AFRICAN *RAUWOLFIA* SPECIES

PART I. THE STRUCTURE OF THE ROOT AND STEM OF *RAUWOLFIA VOLKENSII* STAPF

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A possible substitute or adulterant for the roots of *R. vomitoria* Afz. is the shrubby East African species *R. volkensii* Stapf. The anatomy of the roots and stems is described and illustrated, and compared with published data about other African species.

R. volkensii was first described by Schumann (1895) as *Tabernaemontana volkensii* and recorded as such in Supplement I, Kew Index (1886–95). Later Stapf described the shrub as *R. volkensii* (Thiselton-Dyer, 1904), the Kew Index being amended in Supplement III (1901–1905). Markgraf (1923) described *R. oreogiton* but this species is regarded as doubtfully distinct from *R. volkensii* (Willan, 1960). Pichon (1947), in his classification of the genus *Rauwolfia*, grouped the species together in the section Ophioxylanthus but was unable to examine specimens of *R. oreogiton* as no authentic specimens are available (Greenway, 1955; Willan, 1960).

R. volkensii is a shrub with slender branches, growing to a height of 6 ft., which occurs in Tanganyika in the West Usambara Mountains, the Pare Mountains, the Ulugura Mountains and on Kilimanjaro at elevations in excess of 4,000 ft., and in Mozambique. Although occasionally encountered in dry forest areas, *R. volkensii* normally occurs in typical moist montane forest dominated by *Ocotea usambarensis* and *Podocarpus spp.* (Thiselton-Dyer, 1904; Willan, 1960).

Plant Material

The following material was used in this investigation:—

1. *R. volkensii* roots and stems collected by the Forest Department, Lushoto, Tanganyika and vouched for by the East African Herbarium, Nairobi, from botanical specimens sent under collector's number "Semsei 2827" (March 10, 1959).

2. Further supply from Lushoto under collector's number "Semsei 2948" (January 20, 1960).

MACROSCOPY

Root

The roots occur as somewhat contorted cylindrical or conical, occasionally branched pieces of varying lengths up to about 70 cm. and diameters up to 4 cm. Most samples possess a few side rootlets varying from about 1 to 6 mm. diameter (Fig. 1, A). Externally the soft, buff or yellow brown cork is longitudinally striated. Occasionally, especially on the outer aspect of bends, the cork has rubbed away to reveal the inner

