AFRICAN *RAUWOLFIA* SPECIES

PART II. THE STRUCTURE OF THE ROOT AND STEM OF *Rauwolfia mombasiana* STAPF

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Received September 18, 1961

A substitute or adulterant for the roots of *R. vomitoria* Afz. is *R. mombasiana* Stapf, an East African shrub with a high reserpine yield. The anatomy of the root and stem is described and illustrated, and compared with published data about other African species.

DURING the last decade the root of the African tree *R. vomitoria* has become an important source of reserpine. Its widespread use has prompted the investigation of other African species which have occurred or may occur as substitutes or adulterants. One such species which has occurred in commerce as a substitute for *R. vomitoria* roots is *R. mombasiana* (Trease, private communication). The two species are closely related and Pichon (1947), in his classification of the genus *Rauwolfia*, has grouped them together with *R. cumminsii* Stapf in the section Endolobus. This section is characterised by a curious aestivation and apocarpous gynaecia.

Raymond-Hamet (1940) reported the hypotensive and adrenaline antagonistic activity of *R. mombasiana* extracts. Reserpine was isolated from the root in 1956, the published yields varying from 0·05 to 0·116 per cent (McAleer, Weston and Howe, 1956; Korzun, St. André and Ulshafer, 1957). The highest yield of weakly basic alkaloids occurs in the root bark (Court, Evans and Trease, 1958).

R. mombasiana was first described by Stapf (1894) and recorded in the Kew Index, Supplement I (1886–95) together with *R. monopyrena*, a species described by Schumann (1895) and now regarded as synonymous. The plant was briefly described by Delourme-Houdé (1944) as a false iboga, a substitute for *Tabernanthe iboga* Baill. Few diagrams and no numerical data were presented and, therefore, a detailed description of a range of specimens is given below and compared with the published anatomy of some other African species.

Habitat and Indigenous Use

A shrub growing to a height of 2 m., *R. mombasiana* occurs in coastal swamp forests. It is found in the Mombasa region of Kenya, on the East and West Usambaras and Pugu Hills of Tanganyika, in Zanzibar and in Mozambique (Feuell, 1955; Greenway, private communication).

The East African tribesmen use a preparation of the roots, ground with coconut oil, for the treatment of pimples. A mixed decoction is taken orally as a cure for gonorrhoea (Feuell, 1955).

Plant Material

The following material was used in this investigation:

1. *R. mombasiana* roots supplied by Dr. P. J. Greenway, East African Herbarium, Nairobi, Kenya, 1956.

2. *R. mombasiana* roots; commercial samples supplied by Professor G. E. Trease, Nottingham University, 1958.

3. *R. mombasiana* roots and stems supplied by Dr. P. J. Greenway, Nairobi, 1960.

4. *R. mombasiana* roots and stems collected near the mouth of the Tana River, north of Malindi, Kenya and supplied by the Department of Scientific and Industrial Research, 1960.

Macroscopy

Root

The roots occur as cylindrical or flattened, occasionally branched segments of varying lengths and up to about 6 cm. diameter. Narrower segments 0·5–2 cm. in diameter comprise the bulk of the samples examined. Externally the soft, pale yellowish brown cork shows irregular longitudinal furrowing and irregular buff or greyish patches of exposed cortical tissue. Frequently pieces of bark have broken away revealing the longitudinally furrowed, pale yellowish or reddish brown wood. Some segments bear the remains of side roots either as protuberances or stumps, or pale rootlet scars.

Smoothed transverse surfaces of the roots show a narrow bark seldom exceeding 3 mm. in thickness and an inner pale buff or yellowish, finely radiate wood possessing a few distinct growth rings.

The larger roots are tough and difficult to break but smaller roots break easily, the fracture being short in the bark and splintery in the wood.

Stem

The stems occur as cylindrical branched segments up to 5 cm. diameter. The external greyish-brown cork shows irregular longitudinal ridging and bears buff or pale brown, rounded or tangentially elongated lenticels. Semicircular leaf scars, occurring in whorls of 4 or occasionally 3 or 5, are frequently apparent on smaller stem segments. Smoothed transverse surfaces of the stems exhibit a narrow bark up to 1·5 mm. in thickness, a cylinder of secondary xylem with up to 10 growth rings and a small central pith, or cavity due to contraction of the pith, which may be up to 5 mm. diameter.

The fracture of the stems is fibrous in the bark, bark of small diameter segments being more fibrous than that of larger segments, and the fracture of the wood is splintery.

Sensory Characters

Dried roots and stems are almost odourless, the cork and the wood of root or stem is almost tasteless but the cortical tissue and phloem of each is intensely bitter. Powdered samples and exposed fractured surfaces of

