

# THE STRUCTURE OF THE ROOT, ROOTSTOCK AND STEM-BASE OF *RAUWOLFIA VOMITORIA* AFZ.

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THE use of the whole root and the selected alkaloids of *Rauwolfia serpentina* Benth. for the treatment of hypertension and various psychiatric conditions has prompted the investigation of other members of the Apocynaceæ for similar active constituents. One such species which is now employed commercially as a source of reserpine is *Rauwolfia vomitoria* Afz., a bush or tree sometimes attaining a height of about 10 m. and of common occurrence in tropical Africa from the Guinea coast to Mozambique. This plant, which possesses many local names<sup>1</sup>, has been employed for its vermifugal and insecticidal properties as well as for the treatment of a wide variety of ailments<sup>1,2,3,4,5</sup>. Some of the therapeutic effects of the water-soluble constituents of the drug have been studied by Raymond-Hamet<sup>6,7</sup>; it can act as an emetic and drastic purgative when administered orally.

In 1943, Paris<sup>8</sup> isolated from the root bark of *R. vomitoria*, ajmaline, isoajmaline, small quantities of ajmalicine and ajmalinine and probably serpentinine, five alkaloids contained in *R. serpentina*<sup>9,10</sup>. The roots also contain alstonine<sup>11</sup>, two new alkaloids raumitorine and sederine described by Janot *et al.*<sup>12</sup> and reserpine<sup>13</sup>.

The structure of *R. serpentina* root and rhizome has been fully investigated by Wallis and Rohatgi<sup>14</sup>, several other species have been described by Youngken<sup>15</sup> and *R. parakensis* from Malaya by Wan<sup>16</sup>. No complete histological investigation of *R. vomitoria* root appears to have been published; a brief unillustrated description is given by Paris<sup>8</sup> in which the histology of the root is stated to be analogous to *R. heterophylla* and Pichon<sup>17</sup> includes the species in his classification of the genus based on floral structure. In view of the commercial importance of *R. vomitoria* and the need for differentiating between the common species of this genus a study of the structure of the root and attached rootstock and stem-bases has been undertaken and the results presented below.

## PLANT MATERIAL

The following material was utilised in this investigation:

1. Roots and aerial parts collected in October, 1954, by Dr. W. S. S. Ladell, Oshodi, Nigeria.
2. Roots, twigs and leaves obtained from the Ivory Coast in 1949 and presented by Professor R. Paris, Faculté de Pharmacie de Paris.
3. Various commercial samples obtained from a number of geographical sources and made available by Riker Laboratories, Ltd., England, and Riker Laboratories Inc., Los Angeles, U.S.A.

## RAUWOLFIA VOMITORIA AFZ.

### MACROSCOPY

The roots occur in cylindrical or flattened, slightly tapering and occasionally branched pieces up to 30 cm. in length and 5 cm. in diameter. Segments 0.5–1.5 cm. in diameter and cut into short lengths form the bulk of commercial samples (Fig. 1: A).

The outer surface consists of a greyish brown cork, often deeply longitudinally furrowed or rubbed smooth. If present, the outer cork has a soft texture and is easily removed as irregular flakes by rubbing, thereby exposing the smooth light brown surface of the outer cortex. Small roots and rootlets which occur in some samples are seldom found attached to the larger roots but their remains appear at intervals as oblique protuberances or stumps.

With the exception of the small brittle roots the drug is extremely tough, eventually breaking with a fracture splintery in the wood and short in the bark.

A smooth transverse surface of a root exhibits a narrow pale brown bark, rarely exceeding 3 mm. in thickness in the dried material but swelling considerably on soaking in water. The buff or yellowish coloured finely radiate porous wood, showing in the larger pieces distinct growth rings, forms the majority of the drug.

When dry the root is odourless. The cork is practically tasteless, the phloem and outer cortex very bitter and the wood slightly bitter.

Occasional rootstocks with attached stem-bases occur in commercial samples and will be described below.

### MICROSCOPY

In the following description the symbols R, T and L designate measurements in the radial, tangential and longitudinal planes respectively. Wherever possible, ranges of size have been obtained from a wide variety of material but nevertheless they cannot be considered as absolute.

The transverse sections of roots of different girth show different appearances depending chiefly on the degree of development of stone cell layers within the secondary phloem. These differences range from a few isolated groups of stone cells in some small roots to interrupted concentric bands in some of the larger roots. Figure 1: B–E.

The radially arranged cork cells appear in transverse sections as alternating zones of flattened, unligified suberised cells, 3–4 layers in radial depth and larger lignified cells from one to about 120 layers in radial depth. The largest of the latter cells in any one stratum usually occur immediately interior to the nonlignified zones (Fig. 2: G). For the unligified cells R = 5 to 7 to 11 to 14  $\mu$ , T = 9 to 25 to 36 to 53  $\mu$  and L = 14 to 29 to 40 to 46  $\mu$  and for the lignified cells R = 14 to 32 to 50 to 208  $\mu$ , T = 9 to 28 to 40 to 53  $\mu$  and L = 7 to 22 to 40 to 53  $\mu$ . Some young roots, and older roots deprived of most of their cork, may show only lignified cork cells. In surface view the cork cells appear polygonal and tangentially sectioned, polygonal or elongated (Fig. 2: J).

