industry, is at present making an extended study of *Cracca virginiana* root, with the hope of finding varieties of it which may be of insecticidal value or of value as commercial sources of rotenone. Many samples have been collected by the author from the Southeastern United States, and others in other sections, and sent to the department for tests of their insecticidal value and rotenone-yielding qualities. Through these tests there is being accumulated a large amount of information as to localities, soil types and varieties of the root yielding the best quality of crude drug.

Should the findings of the Department of Agriculture confirm the hope that *Cracca virginiana* root offers an American source for the valuable chemical, rotenone, it will be desirable to know its essential histological characteristics and the histological characteristics of related species in order that they may be differentiated. The object of the present work is to present the histological and morphological characteristics of *Cracca virginiana* root, along with the histological and morphological characteristics of closely related species of Cracca available in this locality.

In addition to the histological and morphological characteristics there are

---

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] From an abstract of a dissertation presented in partial fulfilment of the requirements for the degree of doctor of philosophy at the University of Florida, School of Pharmacy, Gainesville, Florida, June 1935.

[2] Director of the School of Pharmacy, Head of Department of Pharmacognosy and Pharmacology, University of Florida.