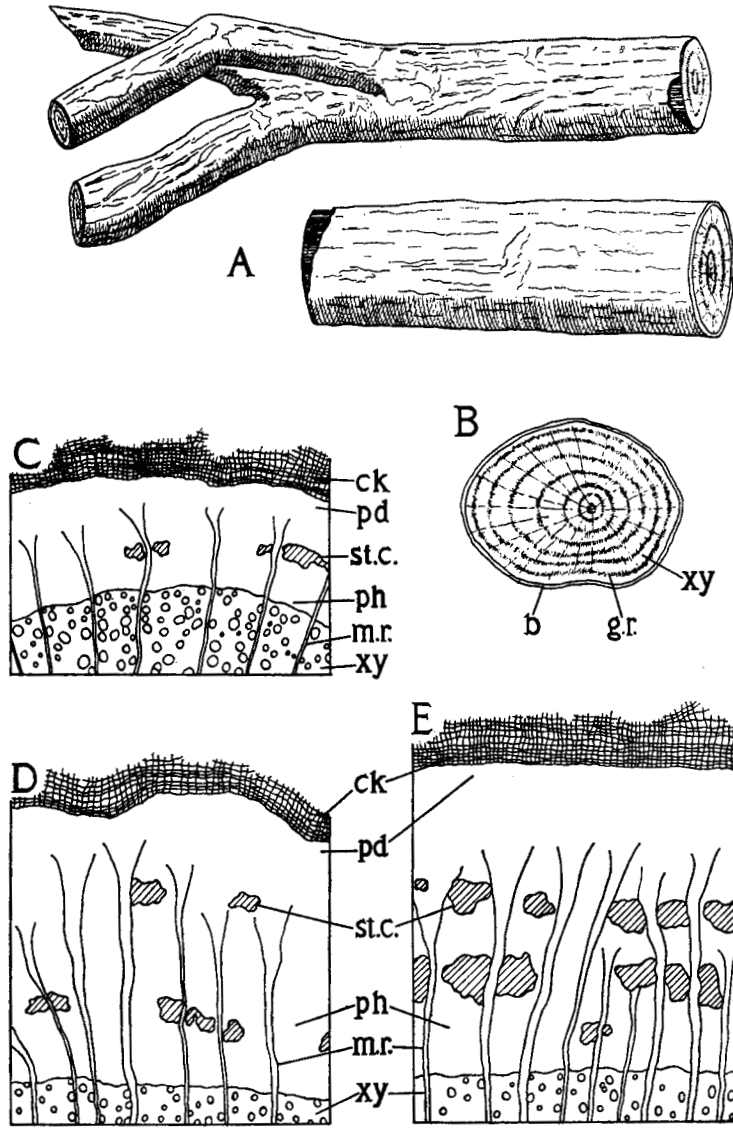

FIG. 1. *Rauwolfia mombasiana* Stapf. Root. A, external appearance, × 1; B, smoothed transverse surface of root, × 3; C, transverse section, root diameter 10 mm., × 15; D, transverse section, root diameter 28 mm., × 15; E, transverse section, root diameter 50 mm., × 15. b, bark; ck, cork; g.r., growth ring; m.r., medullary ray; pd, phelloderm; ph, phloem; st.c., sclereid group; xy, xylem.

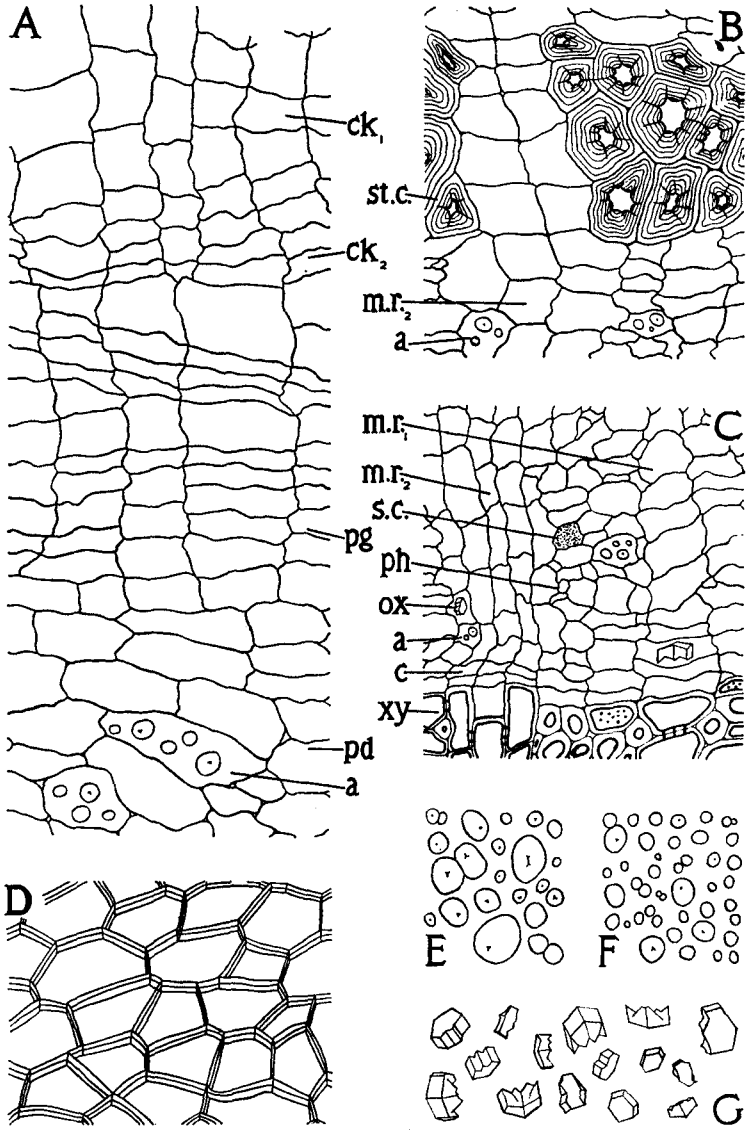

FIG. 2. *Rauwolfia mombasiana* Stapf. Root. A, transverse section of the outer tissues, root diameter 9 mm.; B, transverse section of the middle phloem, root diameter 10 mm.; C, transverse section of the inner phloem, root diameter 50 mm.; D, cork cells in surface view; E, starch grains from the wood; F, starch grains from the bark; G, calcium oxalate crystals from the bark. All × 200. a, starch; c, cambium; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; $m.r._1$, uniseriate medullary ray of upright cells; $m.r._2$, multiseriate medullary ray of procumbent cells; ox, calcium oxalate crystal; pd, phelloderm; pg, phellogen; ph, phloem elements; s.c., secretion cell; st.c., sclereid; xy, xylem.

root or stem exhibit a bluish-green fluorescence in screened ultra-violet light; aqueous extracts fluoresce similarly.