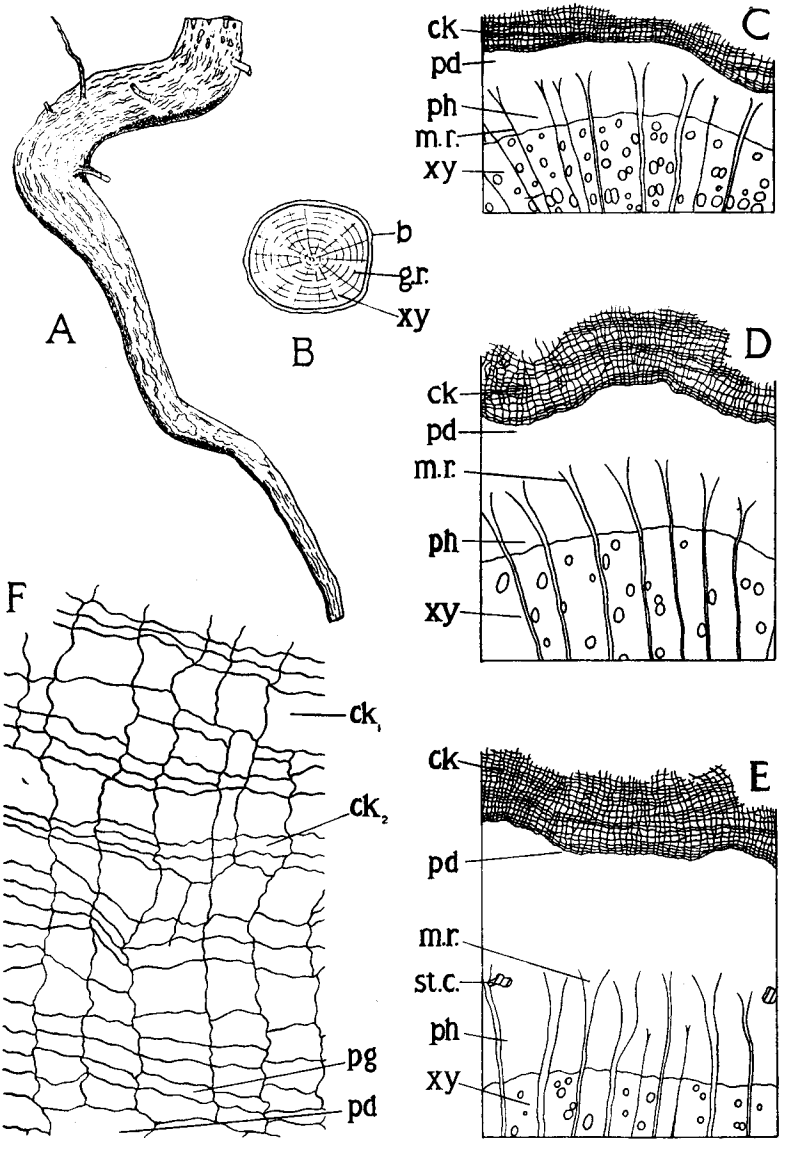

FIG. 1. *Rauwolfia volkensii* Stapf. Root. A, external appearance, × 1/3; B, smoothed transverse surface of root, × 2/3; C, transverse section, root diameter 6 mm., × 25; D, transverse section, root diameter 18 mm., × 25; E, transverse section, root diameter 45 mm., × 25; F, outer tissues, root diameter 17 mm., × 200; b, bark; ck, cork; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; g.r., growth ring; m.r., medullary ray; pd, phelloderm; pg, phellogen; ph, phloem; st.c., sclereid group; xy, xylem.

yellowish phloem tissue or even the yellowish-brown wood. Rootlet scars occur at intervals, but no glistening points could be detected on the outer surface. The cork of the rootlets flakes off readily and longitudinal striations are apparent only on the larger rootlets.

Smoothed tranverse surfaces of the roots show a very narrow bark seldom exceeding 1·5 mm. in diameter. The buff or yellowish finely radiate porous wood possesses a few distinct growth rings, specimens showing up to 16 rings having been examined (Fig. 1, B).

Stem

The stems occur as slender, cylindrical branched pieces up to 1 m. in length and 4 cm. diameter. Externally the yellow-brown or greyish-brown cork is longitudinally striated and bears greyish patches and pale-brown tangentially elongated lenticels. Smoothed transverse surfaces of the stems show a narrow bark 0·25 to 1·0 mm. thick, a cylinder of secondary xylem with up to 12 growth rings and a small central or somewhat eccentric pith or a cavity due to contraction of the pith.

Sensory Characters

Dried stems and roots are odourless. The bark of root or stem is intensely bitter, whereas the wood of each is almost tasteless; the fracture of the bark is short whilst that of the wood is splintery. Exposed fractured surfaces and powdered samples of 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 cork is stratified and consists of alternating layers of larger and smaller cells. The smaller suberised, but unlignified, cells measure about 7 to **11** to **18** to 22 μ radially and the larger suberised and lignified cells measure about 22 to **30** to **44** to 104 μ radially; the other measurements of both kinds of cells are T = 22 to **30** to **48** to 67 μ and L = 22 to **30** to **44** to 56 μ (Fig. 1, F, ck_1, ck_2). In surface view, the cork cells are polygonal (Fig. 2, B).

The cork is followed by a layer of thin-walled phellogen cells. The phelloderm consists of up to 12 rows of cells, those near to the phellogen being arranged in regular radial rows whilst the innermost cells are more oval in shape with intercellular spaces between them (Fig. 2, A). Sclereids are absent. For the phelloderm cells R = 11 to **18** to **26** to 37 μ, T = 30 to **44** to **59** to 96 μ and L = 26 to **37** to **48** to 81 μ. Starch and scattered twinned prisms of calcium oxalate occur in the phelloderm. Starch grains occur singly or occasionally 2 to 4 compound. The single grains are rounded and individual grains measure 1 to 3 to 7 to 30 μ in diameter,

