

THE PHARMACOGNOSY OF RAUWOLFIA

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INTRODUCTION

THE leaves and roots of *Rauwolfia serpentina* Benth. (*Ophioxylon serpentinum* Linn.), family Apocynaceæ, are mentioned in Sanskrit works describing Ayurvedic medicines under the name "sarpagandha" and are commonly known in India by the Hindustani name of "choota chand." This plant is indigenous to India, Burma, Malaya, Siam and Java, and the drug derived from it (hitherto described as root, but actually consisting of root and rhizome mixed) has been in use in indigenous medicine for several centuries in India. Its reputed successful use has attracted the attention of numerous writers on Indian Materia Medica, including Dymock¹, Watt², Chopra³, Nadkarni⁴, Khori and Katrak⁵.

HABITAT

The plant is described^{6,7} as a large climbing or twining shrub, found in the foot-hills of the Himalayas and in the neighbouring plains from Sirhind and Moradabad to Sikkim. It occurs in Assam, Pegu, Tenasserin (at altitudes up to 4,000 feet), in the Deccan peninsula along the ghats to Travancore and in Veylong. It is also widely distributed in the Malaya peninsula and in Java.

CONSTITUENTS

In 1931, Sen and Bose⁸, in Calcutta, isolated from the root two alkaloids having different melting-points. Siddiqui and Siddiqui⁹ in the same year, working in Delhi, found five alkaloids and arranged them in two groups, which they named the ajmaline and the serpentine groups respectively; Van Itallie and Steenhauser¹⁰ confirmed these findings in 1932. Other constituents in the roots are oleo-resins, sterols, unsaturated alcohols, oleic acid, fumaric acid, glucose, sucrose, an oxymethylantraquinone derivative, a fluorescent substance and mineral salts. J. C. Gupta *et al.*¹¹ state that the roots contain about 1.21 to 1.36 per cent. of total alkaloids, and that a standardised alcoholic extract containing 0.5 per cent. of total alkaloids is being made commercially.

PHARMACOLOGICAL ACTION

According to Siddiqui and Siddiqui⁹, the alkaloids of the ajmaline group, as tried on frogs, act as general depressants to the heart, respiration and nerves; while those of the serpentine group paralyse the respiration and depress the nerves, but stimulate the heart. Sen and Bose⁸ found from their experiments on cats that the alkaloids isolated by them brought about a slight fall in blood pressure and the respiration was slightly stimulated. The heart muscle was depressed and the plain muscle, like that of the intestines, uterus, etc., was relaxed. None of these actions represents the characteristic sedative and hypnotic effect for which the drug is valued. For this reason Gupta *et al.*¹¹ in 1947,

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working in India, reinvestigated the drug, giving their attention more especially to the pharmacological action of the oleo-resins present in the root. They report that these oleo-resins possess the specific sedative and hypnotic action of the drug which the alkaloids failed to produce.

DESCRIPTION

A description of the root, with brief notes upon its microscopical structure, was given in Dymock's "Materia Medica of Western India,"



FIG. 1.—*Rauwolfia serpentina* Benth. (= *Ophioxylon serpentinum*, Linn.). Drawing of a flowering plant (about $\frac{1}{2}$ natural size) from Wight, *Icones plantarum Indiæ orientalis*, 1840-53, plate 849. The flowers are in cymes; they have shining red pedicels and calyces, and pure white corollas. The fruits are black berries.

2nd edition, as long ago as 1885. Quite recently the root has been included in the Indian Pharmacopœial List of 1946, where the drug is represented by a brief monograph containing particulars giving information somewhat similar to that in the earlier description of Dymock. These accounts appear to be the only two available descriptions of the structure of the root. It is, moreover, obvious that the data given are not sufficient to characterise the drug properly, either in the unground condition or in the form of powder; also no reference is made to the presence of rhizome in the drug. In view of its promising therapeutic value and increasing importance as an article of commerce, it was decided to make a systematic pharmacognostical study of the drug.

MATERIAL

Four samples from different sources were examined, *viz.*:

1. Sample from the Museum of the Pharmaceutical Society of Great Britain. (July, 1947, S. K. Crews.)
2. Sample from a drug supplier in Saharanpur, U.P., India, obtained in December, 1947.
3. Sample received from the drug market in Calcutta in November, 1947, the identity of which was confirmed by S. N. Bal, Curator, Botanical Survey of India, Calcutta.
4. A fresh plant with the rhizome and a few fibrous roots attached, collected from the foot of the Himalayas near Saharanpur in December, 1947, by a drug dealer. This plant was examined by Dr. Chatterji, in charge of the Indian section of the Herbarium at the Royal Botanic Gardens, Kew, and was confirmed as belonging to the family Apocynaceæ and agreeing in all particulars with the specimen of *Rauwolfia serpentina* Benth. in the Herbarium at Kew.