MICROSCOPY

In the following description the symbols R, T and L refer to measurements made in the radial, tangential and longitudinal directions respectively of material mounted usually in Berlese mountant.

Root

The radially arranged cork cells occur as alternating zones of flattened, unlignified, suberised cells, 3 to 8 cells in radial depth, and larger, lignified, suberised cells from 1 to 14 cells in radial depth. For the smaller cork cells, R = 8 to **16** to **24** to 35 μ, T = 20 to **39** to **55** to 94 μ and L = 23 to **35** to **55** to 86 μ; and for the larger cells, R = 19 to **51** to **74** to 116 μ, T = 31 to **43** to **63** to 79 μ and L = 27 to **39** to **55** to 75 μ (Fig. 2,A). In surface view, the cork cells appear polygonal (Fig. 2,D).

The phellogen, a layer of thin-walled, radially flattened cells, is followed by the phelloderm which consists of 5 to 15 layers of cells. The phelloderm cells adjacent to the phellogen are arranged in regular radial rows whilst the innermost cells are oval in shape with intercellular spaces. The cell walls are cellulosic and sclereids are absent. For the phelloderm cells R = 16 to **24** to **35** to 47 μ, T = 35 to **51** to **74** to 141 μ and L = 23 to **39** to **59** to 86 μ. Starch and scattered twinned prisms of calcium oxalate occur in the phelloderm. The starch consists chiefly of single rounded grains 2 to **4** to **10** to 38 μ diameter. 2 to 4 compound grains also occur and may split into individual plano-convex or angular grains. The hilum usually appears as a central point or star-shaped cleft and many grains show a Maltese cross effect when examined in polarised light (Fig. 2,F).

The phloem is a relatively wide zone internal to the phelloderm and characterised by up to 3 interrupted bands of sclereids dependent on the diameter of the specimen (Fig. 1,C,D,E). The phloem contains secretion cells and is traversed by conspicuous rays (Fig. 2,B,C). The heterogeneous rays consist of groups of small procumbent cells often with wavy walls, 2 to 5 cells wide tangentially and up to 26 cells high with uniseriate upper and lower extensions consisting of 1 to 5 larger cells (Fig. 3,C). For the smaller cells R = 19 to **27** to **39** to 63 μ, T = 15 to **19** to **30** to 78 μ and L = 15 to **19** to **26** to 51 μ, and for the larger cells R = 12 to **20** to **27** to 40 μ, T = 31 to **55** to **75** to 110 μ and L = 23 to **39** to **63** to 99 μ.

The irregular sclereid groups in the outer phloem are up to about 10 cells in radial thickness, 20 cells tangentially and 40 cells in depth. Individual sclereids vary greatly from isodiametric to irregularly elongated fibre-like structures (Figs. 3,C; 5,D) and measure R = 12 to **27** to **51** to 118 μ, T = 16 to **31** to **55** to 130 μ and L = 37 to **68** to **97** to 251 μ. Sclereids isolated by maceration using chromic-nitric acid reagent measured 30 to **52** to **158** to 326 μ in length and 19 to **30** to **56** to 97 μ in breadth. The sclereids are lignified and possess stratified walls with funnel-shaped pits (Fig. 2,B). In the largest diameter roots the sclereids

form an almost continuous layer broken only by the passage of medullary rays.

In radial and tangential longitudinal sections of the secondary phloem, long rows of calcium oxalate crystals are evident in the phloem parenchyma cells, 2-4 crystals occurring in each cell (Fig. 3,C). These crystals consist of monoclinic prisms, usually twinned on one of the hemipyramid faces and exhibit, in polarised light, a bicolouration effect. Length of prisms = 15 to **18** to **26** to 34 μ; breadth = 6 to **7** to **11** to 15 μ (Fig. 3,B,C).

Starch grains are distributed uniformly, although not abundantly, in the outer phloem and are usually less frequent in the inner functional phloem; they resemble those of the phelloderm.

Secretion cells are not numerous and are found occasionally in the phelloderm and, more frequently, in the inner phloem region. The amorphous contents of these cells stain with iodine solution, Sudan III and Tincture of Alkanna.

The primary xylem is indicated by four to six small groups of vessels near the centre of the root. The completely lignified secondary xylem consists of vessels, fibres and wood parenchyma and is traversed by medullary rays. In transverse sections the rounded or rather oval vessels occur solitary or in pairs. R = 27 to **50** to **98** to 165 μ and T = 24 to **49** to **90** to 131 μ. Numerous alternately arranged, bordered pits occur in the relatively thin, lignified vessel walls. Vessel segments isolated by chromic-nitric acid maceration show transverse and oblique perforation plates and peg-like prolongations (Fig. 5,G). For the isolated segments, length = 145 to **435** to **667** to 913 μ. A few nonfunctioning vessels may be occluded by brown amorphous material.

In transverse section the apotracheal wood parenchyma appears in short uniseriate rows connecting the vessels and medullary rays (Fig. 4,A). The cells appear, in longitudinal section, in vertical rows of up to 14 cells and the walls bear simple or half-bordered pits dependent on the nature of the adjacent cell structure (Fig. 4,B,C). R = 16 to **23** to **31** to 47 μ, T = 15 to **19** to **27** to 43 μ and L = 39 to **59** to **90** to 137 μ.