The phellogen consists of thin walled, radially flattened cells and is followed by the phelloderm possessing about 5 to 16 layers of cells

according to the size of the root. The cells near the phellogen often occur in radial rows but the inner ones are generally displaced and oval or much flattened, with intercellular spaces.  $R = 3.6$  to  $14$  to  $29$  to  $43 \mu$ ,  $T = 8.0$  to  $36$  to  $72$  to  $130 \mu$  and  $L = 22$  to  $36$  to  $49$  to  $72 \mu$ . Some of the cell walls are thickened with cellulose and others lignified forming single

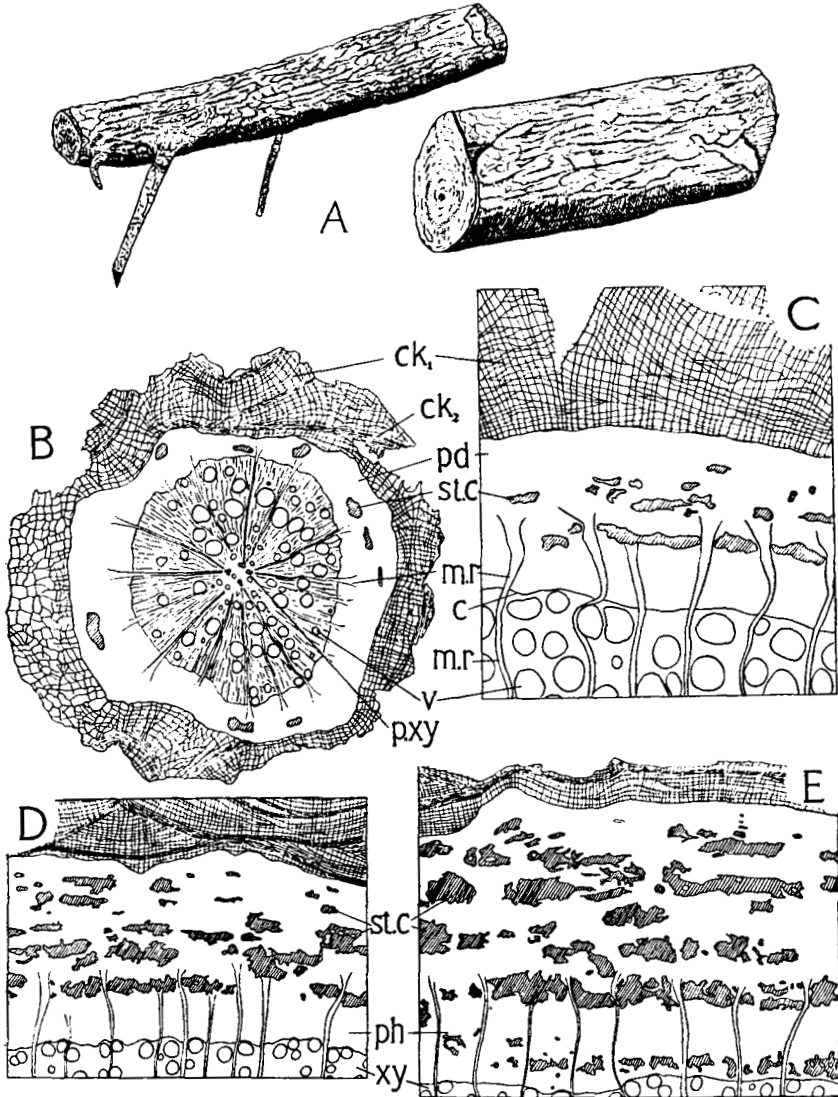
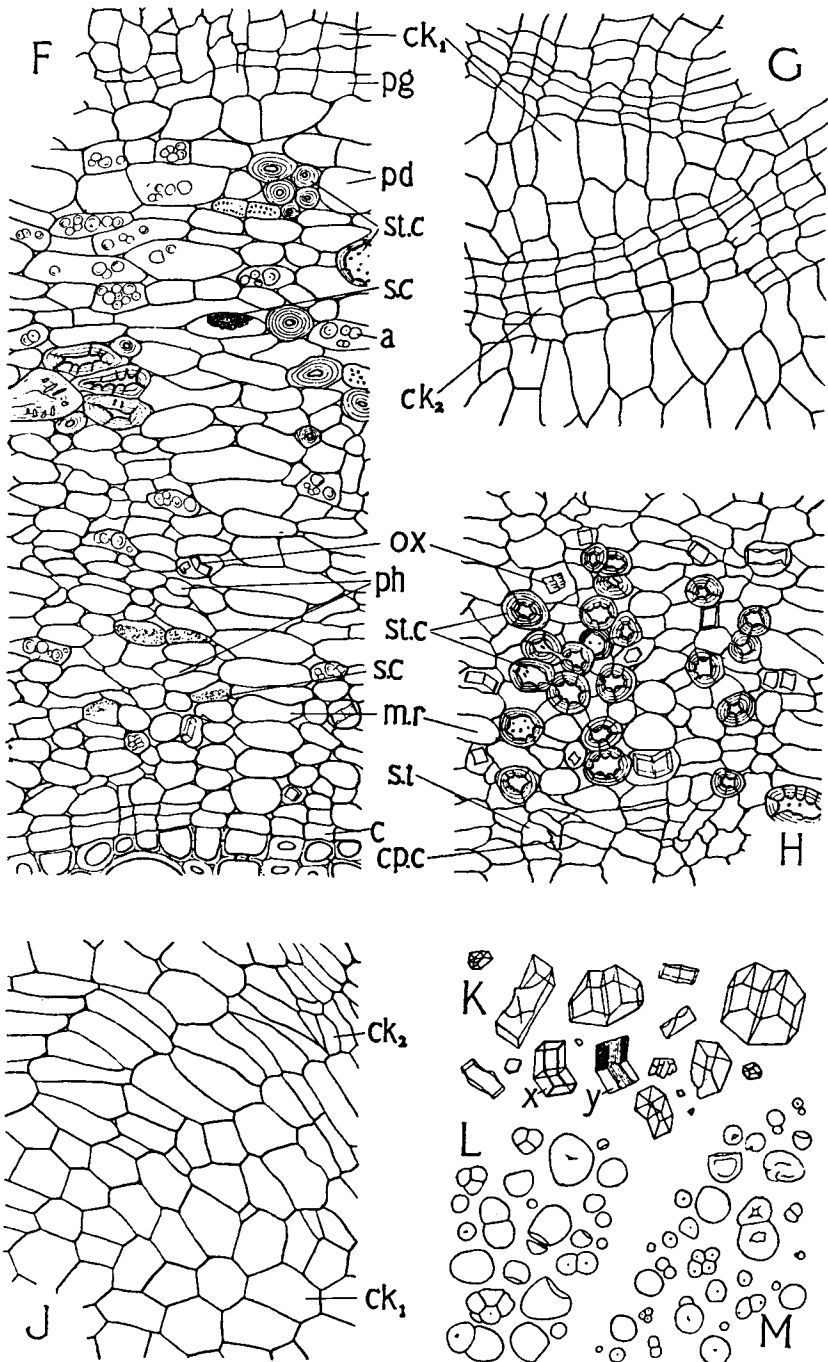


