THE PHARMACOGNOSY OF THE ROOT OF *RAUWOLFIA LIGUSTRINA* ROEM. AND SCHULT.

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The morphological and anatomical characters of *Rauwolfia ligustrina* roots are described and compared with those of other *Rauwolfia* species. The characters of the stem, particularly in so far as they concern its detection when mixed with root, are recorded. Analytical studies on individual plants indicate that reserpine is confined almost entirely to the root-bark, the concentration rapidly decreasing towards the crown and becoming negligible in the stems. Samples of root from different localities may vary greatly in reserpine content.

RECENT investigations (Woodson, 1957; Rao, 1956) have suggested that some 34 species of *Rauwolfia* are indigenous to tropical and sub-tropical America ranging from *R. linearifolia* Brit. and Wils., a small plant of up to 50 cm. in height to *R. praecox* K.Sch., a tree of up to 30 metres. Two species are of widespread distribution, *R. tetraphylla* L. which is found in the Antilles, Central America, Colombia, Ecuador, Peru and Venezuela and *R. ligustrina* R. and S. having a similar geographical distribution. They differ in the number of leaves at each node and the length of the inflorescence relative to the associated large leaf (Rao, 1956). *R. tetraphylla* L. (synonyms: *R. canescens* L., *R. heterophylla* R. and S., *R. hirsuta* Jacq.) (Woodson, 1957; Rao, 1956) is a commercial source of the rauwolfia alkaloids. The pharmacognosy of the roots has been studied by Youngken (1954, 1955) (*R. heterophylla* and *R. canescens*), Esdorn and Nolde (1955) and Esdorn and Schmitz (1956) (*R. heterophylla* and *R. canescens*), and Dillemann and Paris (1958) (*R. tetraphylla*). *R. ligustrina* R. and S. is considered by Rao (1956) to include the species *R. ternifolia* H.B.K., *R. parvifolia* Bert. ex Spreng., *R. parvifolia* var. *cubana* A.DC., *R. parvifolia* var. *tomentella* Muell.-Arg., *R. alphonsiana* Muell.-Arg. and *R. indecora* Woodson, the differences in leaf characteristics originally used as the basis for the establishment of a new species being unreliable. The native names for *R. ligustrina* include Brazil–Paratudo, Mamao de Sapo; El Salvador—San Jose; Mexico—Chirillo, Veneno; Colombia–Contra, Venenito. The plant's natural habitat is chiefly moist situations, near the seashore in coastal thickets, on the plains near rivers, in savannas and wet meadows, with an altitude range of from sea-level to about 1,000 metres. In common with other species of the genus, *R. ligustrina* has been the subject of considerable research and at least 21 different alkaloids have been reported in roots described as *R. ternifolia*, *R. indecora* or *R. ligustrina* (Ishidate, Okada and Saito, 1955; Cardoso and Venâncio, 1956; Korzun, St. André and Ulshafer, 1957; Müller, 1957). Fernandez (1958) estimated the total alkaloidal content of fresh roots of *R. ligustrina* as 2·6 per cent and the reserpine content as 0·03 per cent.

As *R. ligustrina* contains reserpine in the roots, the preparation of a systematic description of the roots seemed desirable, together with an investigation of the distribution of reserpine in the main axis and in samples from different sources. A more limited study of the stem was undertaken when it was observed that its total alkaloidal content was very different to that of the root and that its addition to commercial material would be undesirable.

Plant Material

The following specimens of *R. ligustrina* were utilised in this investigation:

1. Dried plants consisting of roots, rootstock and the attached aerial stems, collected in Trinidad by Dr. F. J. Simmonds, Imperial College of Tropical Agriculture, Trinidad.

2. Large roots and rootstocks with attached stem-bases, collected in N.E. Brazil, identified by Dr. Hürlimann and donated by Ciba Ltd., Basle.

3. Roots of plants collected in Brazil, identified by Dr. A. Ducke and presented by Professor Francisco José de Abreu Matos of the University of Céara, Brazil.

4. Powdered root from Professor H. T. Cardoso, Instituto Oswaldo Cruz, Rio de Janeiro.

5. A small root segment, authenticated by Dr. A. Ducke and donated by Professor R. E. Woodson, Jnr.

Macroscopy

Large roots may be up to 20 cm. long and taper from 10 cm. diameter at the crown to 1·5 cm. in the lower portions of the root system; they bear numerous lateral roots, circular scars and stumps which vary from about 1 mm. to 3 cm. in diameter. The small wiry roots are brittle and the large ones tough and difficult to fracture, eventually breaking with a fracture short in the bark and splintery in the wood. The outer yellowish-brown cork, slightly darker in colour in Brazilian than Trinidad samples is marked by short longitudinal discontinuous ridges and furrows; in old samples it may be scaly and occasionally exfoliates to reveal the orange-brown phloem or yellowish wood. A smoothed transverse surface of a root exhibits a yellow-brown bark rarely exceeding 1·5 mm. in thickness and a yellowish finely radiate, porous wood, showing in the larger pieces faint growth rings. Large roots may show a distinctly darker heartwood or may occasionally be hollow resulting from decay or insect attack. When examined under screened ultra-violet light the cork appears a velvet-brown colour, the phloem fluoresces a yellowish-brown and the xylem pale yellow. The odour is slight and the taste, particularly of the bark, bitter.

Microscopy

Variation in the general appearance of transverse sections of roots is due mainly to the presence or absence of sclereids in the bark and of