MACROSCOPICAL CHARACTERS

Rauwolfia roots (Fig. 2, A) occur in commerce in pale brownish-grey pieces about 4 to 10 cm. in length and 4 to 17 mm. in diameter, cylindrical or slightly tapering, rather tortuous, rarely branched, with occasional small oval or rounded scars of rootlets, usually in a tetrastichous arrangement; the rootlets themselves are very few and when present are broken off short, their diameters varying from 0.5 to 1 mm.; outer surface of the root dull, with somewhat coarse and irregular longitudinal ridges; the outer layers of the cork are soft and tend to scale off from the harder somewhat prominently ridged, yellowish-brown inner bark; patches of entire bark sometimes exfoliate exposing a hard, compact, pale yellowish wood which has a fairly smooth surface and a low density (about 0.3); occasionally pieces of aerial stem or rhizome occur attached to the root. A smoothed transversely cut surface (Fig. 2, B) shows a large pale-yellowish wood, which is compact and finely radiate and usually shows 3 to 8 growth rings. The xylem itself is very finely porous and the woody core occupies about 4/5th of the diameter of the root; surrounding the wood is a narrow yellowish-brown bark about 0.5 to 2 mm.

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wide; the root is starchy throughout and the fracture is short. The drug is almost odourless and has a bitter taste.

Pieces of the rhizome closely resemble the root, but are less uniform in diameter; they are somewhat knotty and tortuous. They are best

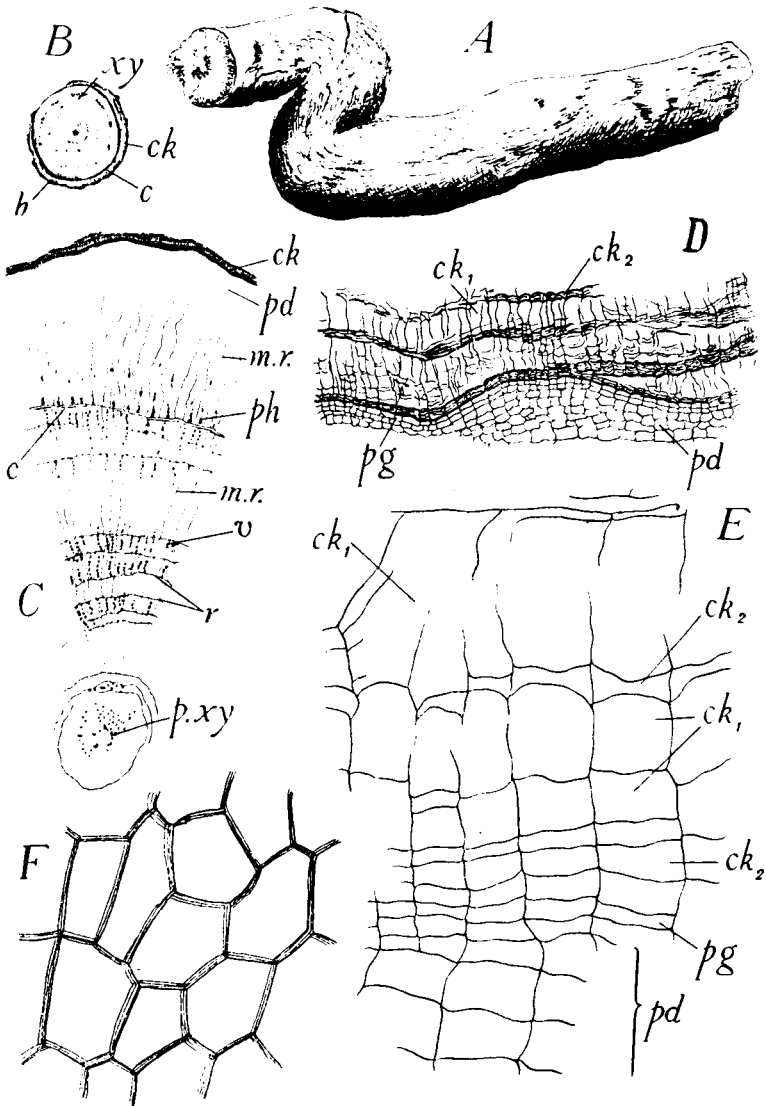


FIG. 2.—*Rauwolfia serpentina* Benth. Root. A, habit sketch of a piece of root $\times 1$. B, smoothed transverse surface of root $\times 1$. C, diagrammatic transverse section $\times 8$. D, transverse section of the tegumentary tissues $\times 40$. E, a portion of D to show details of the cells $\times 200$. F, cork in surface view $\times 200$. *b*, bark; *c*, cambium; *ck*, cork; *ck₁*, wide cork cells; *ck₂*, narrow cork cells; *m.r.*, medullary ray; *pd*, phelloderm; *pg*, phellogen; *ph*, phloem; *p.xy.*, primary xylem bundle; *r*, growth ring; *v*, vessel; *xy*, xylem.

distinguished by the smoothed transversely cut surface which exhibits a central pith having a very small diameter (about 1 to 2 mm.).