The heterogeneous medullary rays resemble those in the bark but are completely lignified and consist of a core of procumbent cells 2 to 5 cells in tangential width and up to 20 cells high with upper and lower uniseriate extensions of 1 to 6 larger upright cells. For the smaller cells R = 31 to **47** to **86** to 133 μ, T = 11 to **15** to **19** to 31 μ and L = 8 to **15** to **23** to 43 μ; and for the larger cells R = 15 to **24** to **43** to 67 μ, T = 20 to **27** to **39** to 55 μ and L = 31 to **47** to **67** to 106 μ. The procumbent cells are, when viewed in longitudinal section, often nearly circular in outline with small intercellular spaces and heavily pitted walls (Fig. 4,B,C), and in transverse sections the uniseriate rays predominate (Fig. 4,A).

The numerous xylem fibres appear in transverse section as rounded or polygonal structures with thick lignified walls. The length of the fibres is 903 to **1,129** to **1,677** to 2,096 μ and the breadth is 16 to **20** to **31** to 47 μ. Most of the fibres are spindle-shaped with tapering apices and bases and the walls bear spirally arranged slit-like pits (Fig. 5,H).

Starch grains, 3 to **6** to **14** to 46 μ in diameter and similar to those in the

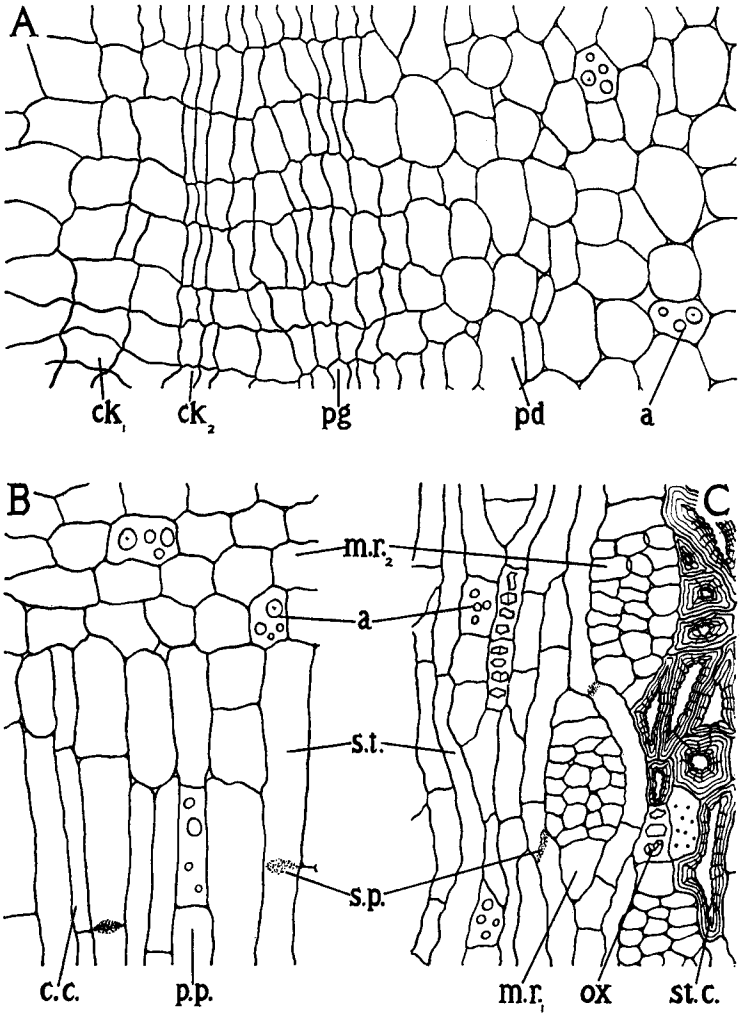

FIG. 3. *Rauwolfia mombasiana* Stapf. Root. A, radial longitudinal section of outer tissues, root diameter 23 mm., × 200; B, radial longitudinal section of inner phloem, root diameter 23 mm., × 200; C, tangential longitudinal section of inner phloem, root diameter 35 mm., × 100. a, starch; c.c., companion cell; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; $m.r._1$, uniseriate medullary ray of upright cells; $m.r._2$, multiseriate medullary ray of procumbent cells; ox, calcium oxalate crystal; pd, phelloderm; pg, phellogen; p.p., phloem parenchyma; s.p., sieve plate; s.t., sieve tube; st.c., sclereid.