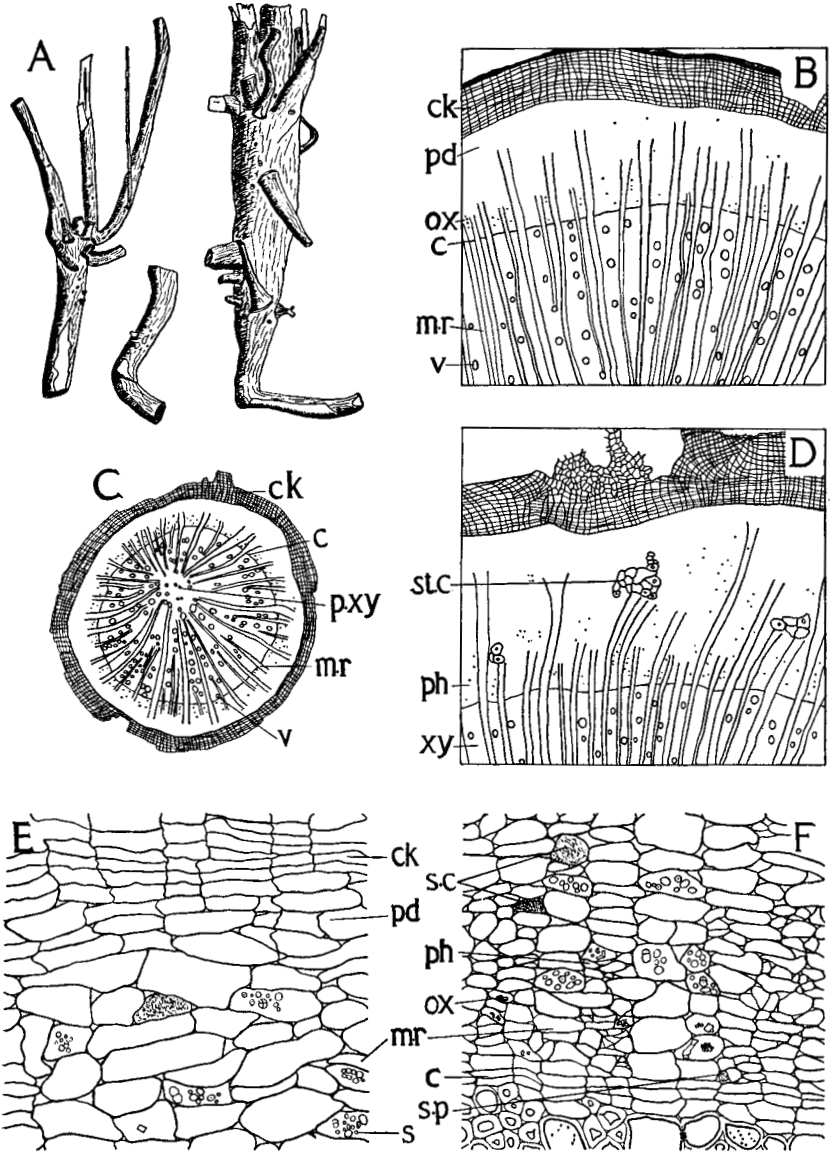

FIG. 1. *Rauwolfia ligustrina* Roem. and Schult. Root. A, roots and attached stems × ¼. B–D, general diagrams of transverse sections of roots. B, 5 mm. diameter × 25; C, 2 mm. diameter × 15; D, 1·8 cm. diameter × 25. E, F, transverse sections of bark of root of diameter 1·5 cm., both × 200. E, outer tissues; F, inner phloem. c, cambium; ck, cork; m.r, medullary rays; ox, calcium oxalate crystal; pd, phelloderm; ph, phloem; p.xy, primary xylem; s, starch; s.c, secretion cell; s.p, sieve plate; st.c, stone cell; v, vessel; xy, xylem.

a rhytidoma. Sclereids occur often in the larger roots but are commonly absent in roots of diameter less than 8 mm. Externally there is frequently a brown layer of collapsed cells and the periphery of the cork layer may show radial clefts containing small areas of ovoid or polygonal, lignified cells. Large roots may possess a rhytidoma-like structure, consisting of oval, tangentially elongated groups of brown lignified ovoid cells and occasional well formed sclereids, surrounded by the cork layer. The cork cells, which usually occur in a single layer of up to about 40 radial cells are suberised and usually, although not invariably unlignified; in surface view they are polygonal, R = 8 to **12** to **20** to 28 μ, T = 12 to **24** to **44** to 68 μ and L = 12 to **20** to **48** to 60 μ (Fig. 1, E; 2, A; 4, ck).

The phellogen consists of a few layers of radially compressed cells (Fig. 2, A). The phelloderm may possess up to about 15 radial rows of cells, the inner layers of tangentially elongated, oval cells being usually displaced by sliding growth; the walls are sometimes collenchymatous. R = 16 to **20** to **32** to 48 μ, T = 16 to **32** to **56** to 80 μ and L = 20 to **36** to **60** to 80 μ (Fig. 1, E; 2, A). Calcium oxalate, usually as twinned prisms, and starch, as single or compound granules of up to four components, occur in variable amounts. Single starch granules are spherical or ovoid, 3 to **4** to **12** to 20 μ in diameter with central hila as points or stellate clefts.

Internal to the phelloderm is a relatively wide zone of secondary phloem composed of radial groups of sieve tubes and companion cells separated by phloem parenchyma, medullary rays, secretory cells and sclereids. The sieve tubes have thin cellulosic walls, a wide lumen and possess sieve plates on the end walls (Fig. 1, F; 2, B; 5,C), the companion cells are narrower and the phloem parenchyma cells are of variable proportions, being up to five times as long as they are wide. Two types of cell are present in the medullary rays. There is a central core of relatively small isodiametric, or slightly radially elongated procumbent cells, 3 to 5 cells wide and up to 15 cells high, with upper and lower extensions of larger erect cells which may be continuous with the cells of the medullary rays immediately above or below (Fig. 1, F; 2, A,B,D; 5, J). For the procumbent cells R = 16 to **20** to **32** to 40 μ, T = 12 to **20** to **28** to 40 μ, L = 12 to **16** to **28** to 32 μ and for the erect cells R = 12 to **16** to **28** to 32 μ, T = 20 to **28** to **44** to 52 μ, L = 28 to **36** to **56** to 60μ. Sclereids may occur in the outer phloem, either as solitary cells or as groups of up to about 4, 7 and 10 cells in the radial, tangential and vertical planes respectively (Fig. 2, C). In longitudinal sections the apices of the groups may be prolonged by narrow elongated sclereids with ill-defined cross walls. In outline, isolated cells may be circular, polygonal or elongated with a sinuous margin; some have solid projections at the apices. Length = 36–**60**–**160** to 280 μ, breadth = 20 to **32** to **72** to 116 μ. Well developed sclereids possess irregular, sometimes branched lumina, with funnel-shaped pits and stratified, lignified walls (Fig. 4, st.c; 5, M). Secretory cells occur with variable frequency in the phelloderm and phloem, being completely absent in some roots and very frequent in others. The contents stain orange-brown with a solution of iodine and