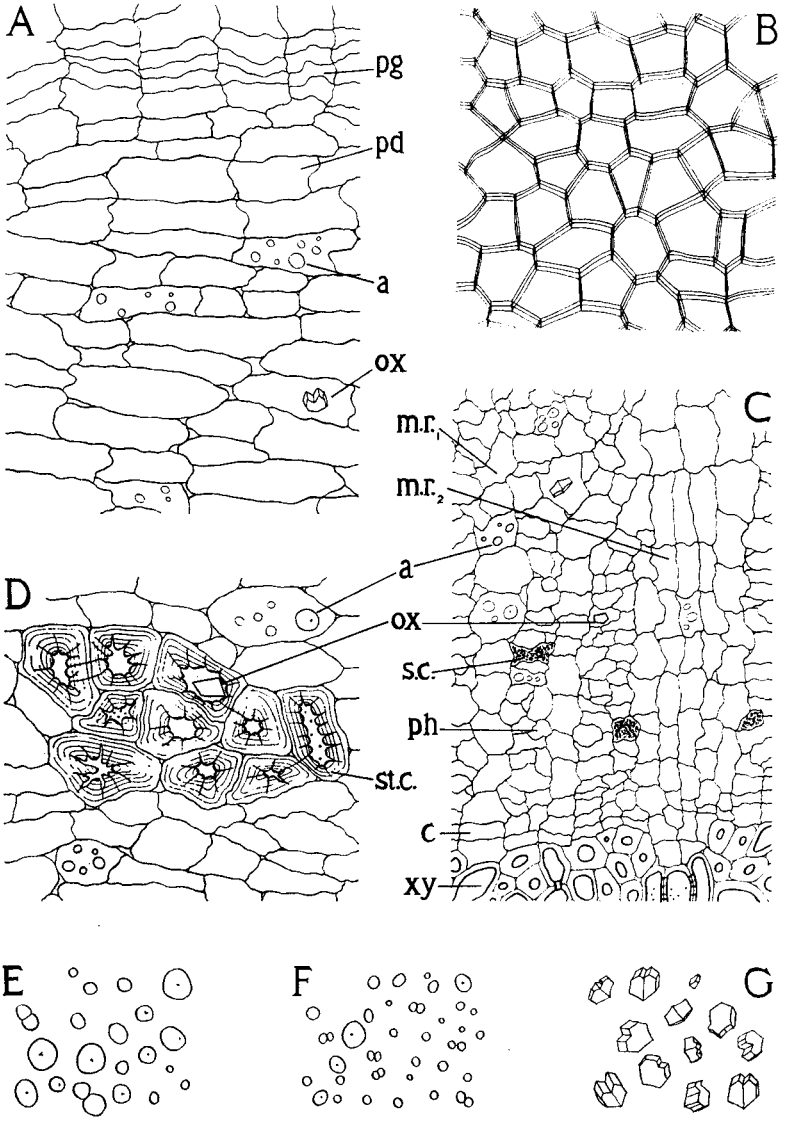

FIG. 2. *Rauwolfia volkensii* Stapf. Root. A, transverse section of the outer tissues; B, cork cells in surface view; C, transverse section of the inner phloem; D, transverse section of the outer phloem; all from 45 mm. diameter root; E, starch grains from the wood; F, starch grains from the bark; G, calcium oxalate crystals from the bark. All × 200. a, starch; c, cambium; $m.r_1$, 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.

the hilum normally appearing as a point or is replaced by a tri-radiate cleft (Fig. 2, F).

The phloem contains secretion cells and is traversed by conspicuous medullary rays. The heterogeneous medullary rays consist of groups of small procumbent cells often with wavy walls, 2 to 4 cells wide and up to 17 cells high, with upper and lower uniseriate extensions consisting of 1 to 4 larger cells (Fig. 3, C). For the smaller cells R = 15 to **19** to **30** to 52 μ, T = 18 to **26** to **37** to 48 μ and L = 18 to **22** to **30** to 48 μ and for the larger cells R = 11 to **19** to **30** to 37 μ, T = 26 to **37** to **59** to 93 μ and L = 30 to **44** to **59** to 100 μ.

Occasional small groups of up to 10 sclereids arranged in 1 or 2 rows were observed in transverse sections of the outer phloem of specimens exceeding 4 cm. diameter (Fig. 1, E). Such specimens represented the rootstock region of the shrub. Individual sclereids measured R = 18 to **30** to **52** to 92 μ, T = 30 to **37** to **52** to 96 μ and L = 30 to **44** to **74** to 255 μ. The maximum length of sclereids isolated by maceration using chromic-nitric acid reagent was 577 μ. All the sclereids are lignified and possess stratified walls with funnel shaped pits (Fig. 2, D).

Vertical rows of calcium oxalate crystals are frequently evident in radial and tangential longitudinal sections through the secondary phloem (Fig. 3, B, C). These crystals consist of monoclinic prisms usually twinned on one of the hemipyramid faces and frequently exhibiting in polarised light a bicolouration effect. Length of prisms = 15 to **22** to **26** to 33 μ; breadth = 7 to **11** to **15** to 19 μ. Starch grains similar to those in the phelloderm occur uniformly although not abundantly throughout the phloem parenchyma and medullary rays. Secretory cells occur in the phelloderm and more frequently in the inner phloem region; their amorphous contents stain with Sudan III, Tincture of Alkanna and iodine solution. No latex canals were observed in the root.