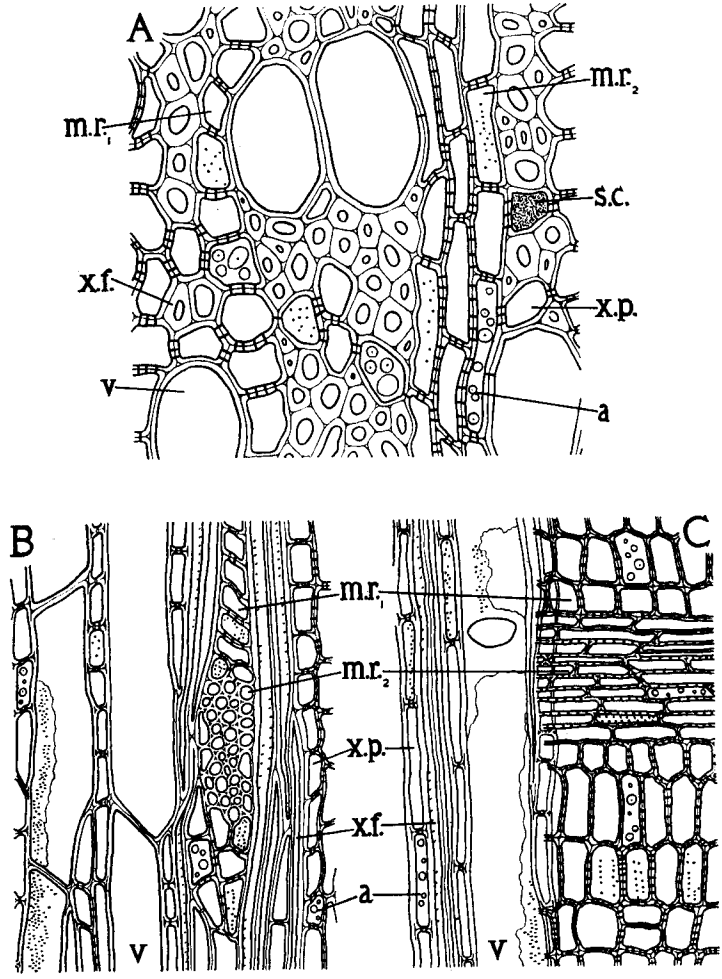

FIG. 4. *Rauwolfia mombasiana* Stapf. Root. Secondary Wood. A, transverse section, root diameter 10 mm., × 200; B, tangential longitudinal section, root diameter 10 mm., × 100; C, radial longitudinal section, root diameter 23 mm., × 100. a, starch; m.r.$_1$, upright medullary ray cells; m.r.$_2$, procumbent medullary ray cells; s.c., secretion cell; v, vessel; x.f., xylem fibre; x.p., xylem parenchyma.

bark, occur freely in the wood parenchyma and medullary ray cells (Fig. 4,A,B,C). Occasional secretion cells containing material staining with iodine and Sudan III and a few calcium oxalate prisms are usually found in the wood.

Stem

The general arrangement of the tissues and the cell dimensions resemble those of the root. The soft outer cork layer is not as extensive as that of the root and the stratification is less obvious. Internal to the phelloderm and cortex, a narrow layer of about 12 rows of cells which are thicker-walled than the corresponding cells in the root, is a zone of highly refractive, unlignified fibres. In specimens of small diameter the fibres form an almost continuous layer of up to 10 fibres in radial thickness and appear uniformly circular in shape, measuring 11 to **26** to **45** to 83 μ diameter (Fig. 6,C). The fibres are more widely scattered in the older and larger specimens and, after isolation by alkaline maceration, many fibres show pronounced swellings 26 to **45** to **64** to 113 μ in diameter (Fig. 7,A,C); hence their appearance in transverse section is variable. The length of these fibres exceeds 12 mm.

The outermost phloem is characterised by one or two interrupted rows of sclereids resembling those in the root bark. The inner secondary phloem is traversed by rays which are usually 2 to 5 cells wide and up to 20 small cells high with uniseriate upper and lower extensions of 2 to 5 larger cells. Typical phloem fibres are absent.

Calcium oxalate prisms and starch grains of the stem bark are similar in dimensions and distribution to those in the root bark.

The stem wood resembles the root wood although the vessels are somewhat smaller. R = 26 to **38** to **75** to 94 μ and T = 26 to **45** to **60** to 75 μ.

The parenchymatous central pith shows a peripheral ring of small-celled groups of perimedullary phloem tissue separated by rays of large-celled parenchyma (Fig. 8,A,B). The central tissue of the pith comprises a large-celled cellulosic parenchyma, individual cells containing starch grains and typical calcium oxalate prisms similar to those in the bark. Isolated sclereids or small groups of about 6 sclereids, resembling those in the bark, occur occasionally (Fig. 8,C).

Laticiferous Tissue

The presence of laticiferous tubes is generally regarded as an important feature of the Apocynaceae and Delourme-Houdé (1944) reported the occurrence of such tubes in the roots of *R. mombasiana.* A careful search for these structures was therefore undertaken.

Most specimens of root and stem showed secretion cells, parenchymatous cells containing granular material staining with iodine solution, Sudan III and Tincture of Alkanna. Such cells are distributed in the phloem and, to a lesser extent, in the phelloderm and wood.

Detailed examination of a wide range of tangential longitudinal sections revealed the presence of narrow, thin-walled, non-articulated laticiferous

tubes in some root specimens. These tubes, which measure 15 to 52 μ diameter and generally occur in the outer phloem, contain granular matter and refractive globules and can be stained rose-pink using aqueous iodine solution followed by aqueous eosin solution and subsequent mounting in 2 per cent aqueous acetic acid (Fig. 8,D).

Similar, but more prominent, laticiferous tubes 19 to **32** to **60** to 90 μ diameter were observed in the stem bark, usually in close association with the unlignified fibres, and also in the pith (Fig. 7,A,C; 8,A,B.C).

The Powdered Root

The principal features of the powdered root are:

1. Thin-walled yellow cork cells of two types—lignified cells and radially compressed unlignified cells, the former being more frequent in occurrence.

2. Thin-walled cellulosic elements of the phelloderm and phloem containing starch grains, occasional calcium oxalate crystals and sometimes yellowish granular material.

3. Rounded, ovoid or plano-convex starch grains 2 to **4** to **14** to 46 μ diameter; occasional 2 to 4 compound grains.