Fig. 1. *Rauwolfia vomitoria* Afz. Root. A, roots taken from a commercial sample  $\times 1$ . B-E, general diagrams of transverse sections of roots. B, 1 mm. diameter  $\times 50$ . C, 8 mm. diameter  $\times 50$ . D, 2.5 cm. diameter  $\times 25$ . E, 4 cm. diameter  $\times 25$ . c, cambium;  $ck_1$ , lignified cork cells;  $ck_2$ , unligified cork cells;  $m.r$ , medullary rays;  $pd$ , phellogen;  $ph$ , phloem;  $p.xy$ , primary xylem;  $st.c$ , stone cell groups;  $v$ , wood vessels;  $xy$ , xylem.



**Fig. 2.** *Rauwolfia vomitoria* Afz. Root. F-H, transverse sections of the bark  $\times 200$ . F, root diameter 6 mm.; G, cork cells; H, inner phloem, root diameter 2.0 cm. J, tangential longitudinal section of cork  $\times 200$ . K, isolated crystals of calcium oxalate  $\times 320$ . L, wood starch  $\times 320$ . M, phelloderm and phloem starch  $\times 320$ . a, starch grains; c, cambial cells;  $ck_1$ , lignified cork cells;  $ck_2$ , unligified cork cells; c.p.c, companion cells; m.r, medullary rays; ox, calcium oxalate crystals; pd, phello-derm; pg, phellogen; ph, phloem; s.c, secretory cell; s.t, sieve tube; st.c, stone cells; x, twin crystal as seen in chloral hydrate mountant; y, the same crystal viewed in polarised light.

or small groups of stone cells (Fig. 2: F; 3: N). Individual stone cells may be isodiametric, tangentially or longitudinally elongated and in the latter case often arranged end to end.  $R = 11$  to  $22$  to  $36$  to  $45 \mu$ ,  $T = 18$  to  $36$  to  $72$  to  $115 \mu$  and  $L = 11$  to  $32$  to  $50$  to  $180 \mu$ . The isodiametric stone cells have relatively thinner walls than the elongated structures and sometimes contain calcium oxalate prisms. All the sclereids possess conspicuous pits in their walls. Scattered crystals of calcium oxalate occur in the phelloderm either as twin or single prisms or smaller irregular masses. Starch is present chiefly as single rounded grains, possessing hila as central points or single or star shaped clefts and generally showing a well marked maltese cross when examined in polarised light. Some of the larger grains appear somewhat gelatinised and fail to show the polarisation effect. Individual grains are  $1$  to  $7$  to  $11$  to  $20 \mu$  in diameter.  $2$  to  $4$  compound grains are also present and these sometimes split to give individual plano-convex or angular grains (Fig. 2: M).

In the smaller roots the secondary phloem consists of sieve tubes, companion cells, phloem parenchyma, secretion cells, medullary rays and isolated groups of stone cells. In larger, older roots there is often an outer non-functioning secondary phloem with up to about five discontinuous bands of stone cells and an inner region in which the sieve elements are clearly discernible. The structure of the phloem is shown in Figure 2: F, H (Fig. 3: O-R).

The heterogeneous rays are  $1$  to  $3$ , occasionally  $4$  to  $5$  cells wide and are composed of groups of small cells, often with wavy walls,  $R = 25$  to  $32$  to  $36$  to  $49 \mu$ ,  $T = 11$  to  $18$  to  $22$  to  $25 \mu$  and  $L = 11$  to  $14$  to  $22$  to  $36 \mu$ , supported in longitudinal rows of deeper cells,  $R = 11$  to  $14$  to  $25$  to  $36 \mu$ ,  $T = 22$  to  $25$  to  $29$  to  $32 \mu$  and  $L = 25$  to  $53$  to  $72$  to  $90 \mu$ . (Figure 3: O, P). The rays pass through gaps in the stone cell layers and due to the tangential elongation of individual cells become funnel shaped near the outer bark.  $R = 14$  to  $22$  to  $32$  to  $36 \mu$ ,  $T = 36$  to  $52$  to  $75$  to  $96 \mu$ . Occasional cells in the vertical extensions of the rays may be lignified.

Calcium oxalate occurs abundantly throughout the secondary phloem in the medullary rays and other parenchymatous cells; it is best studied in the longitudinal sections in which long rows of crystals are evident. The crystals take the form of small irregular structures and larger monoclinic prisms. The latter occur singly or as geniculate twins with the twinning occurring on one of the hemi-pyramid faces. The crystal shape is often complicated by pseudomorphism and in chloral hydrate mounts, often rendered difficult to observe due to the deceptive appearance of many of the twin crystals. The effect is illustrated in Figure 2: K. In certain positions the crystals are not always apparent when viewed in polarised light but the twin crystals can usually be detected due to their bicolouration at certain orientations. Length of the well formed prisms =  $9$  to  $15$  to  $22$  to  $36 \mu$ , breadth =  $7$  to  $11$  to  $14$  to  $18 \mu$ .