The completely lignified secondary xylem consists of vessels, fibres and wood parenchyma and is traversed by medullary rays (Fig 4, A, B, C) and the primary xylem is hexarch. In transverse sections, the rounded or somewhat oval vessels are frequently inconspicuous and occur singly or very occasionally in pairs. The relatively thin, lignified vessel walls bear numerous alternately arranged bordered pits. For the vessels in transverse section R = 26 to **44** to **56** to 96 μ and T = 26 to **37** to **48** to 78 μ. Vessel segments isolated by chromic-nitric acid maceration show transverse and oblique perforation plates and peg-like prolongations. For isolated segments, length = 95 to **392** to **730** to 1,031 μ (Fig. 5, G).

The abundant xylem fibres possess thick lignified walls with spirally arranged slit-like pits (Fig. 5, F). The length of the individual fibres is about 774 to **1,322** to **1,806** to 2,225 μ and the breadth 15 to **22** to **30** to 48 μ.

The apotracheal xylem parenchyma, as seen in transverse sections, is not abundant and occurs in short uniseriate rows connecting the vessels and medullary rays (Fig. 4, A). In longitudinal sections these cells appear in vertical files of up to 12 cells, the cell walls bearing simple or half bordered pits, dependent on the nature of the adjacent cell structure.

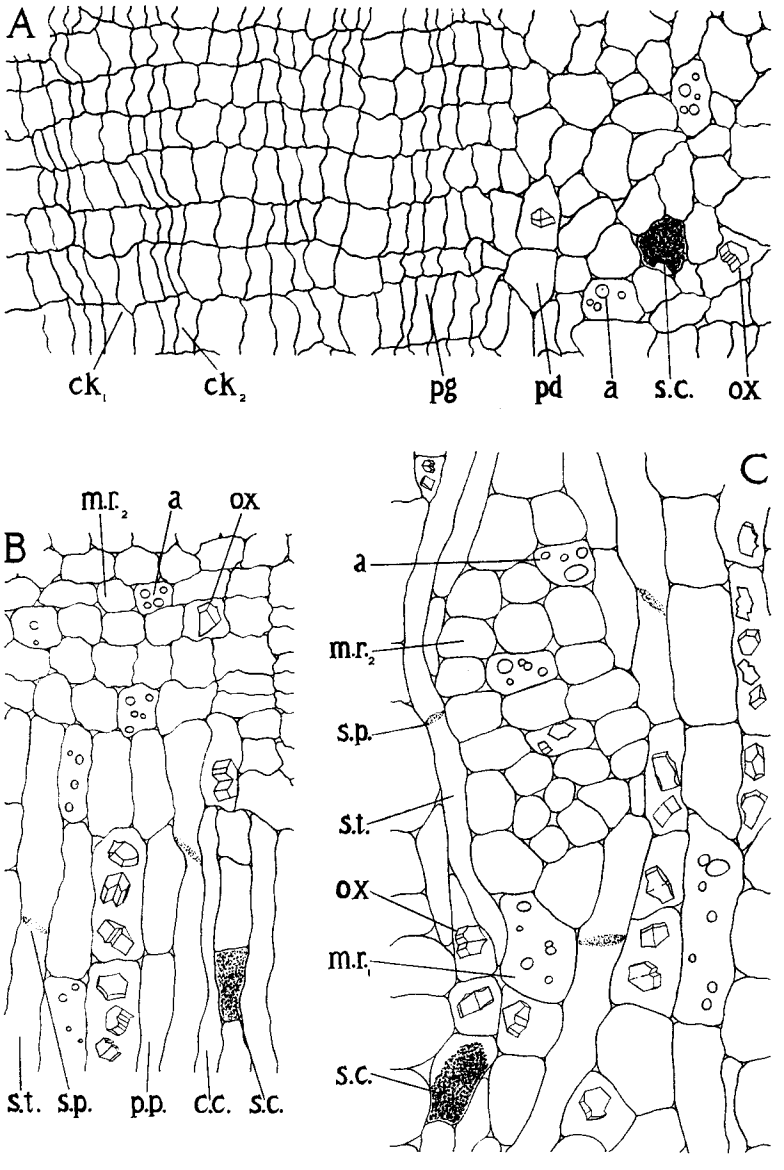

FIG. 3. *Rauwolfia volkensii* Stapf. Root. Longitudinal sections of the bark. A, radical section of outer tissues, root diameter 30 mm.; B, radial section of inner phloem, root diameter 30 mm.; C, tangential section of inner phloem, root diameter 17 mm. All × 200. a, starch; c.c., companion cell; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; ox, calcium oxalate crystal; $m.r_1$, upright medullary ray cell; $m.r_2$, procumbent medullary ray cell; pd, phelloderm; pg, phellogen; p.p., phloem parenchyma; s.c., secretion cell; s.p., sieve plate; s.t., sieve tube.