HISTOLOGY

Root: Externally there are several layers of approximately polygonal-tubular cork cells (Fig. 2, D, E and F) of two kinds, occurring in layers which in transverse sections form bands alternating with each other. One type of band consists of 1 to 5 layers of narrow cells with suberised but unligified walls measuring about *R = 7 to 11 to 18 μ , L = 18 to 35

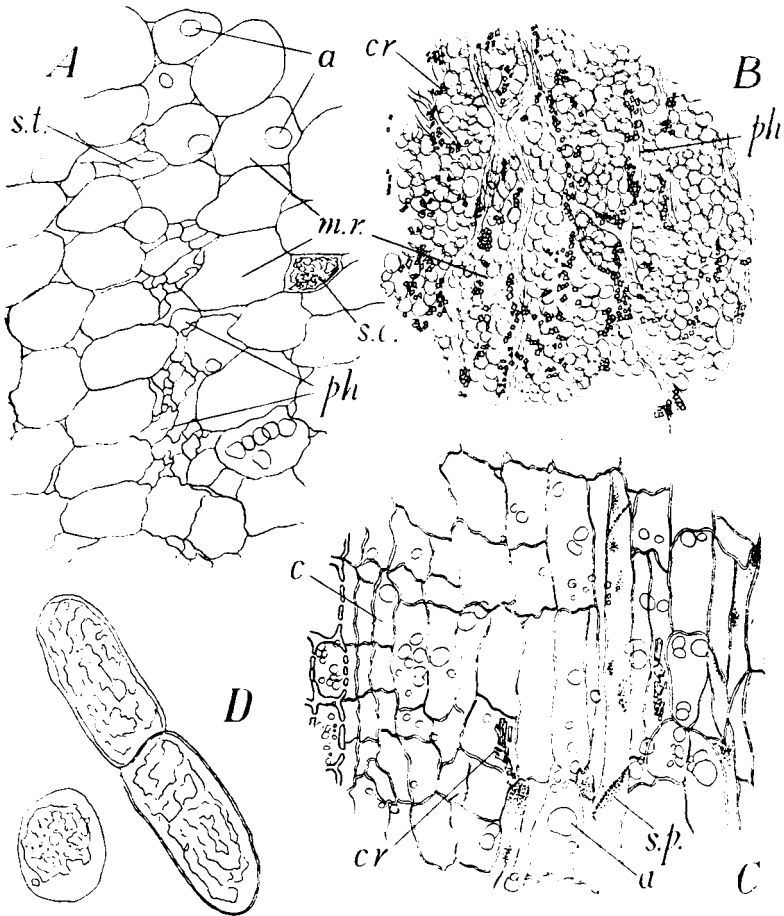


FIG. 3.—*Rauwolfia serpentina* Benth. Root. A, transverse section of the phloem $\times 200$. B, tangential longitudinal section of the phloem $\times 40$. C, radial longitudinal section of the phloem and cambium $\times 200$. D, isolated secretion cells. a, starch grains; c, cambial cells; cr, crystals of calcium oxalate; m.r., medullary ray; ph, phloem; s.c., secretion cell; s.p., sieve plate; s.t., sieve tube.

* R, T and L indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their "Botanical Pen Portraits 1923."

to 56μ and $T = 49$ to **56** to 70μ . Each of these bands is followed by a band consisting of 1 or 2 layers of broad cells with suberised and lignified walls, measuring approximately $R = 28$ to **42** to 49μ , $L = 18$ to **35** to 56μ and $T = 49$ to **56** to 70μ . The radial walls of the broad cells tend to break, and as a consequence the cork frequently peels off in layers. The broad lignified cork cells stain red with phloroglucin and hydrochloric acid, whereas the narrow ones remain yellowish with the same treatment; all the cork cells are insoluble in 80 per cent. sulphuric acid showing that they are suberised.

The cork is followed by a layer of phellogen cells, which have thin cellulosic walls. The phelloderm sometimes consists of about 12 layers of cells, the layers near the phellogen having smaller rectangular tabular cells regularly arranged, whereas the inner layers have larger irregularly shaped cells which appear to have been displaced due to gliding growth during development. The phelloderm cells have walls which are comparatively thicker and more highly refractive than those of the parenchyma of the phloem; they contain numerous starch grains. A few cells of the phelloderm in some specimens contain yellowish granular contents which stain brown with iodine. The fairly wide band of inner bark (Fig. 2, C) consists of numerous broad medullary rays running radially and alternating with comparatively narrow rays of secondary phloem. The cells of the medullary rays (Fig. 3) have thin cellulosic walls and contain numerous starch grains. The medullary rays are 2 to 4 cells wide, the cells being rounded rectangular or ovoid as seen in a transverse section and have small intercellular spaces. The cells measure $R = 18$ to **28** to 49μ , $L = 32$ to **63** to 123μ and $T = 35$ to **63** to 70μ . The path of the ray becomes irregular and indistinct as it approaches the phelloderm. Occasional cells are partly or completely filled with yellowish granular contents which stain brown with iodine or sudan III.