A conspicuous feature of the outer phloem is the numerous irregular stone cell groups up to about five cells in radial thickness and 13 cells in depth. Individual stone cells vary greatly in size and shape ranging

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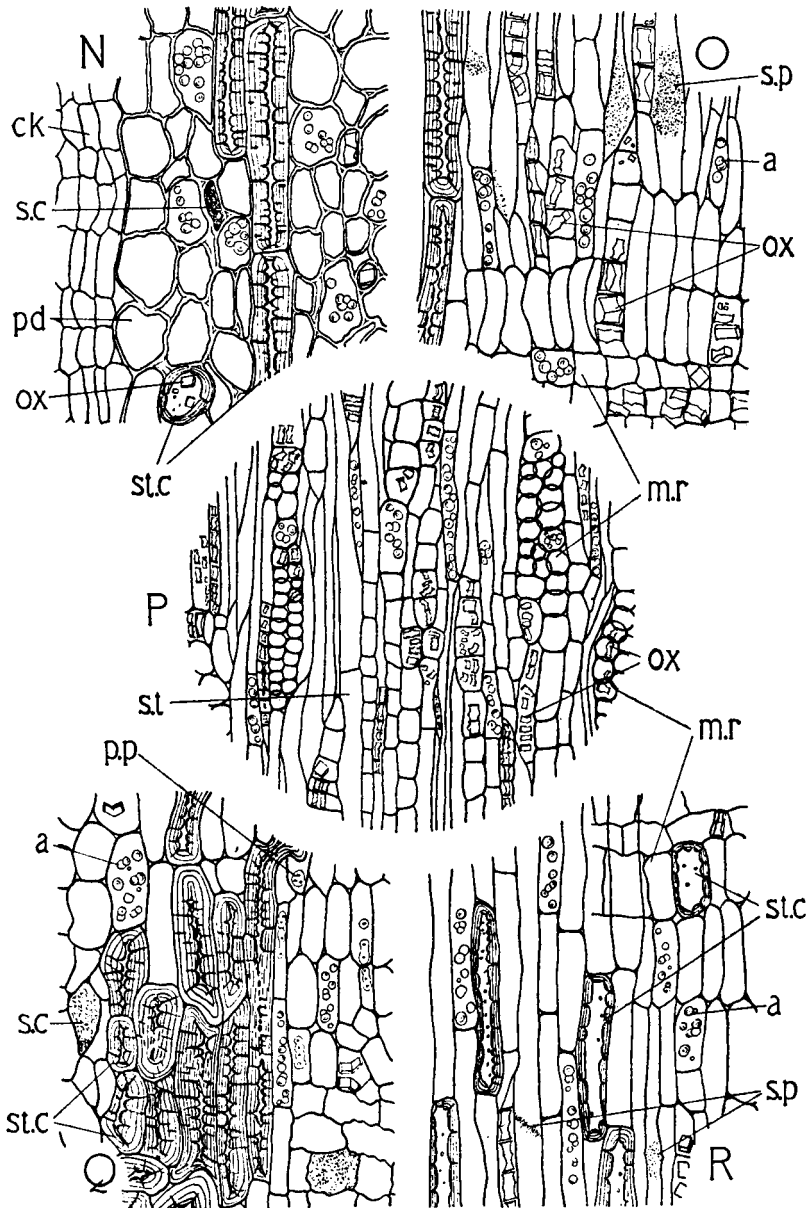


FIG. 3. *Rauwolfia vomitoria* Afz. Root. Longitudinal sections of the bark. N, radial section of outer tissues, root diameter 1.3 cm. O, radial section of inner phloem, root diameter 1.3 cm. Both  $\times 200$ . P, tangential section of phloem, root diameter 6.0 mm.  $\times 85$ . Q, radial section of stone cell group of nonfunctioning phloem, root diameter 2.0 cm. R, radial section of inner phloem, diameter 2.0 cm. Both  $\times 200$ . a, starch grains; ck, cork; m.r., medullary rays; ox, calcium oxalate crystals; pd, pelloderm; p.p., pits in walls of parenchymatous cells; s.c., secretion cells; s.p., sieve plate; s.t., sieve tube; st.c., stone cells.