4. Single or twinned monoclinic prisms and irregular crystalline masses of calcium oxalate.

5. Fragments of narrow, thin-walled laticiferous tubes containing granular matter or refractive globules.

6. Isodiametric, elongated or irregularly shaped lignified sclereids, either singly or in small groups.

7. Abundant fragments of lignified xylem elements derived from thin-walled vessels with alternately arranged bordered pits, xylem fibres and elongated xylem parenchyma and medullary ray cells usually containing starch grains.

8. Amorphous matter staining with iodine solution being the contents of ruptured laticiferous tissue.

Discussion

The histological structure of *R. mombasiana* exhibits the characteristic features of the family Apocynaceae, typical elements being the unlignified fibres in the pericyclic region of the stem and laticiferous canals and vessel segments with large communication pores and peg-like prolongations in the root and stem. Characteristic of the genus *Rauwolfia* is the occurrence of phloem sclereids, non-articulated laticiferous tubes, non-septate fibres and heterogeneous rays. The presence of unlignified fibres in the pericylic region and a well-defined central pith clearly differentiates the stem from the root.

The relatively small vessel diameters, the pronounced radial development of phloem and xylem and the intermediate sclereid development can be related with the shrub-like habit of the species (Woodson, 1957).

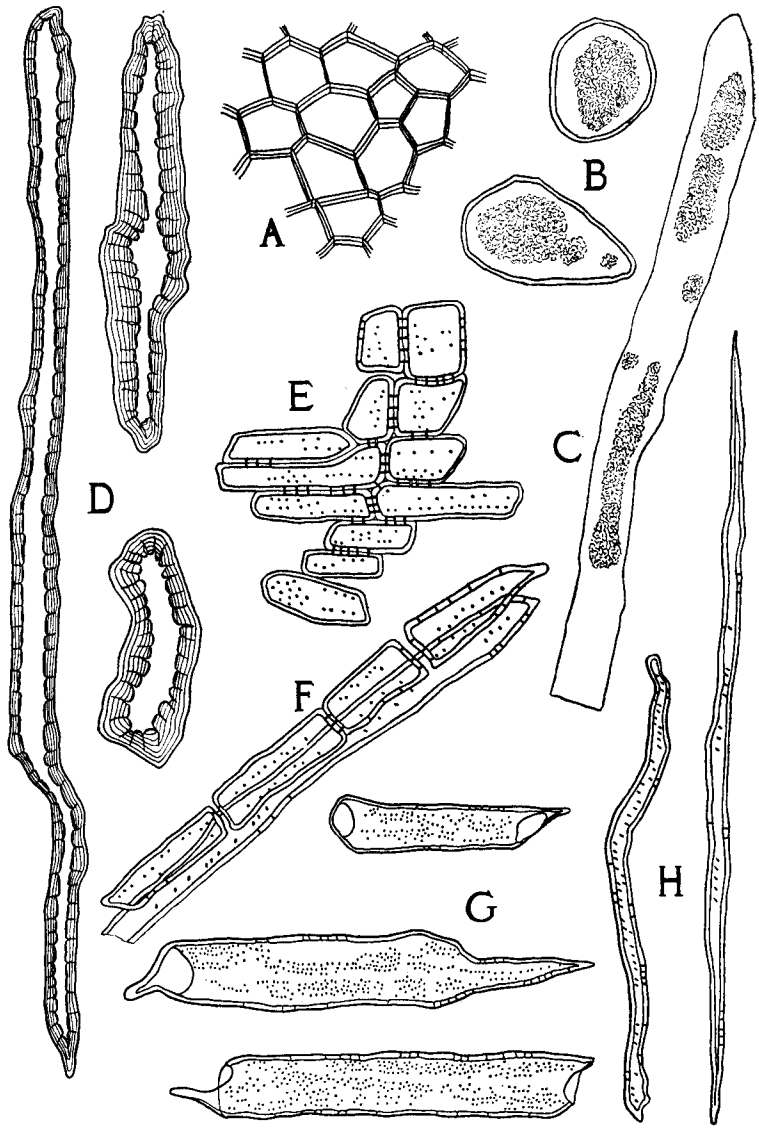

FIG. 5. *Rauwolfia mombasiana* Stapf. Isolated elements of the root. A, cork cells; B, secretion cells; C, laticiferous tube; D, sclereids; E, xylem medullary ray cells; F, xylem parenchyma cells; G, vessel segments; H, xylem fibres. A–F, × 200; G, H, × 100.