The sieve tissue (Fig. 3), consisting of sieve tubes, companion cells, and phloem parenchyma, lies closely packed in the narrow rays of the phloem between the broad medullary rays, as seen in transverse sections. The majority of the cells of the phloem parenchyma contain starch grains, a few cells contain crystals of calcium oxalate. In a tangential longitudinal section of the bark, the sieve tissue forms irregular wavy lines, the interspaces being filled with the cells of the medullary rays as shown in Figure 3, B. The numerous cells containing calcium oxalate crystals are best seen in this section; some of the crystals are well-formed prisms with which are usually associated numerous irregularly shaped angular crystals of different sizes forming groups or clusters. The clusters or groups usually measure about 7 to **11** to 18μ and the well-formed prisms 11 to 14μ .

The simple starch grains of the inner bark are smaller than those of the wood; they measure 4 to **10** to 20μ and are rounded or ovoid in form with a central hilum. Occasional grains are 2- to 3- occasionally 4-compound. Mounted in lactophenol and observed under polarised light they show a well-defined maltese cross.

The cambiform tissue (Fig. 3, C) consists of 2 or 3 layers of well-defined rectangular cells forming a complete ring between the bark and the wood.

Most of the roots exhibit a tetrarch primary xylem (Fig. 4, A), but occasional roots are triarch; the primary xylem groups form narrow

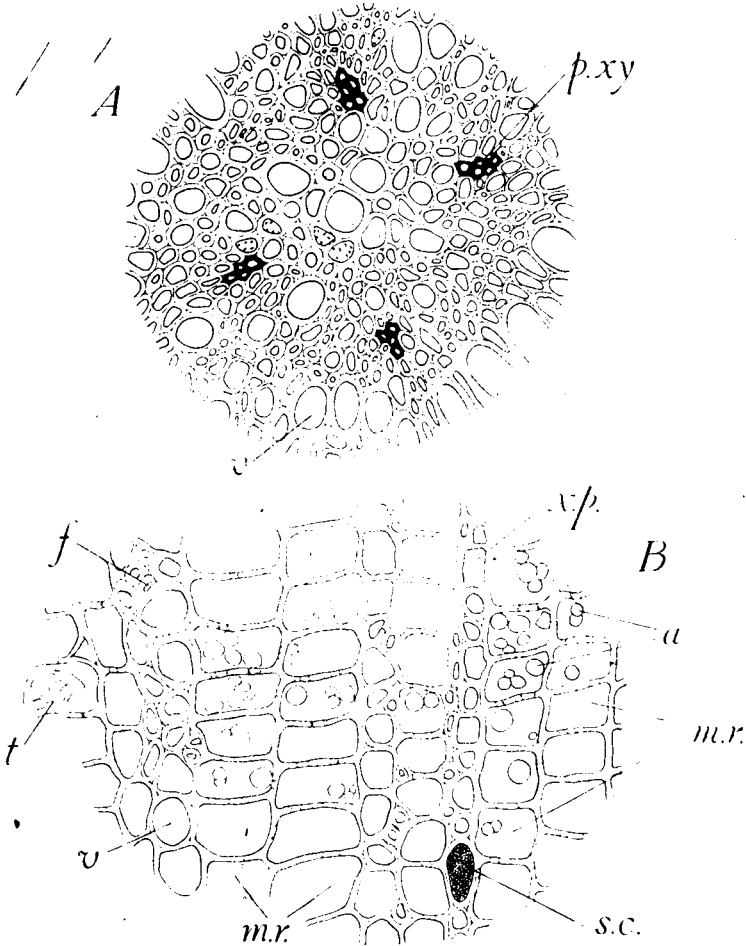


FIG. 4.—*Rauwolfia serpentina* Benth. Root. A, central core of the wood showing primary xylem bundles $\times 200$. B, transverse section of the secondary wood $\times 200$. a, starch grains; f, xylem fibres; m.r., medullary ray; p.xy., primary xylem bundle; s.c., secretion cell; t, resin-like mass; v, vessel; x.p., xylem parenchyma.

triangles as seen in a transverse section. The wood consists of strongly lignified cells (Fig. 2, C), the secondary xylem being arranged in growth rings of varying width, most commercial specimens showing about 3 to 6 seasons' growth. All the cells of the xylem parenchyma (Fig. 4, B) and of the medullary rays contain abundant starch, the grains being simple or 2- to 4-compound; the simple grains measure about 6 to 20 to 46 μ ,

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the components of the compound grains being rather smaller. Occasional cells of the xylem parenchyma are either completely or partly filled with a yellowish granular substance which stains brownish with iodine or sudan III. In a transverse section the medullary rays are well marked, straight, and continuous with the medullary rays of the phloem. The narrow rays of xylem form anastomosing longitudinally undulating bands. The medullary rays are 1 to 5 cells wide alternating with narrow rays of secondary xylem which consists of vessels, fibres and xylem parenchyma. The longest medullary rays start from a point opposite the apices of the protoxylem groups, others originate at points nearer the circumference making the rays more numerous in the outer growth rings. The cells of the medullary rays measure approximately $R = 14$ to 63 to 86μ , $L = 17$ to 42 to 70μ and $T = 14$ to 42 to 60μ , their walls as well as those of the xylem parenchyma having simple pits except when they adjoin a vessel when they bear half-bordered pits.