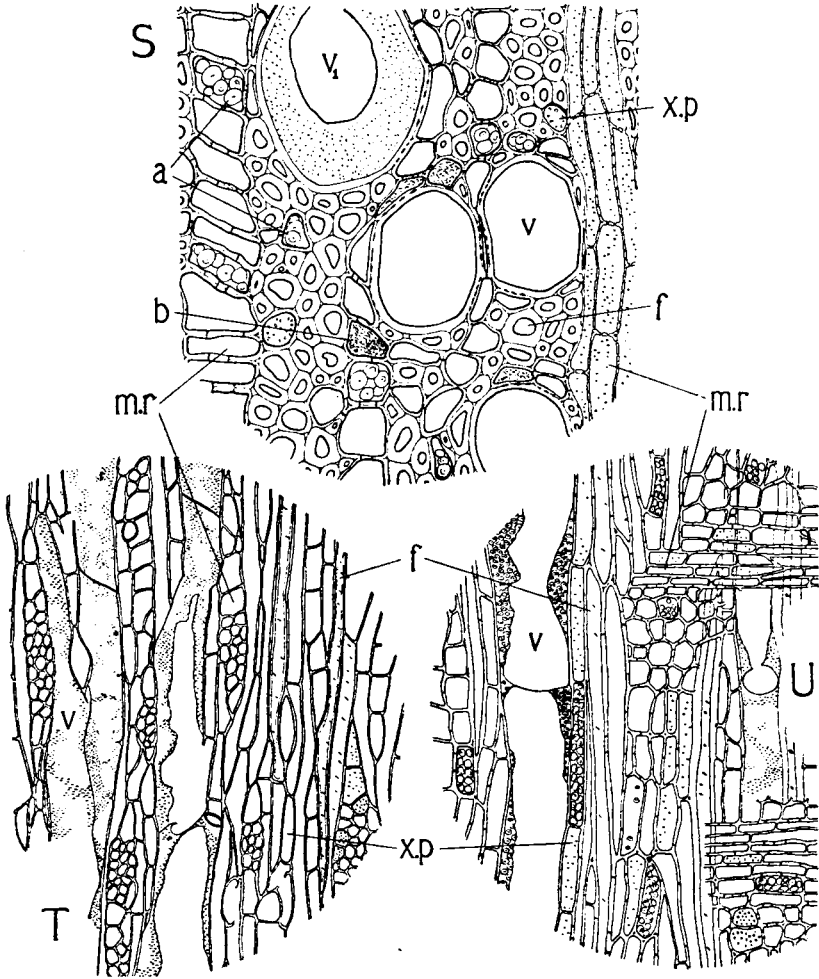


FIG. 4. *Rauwolfia vomitoria* Afz. Root. Secondary wood. S, transverse section  $\times 200$ . T, tangential longitudinal section  $\times 85$ . U, radial longitudinal section  $\times 85$ . a, starch; b, resinous material; f, fibres; m.r., medullary rays; v, vessel;  $v_1$ , vessel showing perforation plate; x.p., xylem parenchyma.

from isodiametric to elongated and irregular structures attached to lignified protuberances. The stratified cell walls are unevenly thickened and bear conspicuous funnel shaped pits. Cells isolated by Schultze's macerating fluid had the following measurements, length = 28 to 71 to 198 to 288  $\mu$ , breadth = 14 to 21 to 36 to 56  $\mu$  (Figures 2: F; 3: A; 5: X). Incomplete stone cells, often with lignin deposited on one wall only, may occur adjacent to the stone cell groups. Other cells of the phloem parenchyma show on their walls pits surrounded by oval areas of thickening. Occasional stone cells contain starch grains and others crystals of calcium oxalate. Occasionally the lignified walls of the stone cells fit so

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closely to the enclosed crystals that in the macerated material, cells with cavities of the exact form of the crystals can be seen (Fig. 5: X).

Secretory cells are not numerous but occur scattered throughout the secondary phloem; their contents appear as amorphous masses when stained with iodine, sudan III or tincture of alkanna (Fig. 2: F; 5: Y).

Variable amounts of starch occur in the phloem. Some samples possess practically none, and others, appreciable quantities in the outer secondary phloem with longitudinal rows of grains in the narrow phloem elements (Fig. 3: O-R). In size and shape the starch grains resemble those of the phelloderm.

The secondary xylem is completely lignified and composed of medullary rays, vessels, fibres and wood parenchyma (Fig. 4: S, T, U). The vessels occur solitary or in pairs and in transverse section are rounded or somewhat radially elongated. R = 36 to 82 to 126 to 180  $\mu$ , T = 36 to 72 to 108 to 134  $\mu$ . Numerous alternately arranged, bordered pits occur in the vessel walls, the latter often separating into longitudinal strips on maceration (Fig. 5: V). Transverse or oblique perforation plates occur at intervals of about 135 to 540  $\mu$ . Yellow or orange tyloses, staining red with phloroglucinol and hydrochloric acid, are rare but may occur in vessels near the centres of roots. Other nonfunctioning vessels may be occluded by brown amorphous material.

In transverse section the apotracheal wood parenchyma occurs in short uniseriate rows; viewed longitudinally the cells are arranged end to end with irregularly pitted cross walls. The anticlinal walls may possess longitudinal rows of bordered pits, depending on the nature of adjacent cells (Fig. 5: W). R = 10 to 18 to 25 to 38  $\mu$ , T = 10 to 18 to 25 to 36  $\mu$  and L = 52 to 72 to 108 to 150  $\mu$ .

The heterogeneous medullary rays consist of one or two, less commonly three groups of small cells, R = 14 to 32 to 41 to 111  $\mu$ , T = 10 to 14 to 21 to 29  $\mu$  and L = 10 to 14 to 21 to 36  $\mu$  embedded in uniseriate longitudinal rows of larger cells, R = 14 to 21 to 36 to 72  $\mu$ , T = 14 to 24 to 36 to 42  $\mu$  and L = 32 to 36 to 72 to 126  $\mu$ . In longitudinal sections the smaller cells are often nearly circular in outline with small intercellular spaces and occur in groups of 3 to 42 cells in rays of up to 25 larger cells in depth. All the walls are heavily pitted. The rays seldom exceed three cells in width (Figs. 4: S, T, U; 5: W).

The medullary ray and wood parenchyma cells are usually packed with starch grains similar in form to those of the bark, but on an average larger in size with a higher proportion of angular and compound grains. Diameter of single grains = 1 to 4 to 18 to 30  $\mu$  (Fig. 2:L). In addition to starch some of these cells possess material staining a pale pink-brown with sudan III.

Numerous wood fibres occur associated with the other wood elements and in transverse section appear as rounded or irregularly outlined angular structures with thick lignified walls. Isolated fibres may be irregular, spindle shaped or arcuate in outline with oblique slit-like pits. Some tracheids and intermediate fibre-tracheids with bordered pits are



also present. Fibres with swollen ends or centres are of frequent occurrence and in macerated material often break across the enlarged thin walled areas. Length = 200 to 540 to 1080 to 1485  $\mu$ , breadth = 6 to 18 to 22 to 32  $\mu$  (Fig. 5: Y).