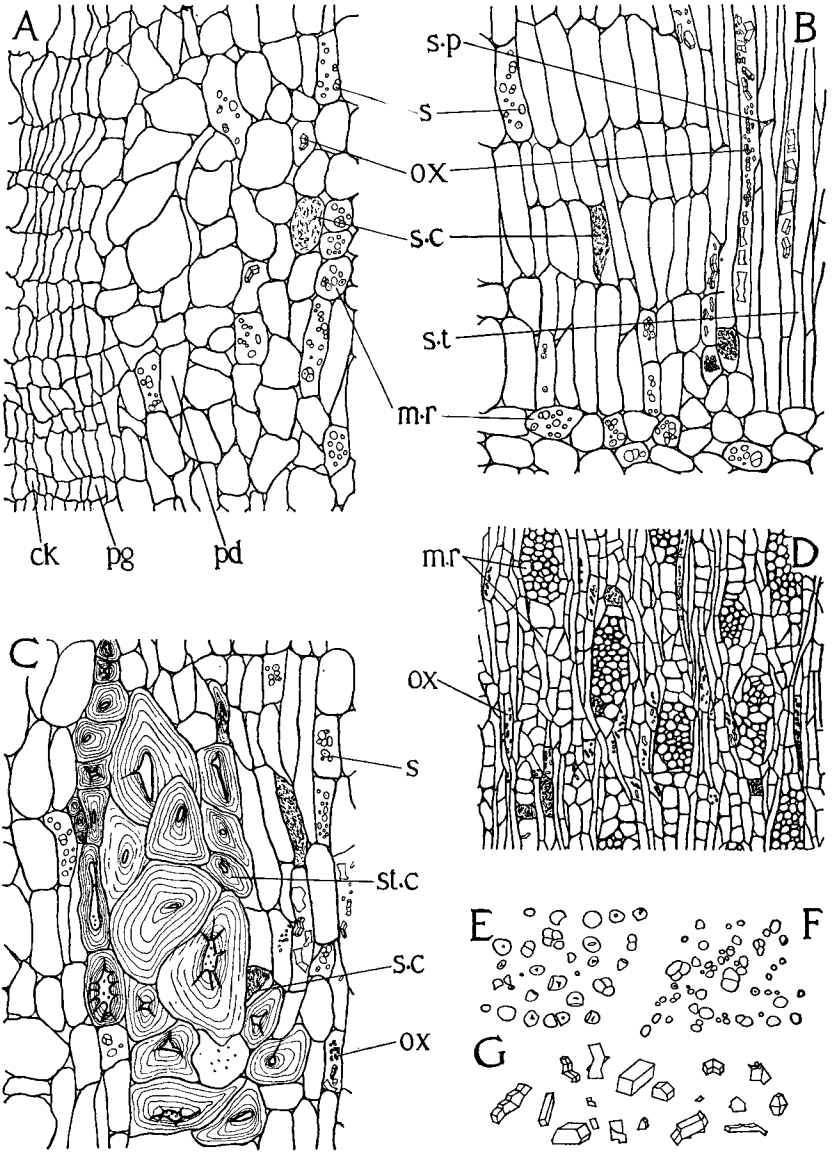

FIG. 2. *Rauwolfia ligustrina* Roem. and Schult. Root bark. A, radial longitudinal section of the outer tissues, root diameter 1 cm. B, ditto inner tissues. C, ditto phelloderm and outer phloem, root diameter 1·8 cm. All × 200. D, tangential longitudinal section of inner phloem, root diameter 1 cm. × 50. E, F, starch grains of wood and bark respectively. G, calcium oxalate crystals from the bark, × 200. ck, cork; m.r, medullary ray; ox, calcium oxalate crystal; pd, phelloderm; pg, phellogen; s, starch; s.c, secretion cell; s.t, sieve tube; st.c, stone cell; s.p, sieve plate.

faintly pink with Sudan III solution (Fig. 1, E,F; 2, A,B,C). Individual cells can be isolated from alkali macerates (Fig. 4, s.c).

Starch granules, similar in size and composition to those in the phelloderm, occur throughout the phloem; they are usually most abundant in the outer region but in some roots may be almost entirely absent (Fig. 1, E,F; 2, A,B,C,F; 5, J). Calcium oxalate occurs abundantly throughout the phloem and is most evident in longitudinal sections in which long vertical rows of crystals may be observed as elongated monoclinic prisms, often twinned on one or more of the hemi-pyramid faces. Large well-formed prisms may be embedded in masses of sandy crystals. When examined in polarised light the twin crystals show a bicolouration effect. For the distinct prisms, length = 6 to **16** to **28** to 36 μ, breadth = 3 to **4** to **8** to 12 μ (Fig. 2, G).

The completely lignified, radial xylem consists of vessels, tracheids, fibres, xylem parenchyma and medullary rays (Fig. 3, A,B,C). The vessels occur solitary or in pairs and in transverse section are radially elongated or rounded, R = 32 to **48** to **64** to 104 μ, T = 28 to **40** to **56** to 80 μ. Isolated vessel segments show transverse or oblique perforation plates also obvious in radial longitudinal sections and, characteristic elongated apices. The walls possess numerous, alternately arranged, bordered pits (Fig. 3, C; 4, V). Yellow and orange tyloses, staining red with phloroglucinol and concentrated hydrochloric acid are frequent; in the larger roots the tyloses may contain masses of starch. Other vessels are occluded with amorphous brown or black material. The apotracheal xylem parenchyma is seen in transverse sections as isolated cells or more usually, short uniseriate, rarely biseriate tangential rows connecting the vessels and medullary rays and, in longitudinal sections as vertical rows of cells (Fig. 3, A,C). Simple, oval or rounded pits are present on the walls except with cells adjacent to vessels when the pits are bordered. R = 12 to **20** to **28** to 40 μ, T = 8 to **16** to **24** to 36 μ and L = 40 to **72** to **94** to 192 μ. Numerous xylem fibres occur associated with the other wood elements and in transverse section appear either rounded or polygonal in outline with thick lignified walls. Fibres isolated by maceration are somewhat irregular in outline with apices which may be acute, obtuse or occasionally bifurcated. Some apices are formed by a cross wall set at an angle of about 60° to the longitudinal plane. The walls bear slit-like or funnel-shaped pits. Tracheids and intermediate fibre-tracheids are also present (Fig. 3, B, C; 4, f, tr). Fibres isolated from wood macerated in potassium chlorate and concentrated hydrochloric acid measured 310 to **620** to **1,050** to 1,387 μ in length and 8 to **16** to **24** to 36 μ in breadth. The heterogeneous medullary ray cells resemble those of the bark being composed of a core of small, radially elongated procumbent cells, three to six cells wide and up to 15 cells in height, with upper and lower extensions of elongated erect cells which extend vertically to connect with other groups of procumbent cells. Groups of the smaller procumbent cells, when viewed in tangential longitudinal sections, where they appear to be almost circular in outline, are partially or completely enclosed by the larger erect cells. For the procumbent cells, R = 20 to **44** to **84** to 128 μ,