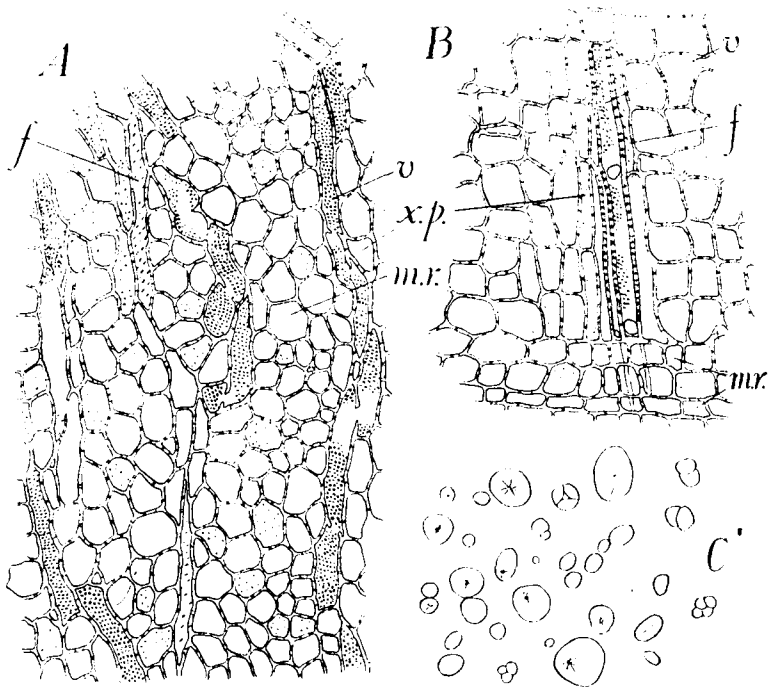


FIG. 5.—*Rauwolfia serpentina* Benth. Root. A, tangential longitudinal section of the wood $\times 100$. B, radial longitudinal section of the wood $\times 100$. C, simple and compound starch grains from powdered drug $\times 200$. f, xylem fibre; m.r., medullary ray; x.p., xylem parenchyma; v, vessel.

The cells of the scanty xylem parenchyma measure about $R = 14$ to 46 to 70μ , $L = 46$ to 53 to 77μ and $T = 14$ to 42 to 56μ .

The vessel elements (Fig. 6), which bear numerous bordered pits, measure about 180 to 234 to 432μ in length and 36 to 54μ in diameter. The transverse or oblique articulations of each element form two rounded

or oval openings either at opposite ends or on the side walls; in the latter case the openings are usually diagonally opposite to each other. The planes of junction of the vessel elements are at right angles to the tangential plane and often lie in the radial plane as shown in Figure 5. Many vessel elements are prolonged at their ends into fibre-like projections. The vessels are associated with numerous xylem fibres having strongly thickened walls and spirally arranged slit-like simple pits. The fibres measure approximately 432 to 576 to 774 μ in length and 18 to