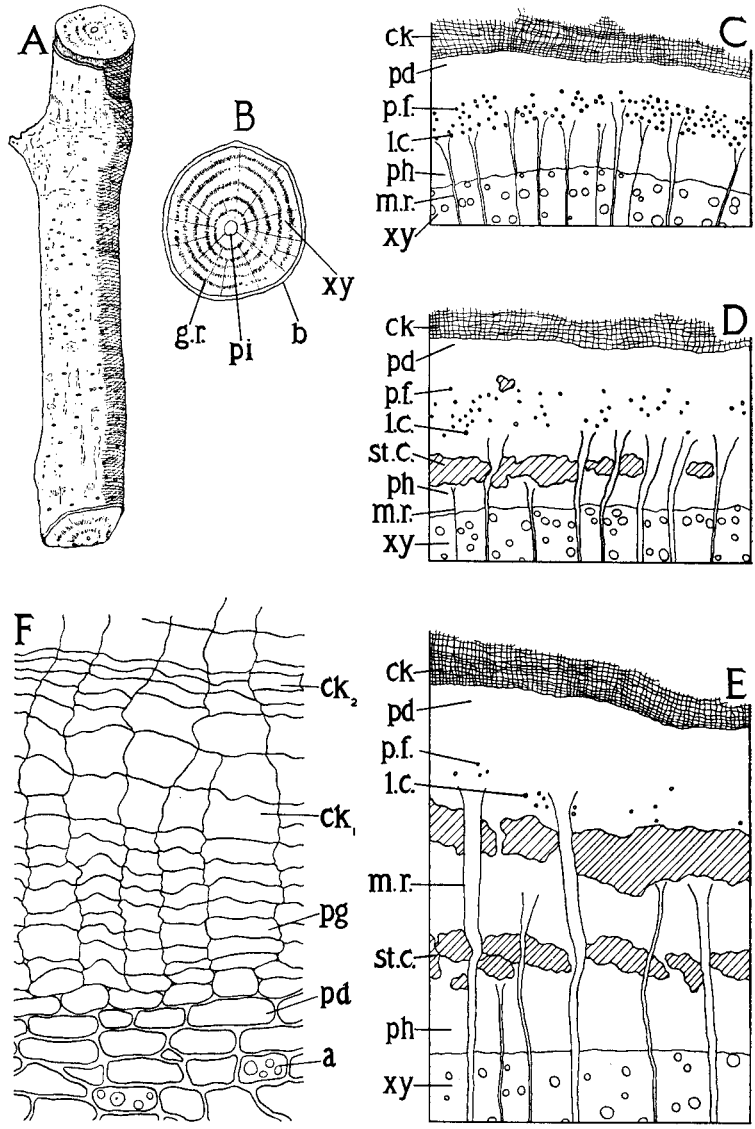

FIG. 6. *Rauwolfia mombasiana* Stapf. Stem. A, external appearance, × ⅓; B, smoothed transverse surface of stem, × ⅔; C, transverse section, stem diameter 9 mm., × 25; D, transverse section, stem diameter 18 mm., × 25; E, transverse section, stem diameter 36 mm., × 25; F, transverse section of outer tissues, stem diameter 9 mm., × 200. a, starch; b, bark; ck, cork; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; g.r., growth ring; l.c., laticiferous canal; m.r., medullary ray; pd, phelloderm; p.f., unlignified fibre; ph, phloem elements; pi, pith; st.c., sclereid group; xy, xylem.

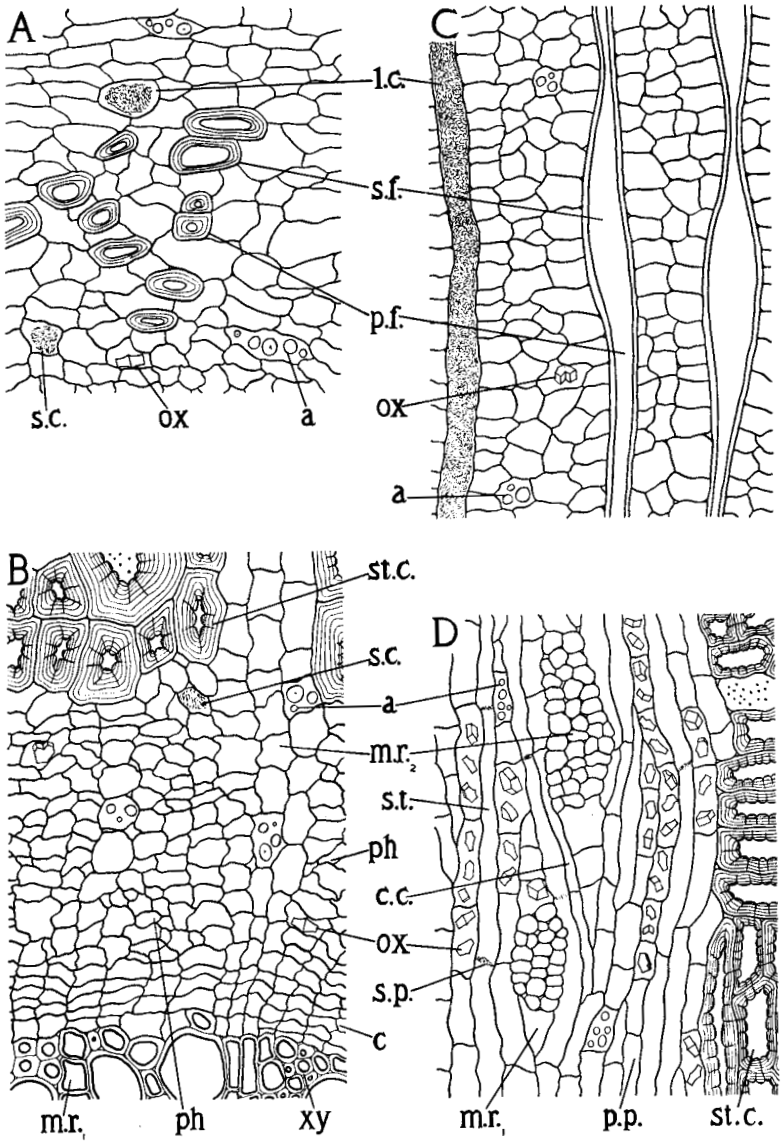

FIG. 7. *Rauwolfia mombasiana* Stapf. Stem. A, transverse section of pericyclic region, stem diameter 19 mm., × 200; B, transverse section of inner phloem, stem diameter 18 mm., × 200; C, longitudinal section of pericyclic region, stem diameter 19 mm., × 100; D, tangential longitudinal section of phloem, stem diameter 19 mm., × 100. a, starch; c, cambium; c.c., companion cell; l.c., laticiferous canal; $m.r._1$, upright medullary ray cells; $m.r._2$, procumbent medullary ray cells; ox, calcium oxalate crystal; p.f., unlignified fibre; ph, phloem elements; p.p., phloem parenchyma; s.c., secretion cell; s.f., swollen fibre; s.p., sieve plate; s.t., sieve tube; st.c., sclereid; xy, xylem.