T = 8 to **12** to **20** to 28 μ and L = 8 to **12** to **24** to 40 μ; for the erect cells R = 16 to **28** to **44** to 56 μ, T = 20 to **24** to **48** to 64 μ and L = 28 to **40** to **72** to 104 μ (Fig. 3, A, B, C; 5, E, L). Single prisms of calcium oxalate are present mainly in the procumbent cells of the medullary rays. Length of prisms = 12 to **20** to **24** to 40 μ, breadth = 8 to **12** to **20** to 36μ.

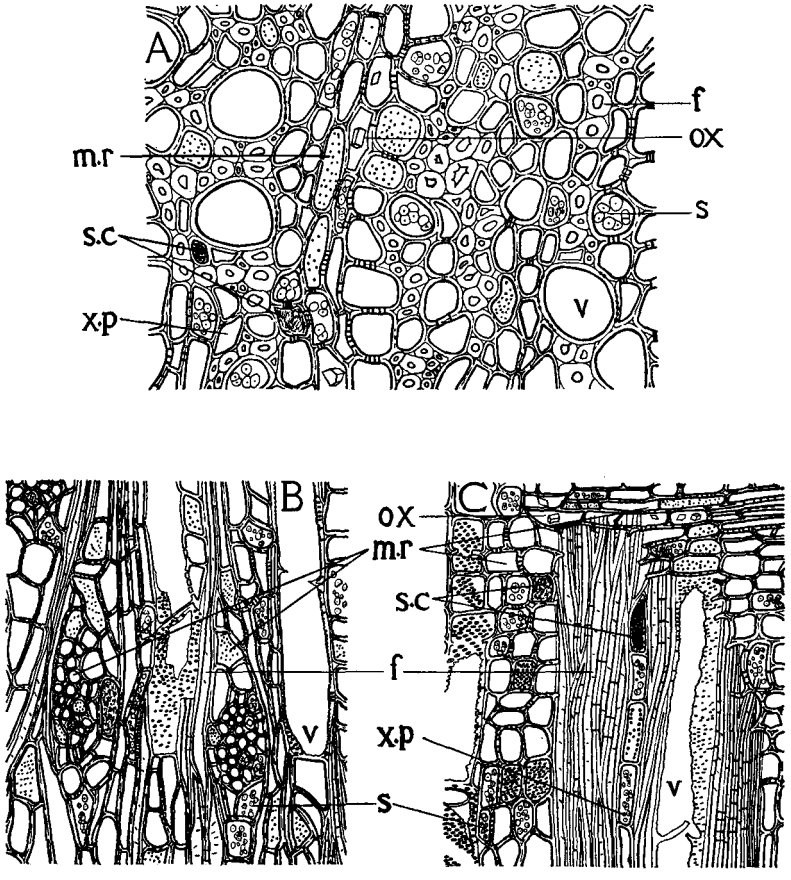

FIG. 3. *Rauwolfia ligustrina* Roem. and Schult. Root wood. Diameter of root 3 cm. A, transverse section × 200. B, tangential longitudinal section × 100. C, radial longitudinal section × 100. f, fibre; m.r, medullary ray; ox, calcium oxalate crystal; s, starch; s.c, secretion cell; v, vessel; x.p, xylem parenchyma.

Xylem parenchyma and medullary ray cells may contain material which with solution of iodine stains orange-brown to purple and other medullary ray cells may possess deep brown amorphous contents. Rounded, oval and plano-convex starch grains, diameter 3 to **8** to **12** to 24 μ, and compound grains of up to five components occur in variable amounts. The hila, as central points or stellate clefts, are more obvious on these grains than on those from the phloem and phelloderm (Fig. 2, E).

Powdered Root

The principal histological features of the powdered root are:

1. Masses of thin-walled, yellow cork cells, polygonal in surface view (Fig. 5, B).

2. Thin-walled cellulosic elements of the phelloderm, phloem and medullary rays containing starch grains, calcium oxalate crystals and resinous material (Fig. 5, C, F, G, J, K).

3. Almost circular or elongated stone cells, either singly or in groups. Length 36 to **60** to **160** to 280 μ, breadth 20 to **32** to **72** to 116 μ (Fig. 5, M).