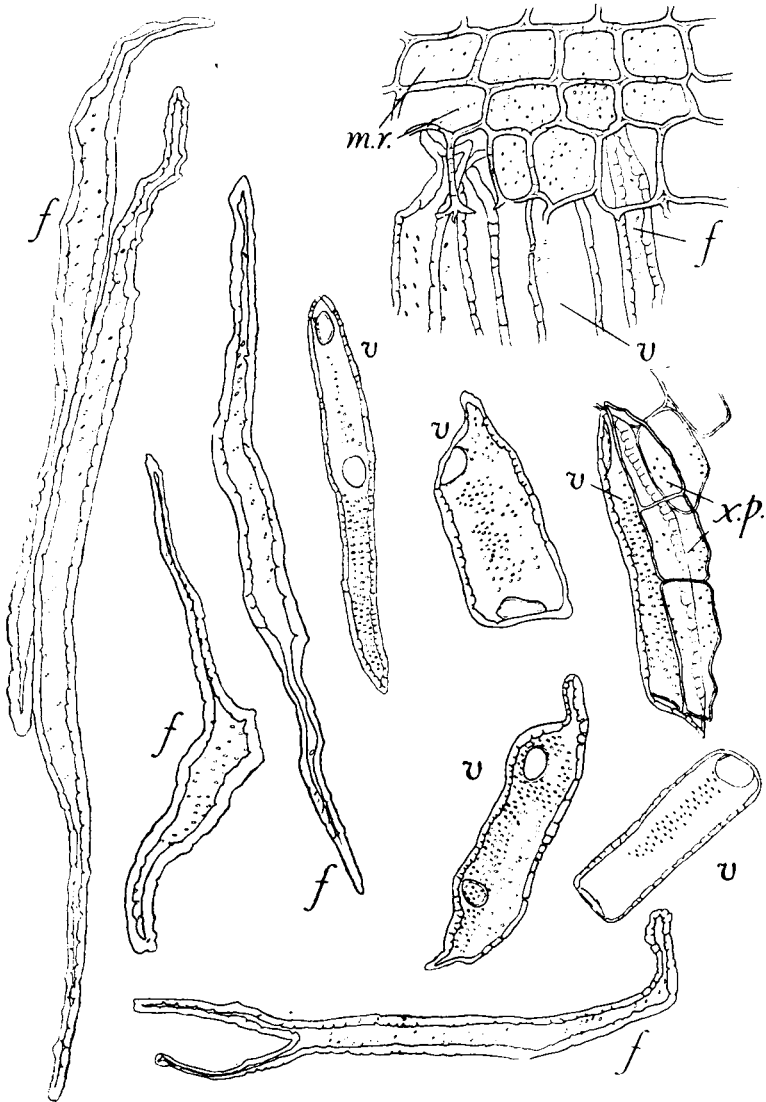


FIG. 6.—*Rauwolfia serpentina* Benth. Root. Isolated elements of the wood from chromic-nitric acid preparation $\times 200$. *f.* xylem fibre; *m.r.*, medullary ray; *x.p.*, xylem parenchyma; *v.* vessel.

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36 μ in diameter. Many fibres possess one, or sometimes two, long tapering and often contorted ends. There are very small intercellular spaces between the elements of the xylem.

STRUCTURE OF THE RHIZOME

Histologically, the rhizome differs from the root in certain respects only. The cork of the rhizome (Fig. 7, B) closely resembles that of

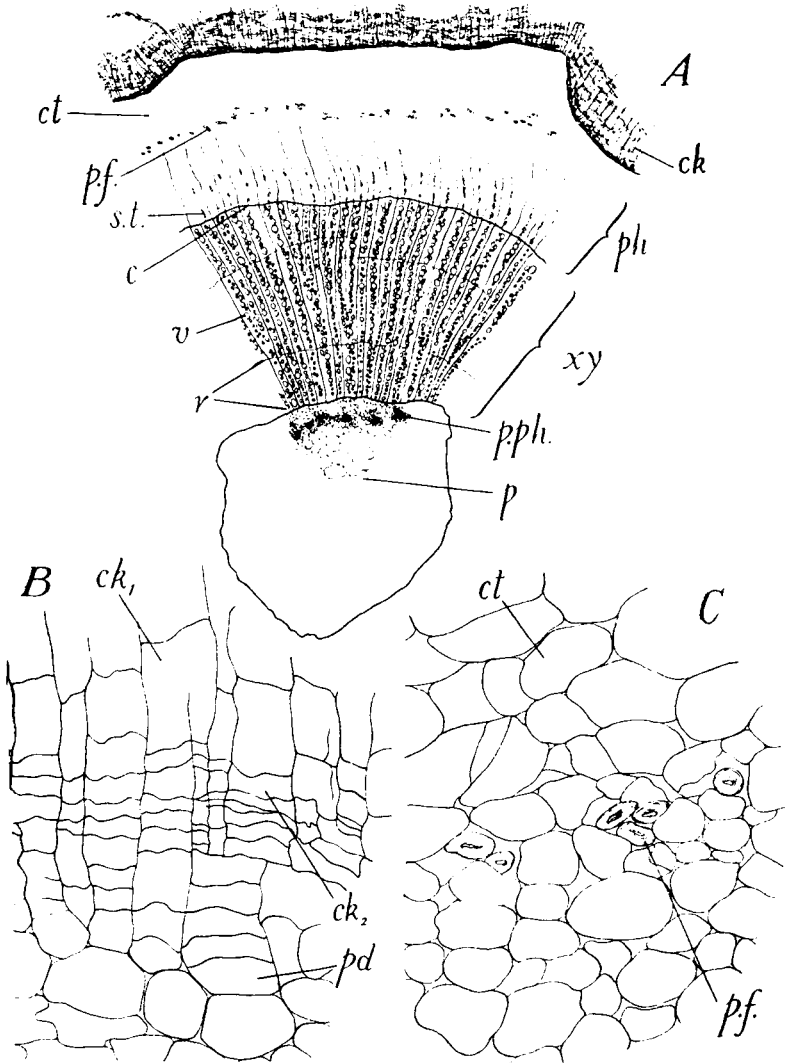


FIG. 7.—*Rauwolfia serpentina* Benth. Rhizome. A, diagrammatic transverse section $\times 25$. B, transverse section of the tegumentary tissues $\times 200$. C, transverse section of the pericyclic region; all drawings $\times 200$. c, cambium; ck, cork; ck₁, wide cork cells; ck₂, narrow cork cells; ct, cortex; p, pith; pd, phelloderm; ph, phloem; p.ph., peri-medullary phloem; p.f., pericyclic fibres; r, growth ring; s.t., sieve tissue; xy, xylem.

the root but, owing to the presence of some cells intermediate in radial width between those of the wide and the narrow cells, the layering is less distinct. The phelloderm in the rhizome is narrow and consists of about 3 to 6 layers only, as compared to about 12 layers present in