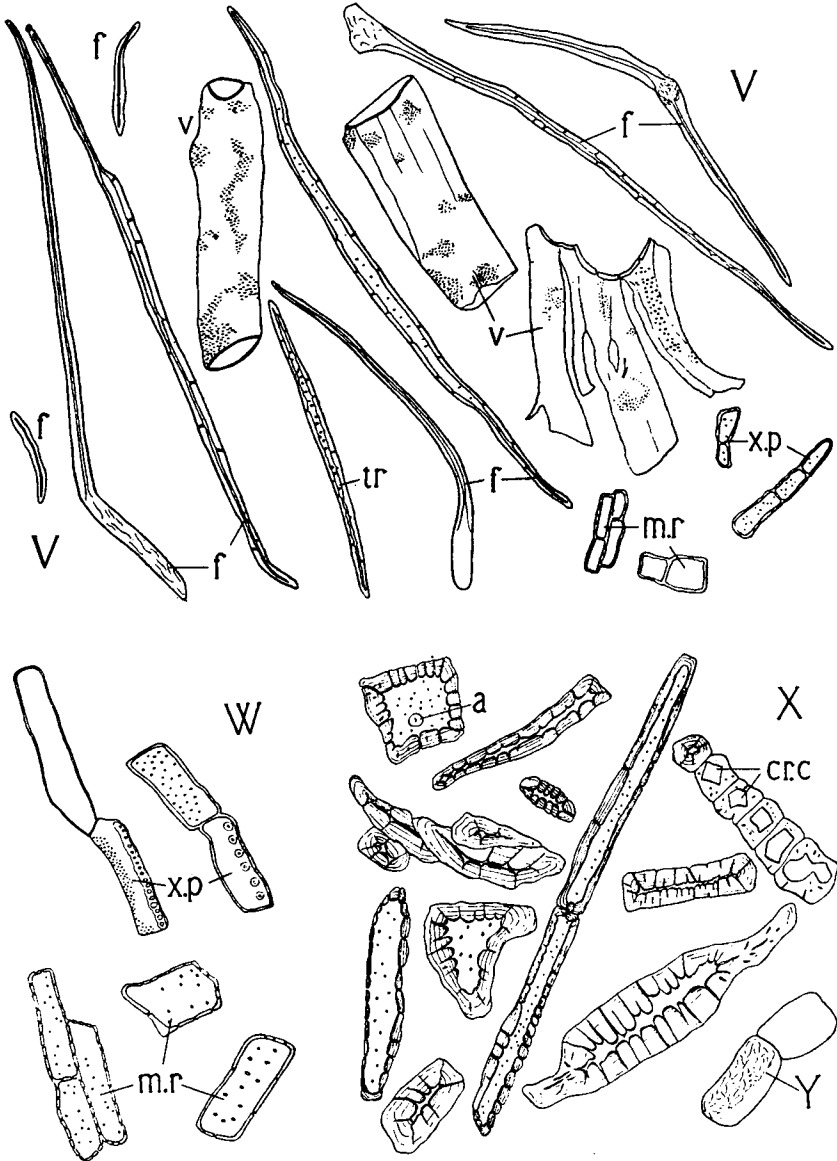


FIG. 5. *Rauwolfia vomitoria* Afz. Isolated elements of the root. V, wood elements  $\times 85$ . W, ditto  $\times 200$ . X, stone cells  $\times 200$ . Y, secretion cell  $\times 200$ . a, starch; cr.c, cavities formerly occupied by crystals; f, xylem fibres; m.r, medullary ray cells; tr, tracheid; v, vessel; x.p, xylem parenchyma.

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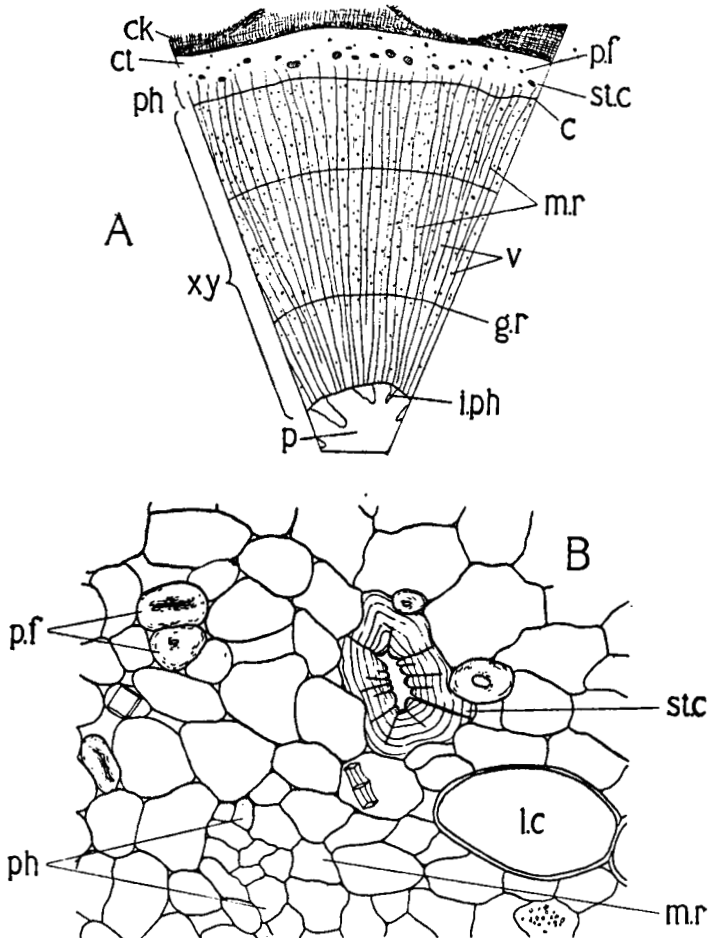


FIG. 6. *Rauwolfia vomitoria* Afz. Stem base. A, transverse section general diagram  $\times 15$ . B, transverse section in the region of the pericycle  $\times 200$ . c, cambium; ck, cork; ct, cortex; g.r, growth ring; i.ph, internal phloem; l.c, latex canal; m.r, medullary ray; p, pith; p.f, pericyclic fibres; ph, phloem; st.c, stone cells; v, wood vessels; xy, xylem.