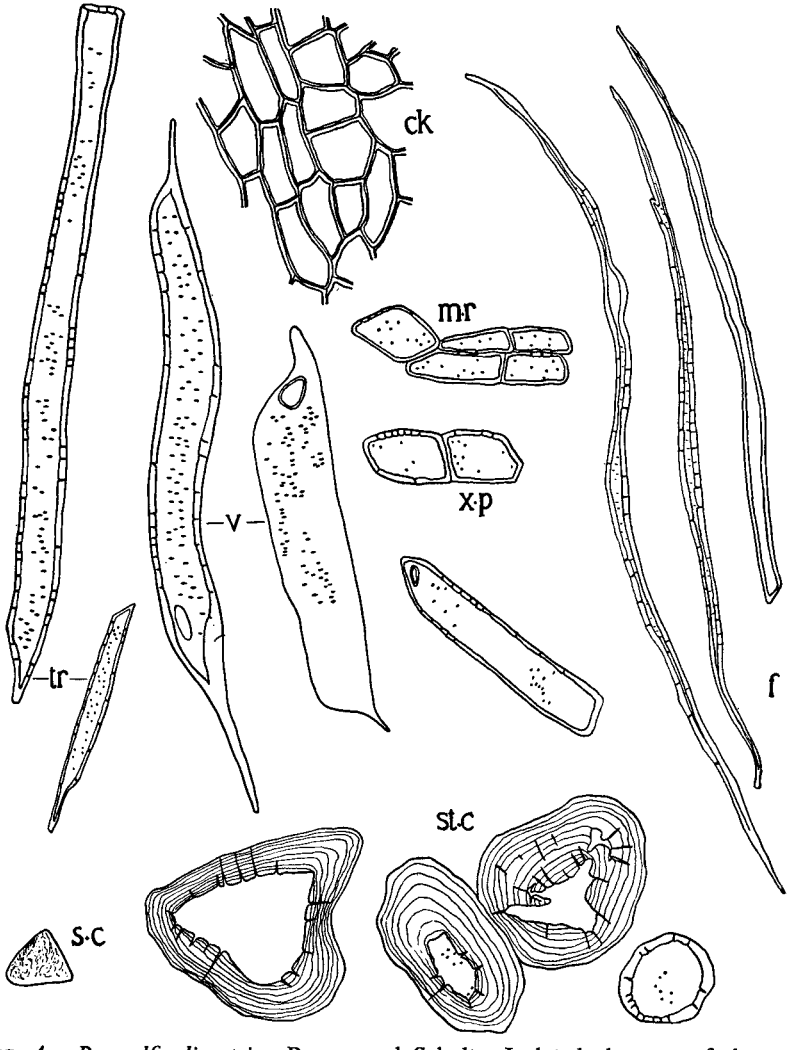

FIG. 4. *Rauwolfia ligustrina* Roem. and Schult. Isolated elements of the root. ck, cork cells; m.r, medullary ray cells; s.c, secretion cell; st.c, stone cell, tr, tracheids; v, vessels; x.p, xylem parenchyma; all × 200. f, xylem fibres × 100.

4. Large quantities of lignified xylem elements composed of fibres, elongated xylem parenchyma cells, two types of medullary ray cell and a relatively few vessels with bordered pits. The parenchyma and medullary ray cells usually contain starch grains (Fig. 5, A, D, E, H, I, L).

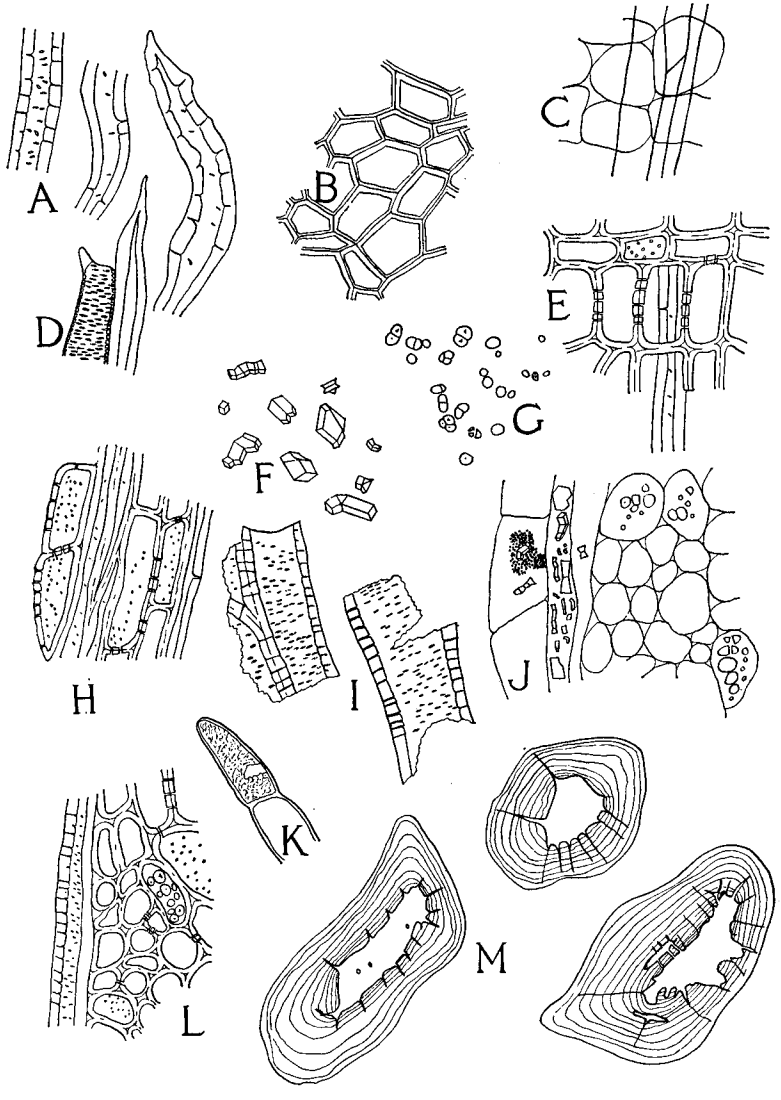

FIG. 5. *Rauwolfia ligustrina* Roem. and Schult. Elements of the powdered root. A, fragments of xylem fibres. B, cork cells. C, portion of medullary ray (as in radial longitudinal section) and sieve tubes. D, vessel and fibre. E, medullary ray cells (as in radial longitudinal section). F, calcium oxalate crystals. G, starch grains. H, xylem parenchyma and fibres. I, vessel fragments. J, medullary ray cells (as in tangential longitudinal section), parenchyma and sieve tube, K, secretion cells. L, medullary ray (as in tangential longitudinal section). M, stone cells. All × 200.

STEM

The stems are cylindrical and, in the samples examined, 0·5 to 3 cm. in diameter at the junction with the rootstock. The buff coloured cork, showing numerous lighter circular scars, is somewhat smoother than that of the root with continuous slight furrows and ridges along its length. A smoothed transverse surface of the stem is similar to that of the root but shows in addition a pith, growth rings which are more distinct. The taste, particularly of the bark, is bitter.

Transverse sections of the stem show variations depending on the presence or absence of sclereids in the phelloderm, outer phloem and pith and upon the number of pericyclic fibres. The brown cork consists of radially arranged, usually unlignified, suberised tabular cells, polygonal in surface view, R = 6 to **8** to **19** to 23 μ, T = 12 to **19** to **38** to 46 μ and L = 15 to **19** to **38** to 55 μ (Fig. 6, A), and in older stems there are intermittent tangentially, elongated groups of lignified cells surrounded by cork cells. The phelloderm is a narrow layer of up to about 12 cells in radial width. Corners of individual cells may be lignified and contiguous cell walls may possess brown unlignified thickening giving the appearance of tangential brown bands in transverse section. R = 11 to **15** to **30** to 38 μ, T = 19 to **34** to **62** to 76 μ and L = 15 to **27** to **40** to 46 μ (Fig. 6, B). In transverse section the pericycle is indicated by scattered, highly refractive fibres, circular, oval or subreniform in outline and giving either no or a slight reaction for lignin. Diameter = 16 to **19** to **27** to 31 μ. In longitudinal sections or in macerated material, the fibres show intermittent swollen portions, 23 to **31** to **58** to 105 μ in width (Fig. 6, B, I). Groups of sclereids, similar to those found in the root may occur in the phelloderm and outer phloem (Fig. 6, G, H) and a crystal sheath occasionally surrounds them.