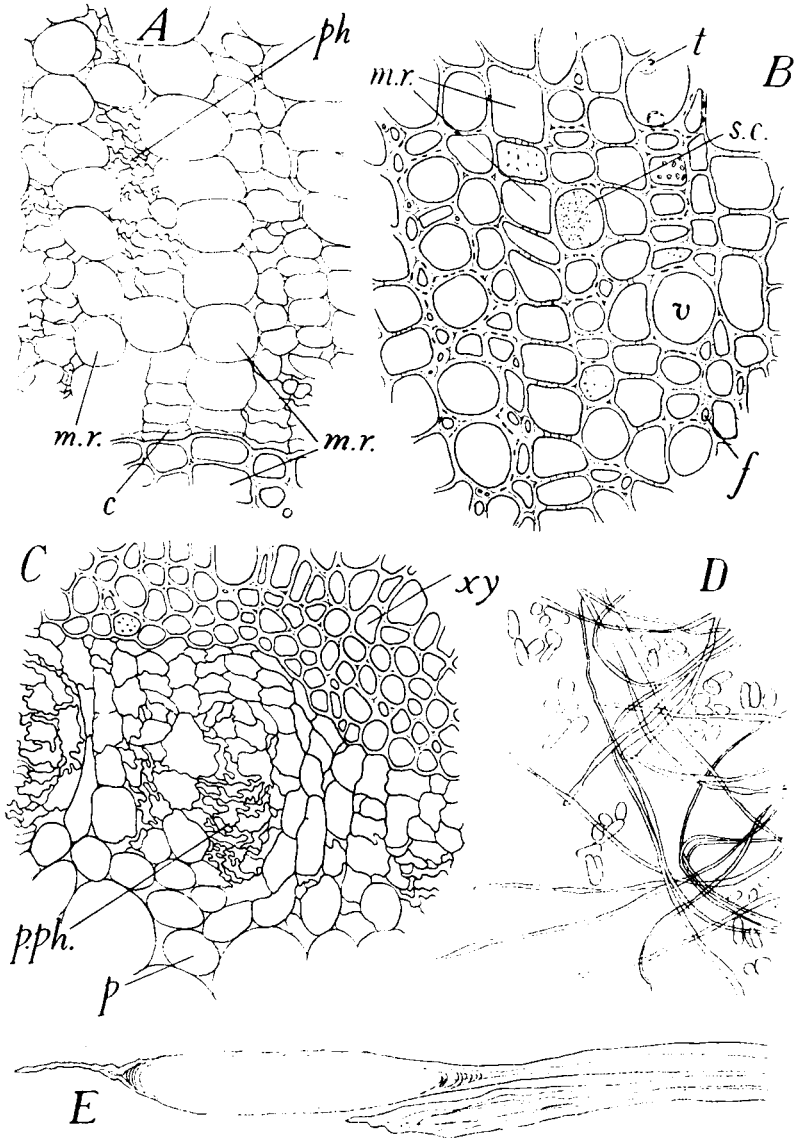


FIG. 8.—*Rauwolfia serpentina* Benth. Rhizome. A, transverse section of phloem adjacent to the cambial tissue $\times 200$. B, transverse section of the wood $\times 200$. C, peri-medullary phloem and pith $\times 200$. D, pericyclic fibres and cells from potash preparation $\times 40$. E, tip of pericyclic fibre showing local enlargement $\times 200$. c, cambium cells; f, xylem fibre; m.r., medullary ray; p, pith; ph, phloem; p.ph., peri-medullary phloem; s.c., secretion cell; t, tylose; v, vessel; xy, xylem.

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the root. This is followed by a wide cortex and a pericycle (Fig. 7, C), the cell walls of which are very refractive and in which are present pericyclic fibres, either solitary or in groups of 2 to 4. The fibres (Figs. 8, D and E) have very thick walls and a correspondingly narrow lumen. Many of these fibres show a few scattered elongated ovoid enlargements especially near the ends, a feature which is typical of the Apocynaceæ. These fibres measure about 1.5 mm. in length and 7 to 20 μ in diameter with local enlargements about 50 μ in width. Secretion cells are more numerous in the rhizome than in the root and are found chiefly in the cortex and in medullary rays of the phloem. The medullary rays of the phloem (Fig. 8, A) are 1 to 3 cells wide, and the cells measure R = 18 to 28 to 49 μ , L = 46 to 63 to 125 μ and T = 18 to 35 to 77 μ . The starch grains in the rhizome are smaller than those in the root, measuring 2 to 11 to 21 μ . The medullary rays of the xylem (Fig. 8, B) are 1 to 4 cells wide, and the cells measure R = 7 to 32 to 53 μ , L = 14 to 46 to 92 μ and T = 7 to 21 to 49 μ ; the xylem parenchyma is more abundant than in the root (Fig. 8). The vessels closely resemble those of the root, measuring 105 to 308 to 490 μ in length and 25 to 46 to 60 μ in diameter. A peculiar feature of the rhizome, particularly in the older pieces, is the presence, in many of the vessels, of numerous tyloses