ROOTSTOCK AND STEM-BASE

Pieces of rootstock with attached stem-bases occasionally occur in the drug and macroscopically they resemble the roots but possess a small central pith. In the samples examined, the rootstocks measured 2 to 3 cm. in diameter and the aerial stem-bases 0.5–2.0 cm. in diameter but much larger sizes must be possible. The cork of the stem-bases is much smoother than that of the roots and does not easily crumble. Well marked growth rings occur in the very hard, compact, finely radiate wood. The taste is bitter, and as with the root, aqueous decoctions show a marked blue fluorescence and give precipitates with the common alkaloid reagents.

Histologically, the rhizome and stem-bases show certain differences from the root. In all three the cork cells are similar and may exhibit the typical stratified structure of lignified and unligified layers. In the stem-base the stratification may be absent with only a few layers of one type of cell; in the rhizome, up to seven alternating bands have been observed. Generally, however, the amount of cork is much less than on the root.

Beneath the cortex, consisting of about five layers of tangentially elongated phelloderm cells and the remains of the primary cortex is an incomplete ring of highly refractive, thick walled unligified pericyclic fibres occurring either singly or in small groups often associated with stone cells (Fig. 6: A, B). These fibres are particularly numerous in the stems and in transverse sections they appear either circular or oval in outline with a small, often flattened lumen; fibres isolated from a 5 per cent. potassium hydroxide solution macerate show stratified walls with oblique pits and at intervals, enlargements of the fibre walls. Length = up to at least 6.5 mm., breadth = 14 to 18 to 32 to 54  $\mu$  and at the swellings, 36 to 90  $\mu$  (Fig. 8: F).

Stone cells and calcium oxalate are of the same size, shape and distribution as in the roots. The occurrence of crystals within the stone cells is quite common.

The cortex and outer phloem are permeated by latex canals seen in transverse section as circular or ellipsoidal structures with yellow walls (Fig. 6: B). R = 50 to 110  $\mu$ , T = 110 to 340  $\mu$ . Isolated by an alkali maceration, these canals vary considerably in length (Figure 8: H), and slight pressure on the coverslip of a temporary mount will extrude globules of granular appearing latex from their broken ends.

The wood is much harder than the root wood due to the absence of large vessels and a correspondingly larger number of fibres. Small wood vessels with bordered pits measure 18 to 33 to 48 to 70  $\mu$  in diameter and vessels with spiral thickenings occur in the protoxylem (Fig. 7: C). Viewed in tangential longitudinal sections, the heterogeneous medullary rays usually consist of one or two groups of up to 70 small cells with one or two longitudinal rows of larger cells. Individual cells of the rays have similar measurements to those of the root and the maximum width of a ray is about eight cells (Fig. 7: D).

Occasional cells of the medullary rays and wood parenchyma contain granular contents staining brown with iodine and others contain single and compound starch grains.

A small central core about 1 mm. in diameter consists of a ring of collapsed internal phloem groups and the central pith (Fig. 8: E). The pith is mainly parenchymatous with a few slightly lignified cells arranged in longitudinal rows, latex tubes similar to those of the bark and calcium oxalate crystals. A few stone cells, 11 to 72  $\mu$  in length, occur either isolated or in small groups at the periphery of the internal phloem (Fig. 8: G).

#### THE POWDERED ROOT

The principal features of the powdered root are:

1. Large quantities of lignified tissue derived from the secondary

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xylem consisting of fragments of relatively thin walled vessels with bordered pits, libriform fibres, fibre-tracheids with bordered pits, isodiametric and elongated pitted medullary ray cells and elongated wood parenchyma cells. The two latter are usually packed with starch grains.

2. Isodiametric, elongated or irregularly shaped stone cells occurring either singly or in groups. A few may contain calcium oxalate crystals or starch grains.

3. Thin walled yellow cork cells of two types—lignified and radially compressed unligified cells.

4. Thin walled cellulosic elements derived from the phelloderm and phloem, usually much broken but sometimes containing calcium oxalate crystals, starch grains and resinous material.

5. Crystals of calcium oxalate in the form of single or twin monoclinic prisms, irregular masses, aggregates and irregular structures.

6. Numerous rounded, oval, plano-convex and concavo-convex single starch grains about 1 to 4 to 18 to 30  $\mu$  in diameter. (Occasional samples possess practically no starch).

7. Yellowish irregular masses of amorphous material derived from the lumina of older vessels.

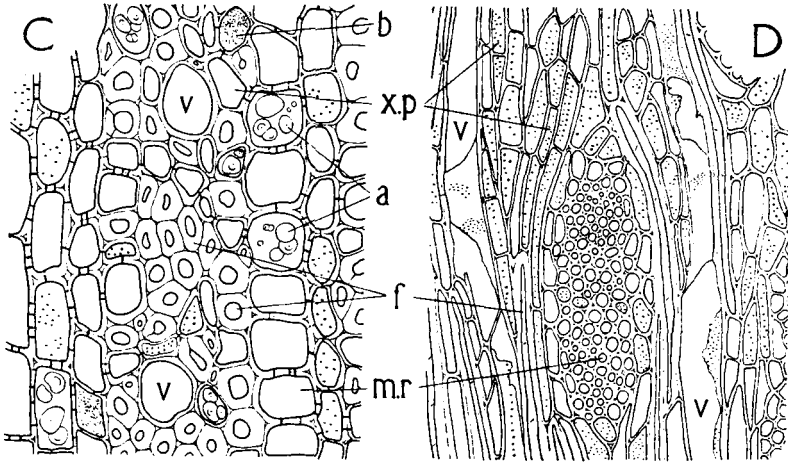


FIG. 7. *Rauwolfia vomitoria* Afz. Stem base. Wood. C, transverse section  $\times 200$ ; D, tangential longitudinal section  $\times 85$ . a, starch grains, b, resinous material; f, fibres; m.r., medullary rays; v, vessel; x.p., xylem parenchyma.