The inner phloem consisting of thin-walled sieve tubes, companion cells and phloem parenchyma together with the medullary rays resemble the corresponding tissue of the root (Fig. 6, B). The rows of calcium oxalate crystals, often embedded in granular material, are again evident. Starch occurs in variable amounts and is completely absent from some stems. Single grains are circular, ovoid or plano-convex in outline with a central hilum appearing as a point or stellate cavity. Diameter = 3·8 to **6** to **14** to 19 μ. Compound grains of up to five components are frequent. Secretion cells, having somewhat granular contents which stain yellow-brown or purplish with an iodine solution, occur throughout the phloem and phelloderm (Fig. 6, B). The number of cells containing such material was very variable in the different plants examined. Latex ducts occur infrequently as circular or oval cavities and in the specimens examined were much more numerous in the pith.

The stem-wood resembles the root-wood in structure and cell contents (Fig. 7, A, B, C). The main differences lie in the diameter of the vessels, R = 24 to **32** to **48** to 56 μ, T = 20 to **28** to **44** to 60 μ, the somewhat larger fibres, length = 474 to **632** to **1027** to 1454 μ, breadth 11 to **15** to **21** to 30 μ and the generally smaller cells of the wood parenchyma

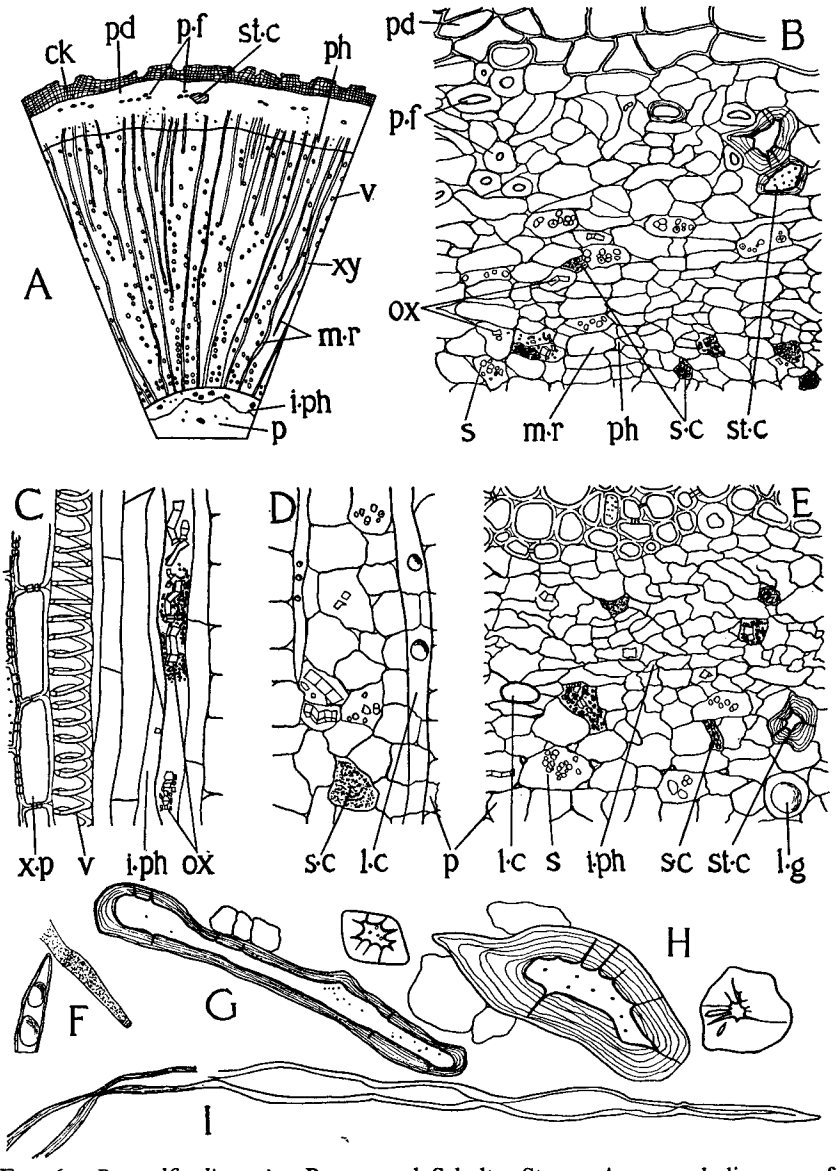

FIG. 6. *Rauwolfia ligustrina* Roem. and Schult. Stem. A, general diagram of transverse section × 15. B, transverse section of phelloderm and phloem. C, radial longitudinal section of xylem and internal phloem. D, ditto pith. E, transverse section of xylem, internal phloem and pith. B–E, all × 200. F–I, isolated elements of bark and pith. F, latex tissue × 200. G, elongated stone cell × 100. H, stone cells × 200. I, pericyclic fibre × 100. ck, cork; i.ph, internal phloem; l.c, latex canal; l.g. latex globule; m.r, medullary ray; ox, calcium oxalate crystal; p, pith; pd, phelloderm; p.f. pericyclic fibre; ph, phloem; s, starch; s.c, secretion cell; st.c, stone cell; v, vessel; x.p, xylem parenchyma; xy, xylem.

and medullary rays. Small groups of spiral vessels constitute the primary xylem (Fig. 6, C).

An unlignified central area consists of large parenchymatous cells of the pith and an internal peri-medullary phloem arranged either as an almost continuous circle or as isolated groups of elements. Calcium oxalate, similar to that of the outer phloem is present in vertical rows (Fig. 6, C, E). Latex tubes permeate between the pith cells, the contents appearing as refractive globules in Berlese mountant and staining yellow-brown with iodine solution. Isolated sclereids or groups of up to three cells radially, five tangentially and eight vertically occur in the pith and are similar to those of the outer phloem (Fig. 6, D, E, F).