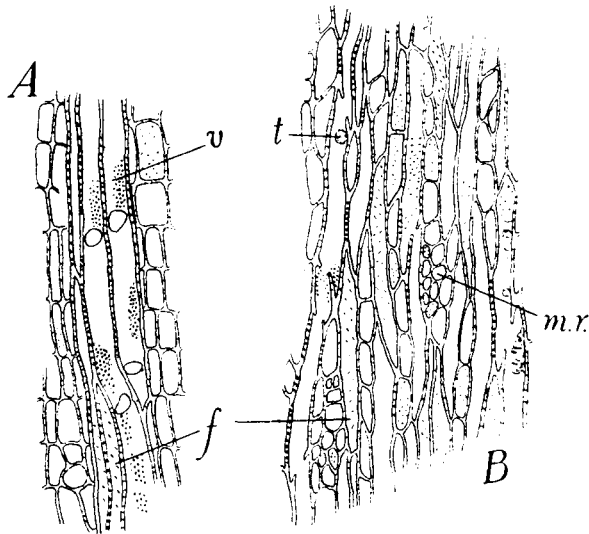


FIG. 9.—*Rauwolfia serpentina* Benth. Rhizome. A, radial longitudinal section of the wood. B, tangential longitudinal section of the wood, both drawings $\times 100$. f, xylem fibre; m.r., medullary ray; t, tylose; v, vessel.

(Figs. 8, B and 9, B) of varying sizes which sometimes almost block the lumen. In some of the vessels exhibiting tylosis, there are granular masses which stain bright red with phloroglucin and hydrochloric acid. Xylem fibres are comparatively abundant in the rhizome and are almost straight; they measure 193 to 560 to 753 μ in length and 11 to 21 to 35 μ in diameter.

The central pith (Figs. 7, A and 8, C) is small, about 0.75 to 1.5 mm. in diameter, and contains in its periphery about 20 small bundles of perimedullary phloem; the remainder of the pith consists of cellulosic parenchyma, the cells of which vary much in size and some of them are filled with a yellowish secretion similar to that in the cortex and phloem.

LATICIFEROUS TISSUE

As the presence of laticiferous tubes is usually regarded as an important characteristic of the Apocynaceæ, a careful search was made for these. No laticiferous tubes were found in the root, but occasional parenchymatous cells in the phloem were found to be filled with yellowish granular contents which stained brown with iodine (Fig. 3, D).

Examination of the rhizome also showed an absence of typical laticiferous tubes; there were, however, a number of secretion cells occurring in the cortex, in the phloem and in the pith, their granular contents staining yellow with iodine or sudan III. Occasionally the secretion cells are arranged in longitudinal rows of 2 to 4, but the transverse walls remain intact.

POWDERED RAUWOLFIA

The salient features of the powdered drug are:--

1. Very numerous rounded and ovoid starch grains about 4 to 20 to 50 μ in diameter, occasional grains being 2- to 4-compound. The starch is rapidly gelatinised in the cold by the action of 0.9 per cent. aqueous solution of potassium hydroxide, the action being more rapid than the similar action of the same solution on potato starch. Strong hydrochloric acid (sp. gr. 1.18) gelatinises the starch instantaneously in the cold.

2. Much lignified rectangular parenchyma having moderately thickened walls bearing simple pits; most of the cells contain numerous starch grains.

3. Fragments of xylem vessels about 36 to 54 μ in diameter, with bordered pits and, associated with them, fragments of the characteristic xylem fibres.

4. Fragments of yellowish cork composed of polygonal-tabular cells of two types, broad and narrow.

5. Small quantities of cellulosic parenchyma, usually filled with starch grains, and occasional unligified pericyclic fibres showing local swellings.

6. Occasional rounded rectangular secretion cells with yellowish granular contents; and scattered prismatic or irregularly triangular crystals of calcium oxalate.

SUMMARY

1. Rauwolfia of Indian commerce consists of the dried rhizomes and roots, with occasional small pieces of attached aerial stem, of *Rauwolfia serpentina* Benth.

2. The important histological features are the cork, composed of alternating layers of broad and narrow cells, giving a somewhat spongy

and friable exterior to the drug; the narrow bark and the wide central mass of wood in the root or broad ring in the rhizome, showing about 3 to 8 growth rings; a tetrarch, or occasionally triarch, primary xylem in roots or a very small pith at the centre of rhizomes; in the periphery of the pith a ring of about 20 small groups of perimedullary phloem.

3. All the cells of the wood, including those of the medullary rays are lignified. The xylem vessels, fibres and parenchyma form narrow undulating radially arranged bands, separated by large medullary rays in the root and by smaller ones in the rhizome. The vessel segments are small and narrow and the xylem fibres are often irregularly shaped with long, usually much contorted, tapering ends; many of the vessels, especially in the rhizome, show tylosis and occasionally contain resinous masses. The phloem also occurs in undulating bands similar to those of the xylem; phloem fibres are absent.

4. In the rhizome, unligified pericyclic fibres occur, either singly or in groups of 2 to 4; they show the local and sub-terminal elongated-oval enlargements characteristic of the Apocynaceæ.

5. All the parenchymatous tissues, except the cork, contain numerous starch grains, about 4 to 20 to 50 μ in diameter. Cells containing a brownish yellow secretion occur in small numbers, either singly or in short longitudinal rows, in the bark, wood and pith, being most frequent in the cortex of pieces of rhizome. Typical laticiferous tubes are absent. Calcium oxalate occurs in numerous cells of the phloem and of the medullary rays of the bark; the crystals are either well-formed prisms or aggregates of irregular angular components.

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