DISCUSSION AND SUMMARY

The structure of *R. vomitoria* root is such that it can readily be distinguished from *R. serpentina* root in both the whole and powdered conditions. The main differences lie in the composition of the woods and in the presence or absence of stone cells.

Most other species of *Rauwolfia* examined to date, differ macroscopically from *R. vomitoria* and can be distinguished by microscopical sections in which the type of cork, phloem modifications and wood structure are

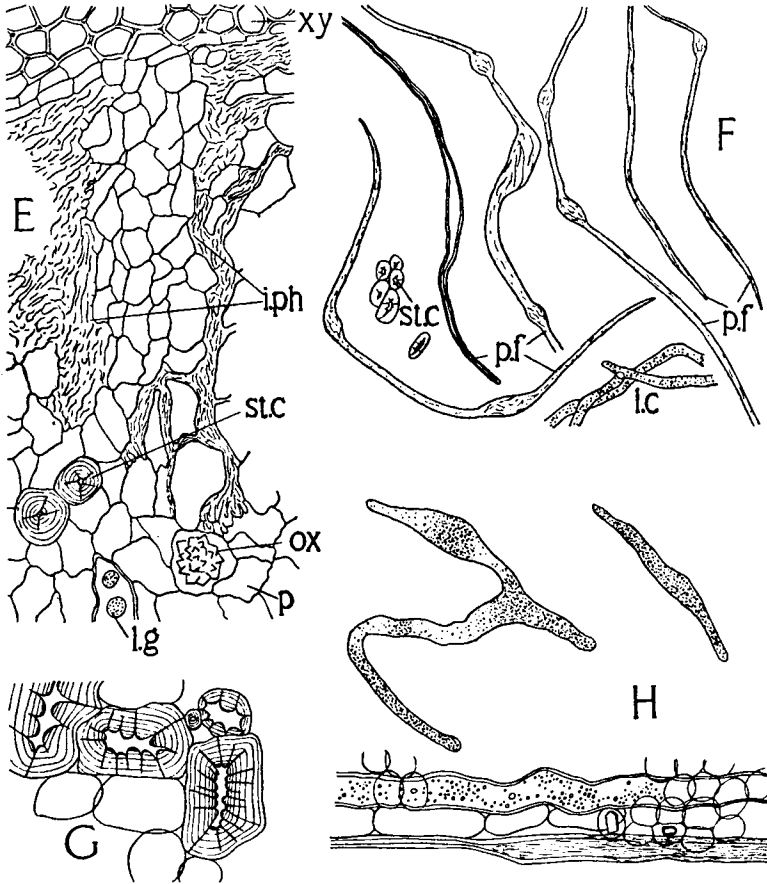


FIG. 8. *Rauwolfia vomitoria* Afz. Stem base. E, transverse section of the pith and internal phloem  $\times 200$ . F, isolated elements from the bark  $\times 45$ . G, isolated stone cells from the pith  $\times 200$ . H, latex vessels from the phloem, lower diagram with attached pericyclic fibre  $\times 85$ ; *i.ph*, internal phloem; *l.c.*, latex canal; *l.g.*, latex globule; *ox*, calcium oxalate crystal; *p*, pith; *p.f.*, pericyclic fibres; *st.c.*, stone cell; *xy*, xylem.

particularly useful. These species present some difficulty when in the ground condition since wood structure is not obvious and the presence or absence of alternating zones of lignified and unlignified cork cells may be difficult to ascertain. The micromeasurements available<sup>15</sup> show certain differences from those of *R. vomitoria* and on further investigation, may provide an additional tool for the differentiation of co-generic species.

The general structure of *R. vomitoria* root and rootstock appear typical of the genus, in so far as it has been examined. The presence of latex canals in the stem structures is characteristic of the Apocynaceæ as a whole, although they do not occur in the rhizome of *R. serpentina*.<sup>14</sup> One feature of interest is the almost complete absence of starch in a few of the commercial samples examined. Since no pre-treatment of the

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material was obvious it is possible that the starch content of the living roots undergoes some natural variation.

The principal histological characters of the drug are summarised below:

1. CORK. A light spongy layer composed of alternating zones of lignified cells and radially flattened, unlignified cells; easily removed as powdery flakes due to the rupture of the larger fragile elements.

2. PHELLODERM. Chiefly parenchymatous, contains starch grains and calcium oxalate. Isodiametric or longitudinally elongated stone cells occur singly or in small groups.

3. PHLOEM. Larger roots possess an outer nonfunctioning secondary phloem with numerous stone cell groups arranged in interrupted tangential rows. An inner secondary phloem consists of sieve tubes, companion cells, phloem parenchyma and secretory cells arranged between the medullary rays. Longitudinal rows of calcium oxalate crystals and starch grains are common.

4. XYLEM. A tough lignified secondary wood comprises the majority of the drug and numerous relatively large vessels give the wood a porous structure. Most vessels possess walls with bordered pits, towards the centre of the root they may be occluded with yellowish brown material. Libriform, thick walled fibres are numerous together with fibre-tracheids and some tracheids. The heterogenous lignified medullary rays and the lignified wood parenchyma contain single and compound, often angular, starch grains and sometimes resinous material.

5. ROOTSTOCK AND STEM-BASE. These differ from the root in the following respects. An incomplete ring of isolated, or small groups of, unlignified pericyclic fibres occurs between the cortex and phloem. The wood is very dense and exhibits no large vessels. A central, almost unlignified zone up to 1 mm. in diameter, consists of a ring of internal phloem groups, a few stone cells and pith. Latex canals occur in the pith and bark.

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