Distribution of Reserpine throughout the Main Axis

For the determination of the reserpine distribution, roots and stems for assay were divided into segments corresponding with the numbered

TABLE I

Reserpine content of *R. ligustrina* Roem and Schult

	Plant	Segment No. of bark, Fig. 8	Reserpine per cent
A.	Main root	1	0·05
	" "	2	0·05
	Crown	3	0·03
	"	4	0·01
	Stem	5	0·01
	"	6	0·01
	"	7	0·02
	"	8	0·02
	"	9	0·01
B.	Main root	1	0·02
	" "	2	—
	Crown	3	0·01
	"	4	0·01
	Stem	5 to 12	All <0·01
C.	Main root	1	0·06
	" "	2	0·05
	" "	3	0·05
	Crown	4	0·02
	"	5	0·01
	"	6	<0·01
	Lateral root	7	0·04
	" "	8	0·09
	" "	9	0·02
	Stem	10	0·03
	"	11	0·01
D.	Main root	1	0·32
	" "	2	0·32
	" "	3	0·44
	" "	4	0·46
	" "	5	0·21
	" "	6	0·22
	" "	7	—
	" "	8	0·19
	" "	9	—
	" "	10	0·16
	" "	11	0·18
	" "	12	0·18
	" "	13	0·13
	" "	14	0·11
	" "	15	0·09
	Crown	16	0·02
	"	17	0·01
	"	18	0·01
	Lateral root	19	0·29
	" "	20	0·21
	" "	21	0·18
	" "	22	0·22
E.	Main root	1	0·01
	" "	2	0·11
	" "	3	0·01
	Crown	4	<0·01
	Stem	5	0·01
	"	6 to 12	<0·01
	Lateral root	13	0·02

sections shown in Fig. 8. As preliminary experiments employing paper chromatography had shown the reserpine content of the wood of both roots and stems to be negligible, quantitative determinations were made on the bark only, using a modification of the fluorimetric procedure described for the evaluation of *R. caffra* (Court, Evans and Trease, 1958). With *R. ligustrina* paper chromatography indicated that the reserpine fractions contained other fluorescent substances which interfered with the assay. It was therefore necessary to submit each fraction to paper chromatography and to elute the reserpine band from the chromatogram

with 5N acetic acid. These operations were carried out in the dark and the conditions were standardised. A standard curve was prepared by subjecting pure reserpine to the same procedure. Any rescinnamine present, which under these conditions of chromatographic separation has an R_F value similar to that of reserpine, would also be determined by the method and is consequently included in the reserpine figures. The results of the assays are recorded in Table I. Two other Brazilian root samples were analysed, the bark of one from a root 1 cm. diameter contained 0·17 per cent reserpine, and the other consisting of powdered whole roots, 0·13 per cent.

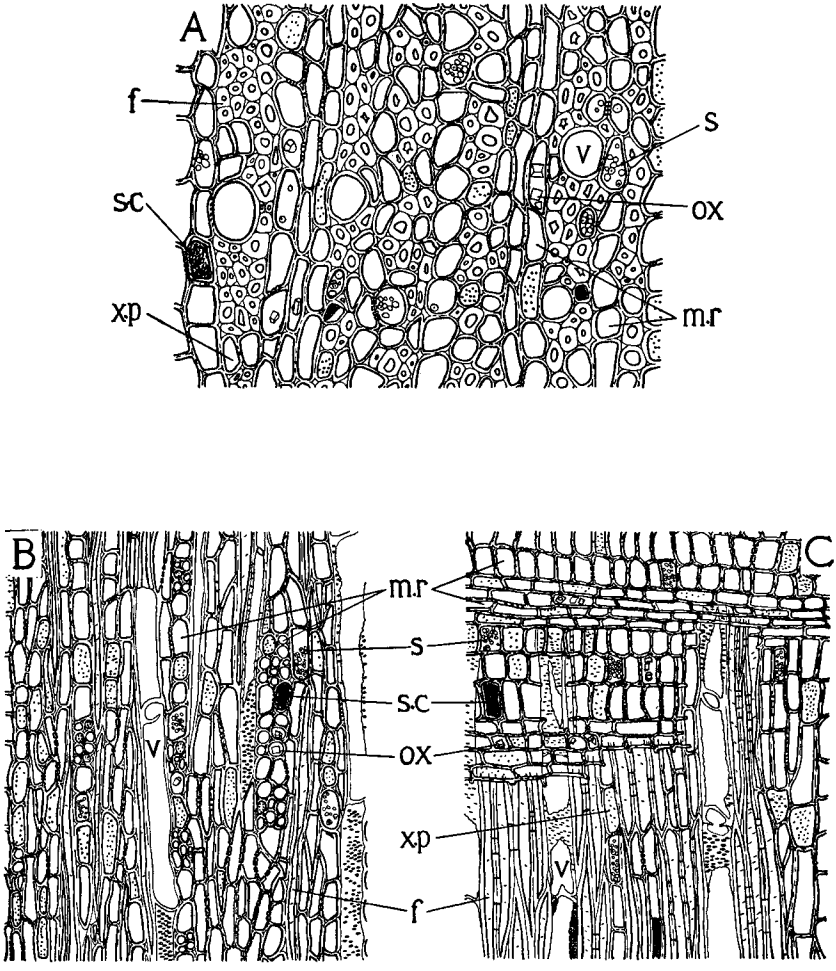

FIG. 7. *Rauwolfia ligustrina* Roem. and Schult. Stem wood. Diameter of stem, 8 mm. A, transverse section × 200. B, tangential longitudinal section × 100. C, radial longitudinal section × 100. f, fibre; m.r, medullary ray; ox, calcium oxalate crystal; s, starch; s.c, secretion cell; v, vessel; x.p, xylem parenchyma.