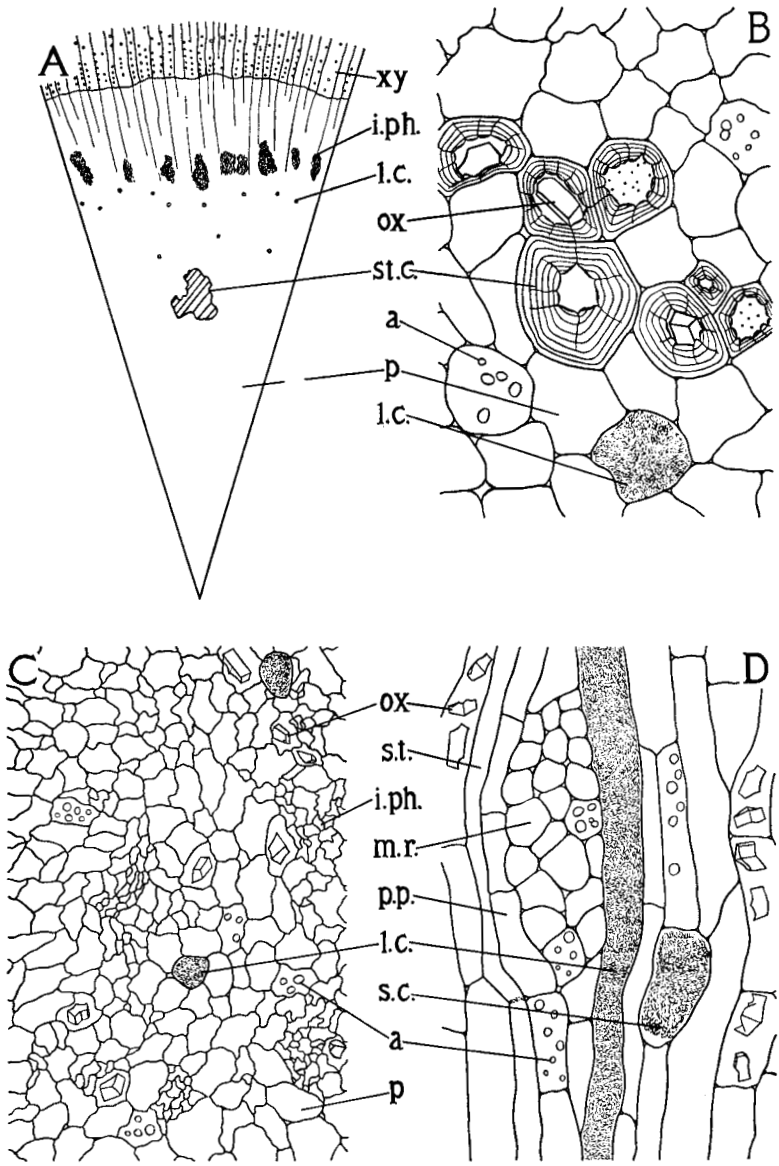

FIG. 8. *Rauwolfia mombasiana* Stapf. Stem and Root. A, transverse section of pith, stem diameter 42 mm., × 25; B, transverse section of central pith, stem diameter 42 mm., × 200; C, transverse section of outer pith, stem diameter 42 mm., × 200; D, tangential longitudinal section of root showing laticiferous tissue, root diameter 40 mm., × 200. a, starch; i.ph., perimedullary phloem; l.c., laticiferous canal; m.r., medullary ray; ox, calcium oxalate crystal; p, large-celled parenchyma; p.p., phloem parenchyma; s.c., secretion cell; s.t., sieve tube; st.c., sclereid group; xy, xylem.

R. mombasiana roots cannot readily be distinguished from other African *Rauwolfia* species by macroscopical examination. External colour is not a reliable criterion, colour variations often being dependent on the type of soil in which the plant has grown. Such variations have already been observed in samples of *R. tetraphylla* L. (Woodson, 1957) and *R. caffra* Sond. (Court, 1958).

Transverse sections of *R. mombasiana* can easily be distinguished from those of *R. caffra* (Court, Evans and Trease, 1957) and *R. macrophylla* Stapf (Paris, Dillemann and Chaumelle, 1957) as these latter two African species exhibit prominent sclereid groups in the phelloderm, extensive sclereid development in the phloem and larger vessel diameters, features associated with their arboreal form.

Sections of the roots of the shrubby species *R. obscura* K. Schum. (Paris and Dillemann, 1956) and *R. volkensii* Stapf (Court, 1961) reveal small diameter vessels and seldom exhibit sclereid groups, facts which differentiate them from the foregoing African species but not from each other.

R. vomitoria root is more difficult to distinguish from *R. mombasiana* root but, by comparison of sections from specimens of a similar diameter, the more extensive sclereid development and greater vessel sizes in *R. vomitoria* (Evans, 1956) become apparent.

Although *R. mombasiana* roots in the entire condition can be differentiated from the roots of the 5 African species about which data is available, their detection in the comminuted form as a substitute or adulterant for *R. vomitoria* roots presents a complex problem requiring further investigation.

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