Discussion

The histological characters of *R. ligustrina* are typical of the family Apocynaceae, characteristic features being latex canals and pericyclic fibres in the stem and vessel segments with peg-like projections and large communication pores in the root and stem woods. Characteristic of the genus *Rauwolfia* are the non-septate fibres, phloem sclereids,

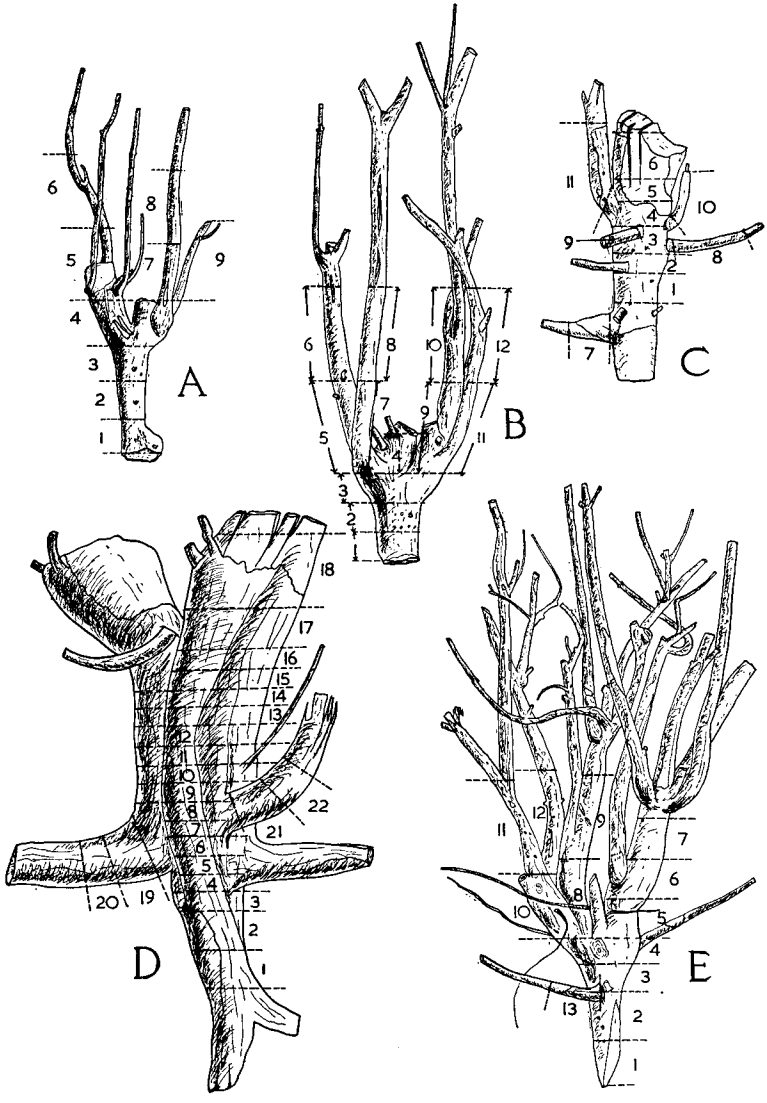

Fig. 8. *Rauwolfia ligustrina* Roem. and Schult. Roots, crowns and attached stems × ¼. Numbers indicate segments analysed for reserpine (see Table I). A, B, E Trinidad samples. C, D Brazilian samples.

laticiferous tubes and heterogeneous rays. The anatomy of the root shows features that are intermediate between those described by Woodson (1957) as being characteristic of arboreal and those of subherbaceous tendency; these include distribution of phloem and xylem, nature of phloem components and vessel diameter.

A considerable variation in cell contents, particularly with regard to starch and resinous secretory material was noted between various specimens, similar variations having been recorded for *R. caffra* (Court and others, 1958) and *R. vomitoria* (Evans, 1956). Woodson has suggested that the starchy tyloses of the vessels of *R. ligustrina* might be used as a diagnostic feature of the root. We have, however, by the examination of more samples than were available to Woodson (private communication), found that such tyloses were only present in the larger roots and were not of such uniform occurrence as to be of great diagnostic value.

R. ligustrina can be readily differentiated from *R. serpentina* as the latter has small vessels of about 36 to 54 μ diameter, no sclereids and an extensive stratified cork consisting of alternating zones of lignified and non-lignified cells (Wallis and Rohatgi, 1949). *R. vomitoria* root possesses larger and more sclereids than *R. ligustrina* root, length 28 to 288 μ and breadth 14 to 56 μ, and vessels of greater diameter, 36 to 180 μ (Evans, 1956). The difference between the roots of *R. ligustrina* and those of the closely related *R. tetraphylla* is less marked and comparison is complicated by the fact that *R. canescens*, *R. heterophylla* and *R. hirsuta* although considered synonymous on gross morphological characters (Woodson 1957; Rao, 1956), do differ in certain histological details (Youngken, 1954, 1955; Dillemann and Paris, 1958; Paris, private communication), particularly vessel size. Like *R. ligustrina*, all have a single unstratified cork layer, although large roots of *R. ligustrina* may exhibit a rhytidoma. Sclereids are present in these species and elongated forms, intermediate between sclereids and fibres, are recorded for *R. heterophylla* and *R. canescens*; these are found only rarely in *R. ligustrina* as are the irregularly lobed sclereids having pointed projections and interrupted lumina which Youngken records (1954, 1955) for *R. canescens*. Compared with the results of Dillemann and Paris (1958), the vessel diameters of *R. ligustrina* (32–**48**–**64**–104 μ) are larger than those of *R. canescens* (56–73 μ maximum sizes) and slightly smaller than those of samples labelled *R. tetraphylla*, *R. heterophylla* and *R. hirsuta* (maximum sizes 100 to 120 μ). Intermediate forms of *R. tetraphylla* would therefore be impossible to distinguish from *R. ligustrina* on this basis. Other S. American species, including *R. biauriculata* Muell.-Arg., *R. cubana* A.DC., *R. littoralis* Rusby, *R. nitida* Jacq. and *R. viridis* R. and S., having a similar habit and geographical distribution to *R. ligustrina* do not yet appear to have been examined in detail.

R. ligustrina stems admixed with root can be detected by the smaller vessels of the xylem, a pith possessing sclereids and latex tubes and, a ring of scattered pericyclic fibres either unlignified or slightly lignified, the individual fibres usually showing well marked swellings along their length.

The assays for individual plants indicate that the reserpine content of the root bark increases with distance from the crown and, in one instance (Plant D), reaches a maximum at about 15 cm. below the bifurcation of the crown and then decreases. From the crown to the stem-bases the reserpine content falls to a negligibly low proportion. Should *R. ligustrina* be used as a commercial source of reserpine, it would be desirable that roots only be collected. It is evident, too, that some plants are richer in alkaloids than others, thus Plants A, B and E would be of little economic use but in contrast the powdered root from Brazil supplied by Professor H. T. Cardoso has a very high reserpine content considering that the bark, which contains the majority of the alkaloids, would form probably only 5–10 per cent of the powder. Thus factors such as habit, time of collection and variety may have a marked effect on the pharmacological activity of the plant.

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