R = 11 to **22** to **30** to 41 μ; T = 15 to **22** to **30** to 37 μ; L = 37 to **67** to **85** to 133 μ.

The heterogeneous medullary rays resemble the rays in the phloem but are completely lignified. Typical medullary rays in tangential longitudinal section show a central core of procumbent cells usually 2 to 4 cells wide and up to 23 cells high with uniseriate upper and lower extensions of

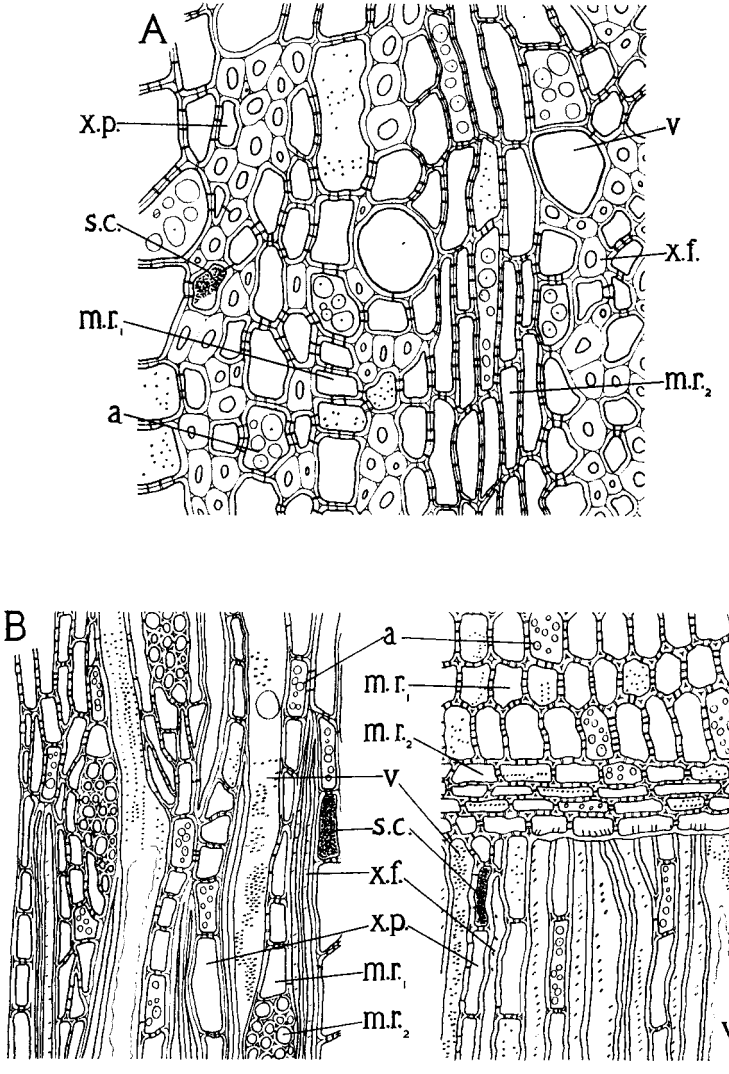

FIG. 4. *Rauwolfia volkensii* Stapf. Root. Secondary Wood. A, transverse section, root diameter 17 mm. × 200; B, tangential longitudinal section, root diameter 10 mm. × 100; C, radial longitudinal section, root diameter 30 mm. × 100. a, starch; $m.r_1$, upright medullary ray cells; $m.r_2$, procumbent medullary ray cells; s.c., secretion cell; v, vessel; x.f., xylem fibre; x.p., xylem parenchyma.

1 to 6 larger upright cells. In transverse sections the uniseriate rays are predominant, the cells often appearing larger than adjacent vessels. For the procumbent cells R = 41 to **67** to **82** to 126 μ, T = 11 to **15** to **19** to 37 μ and L = 11 to **15** to **26** to 48 μ; for the upright cells R = 18 to **30** to **48** to 74 μ, T = 22 to **30** to **48** to 67 μ and L = 30 to **44** to **59** to

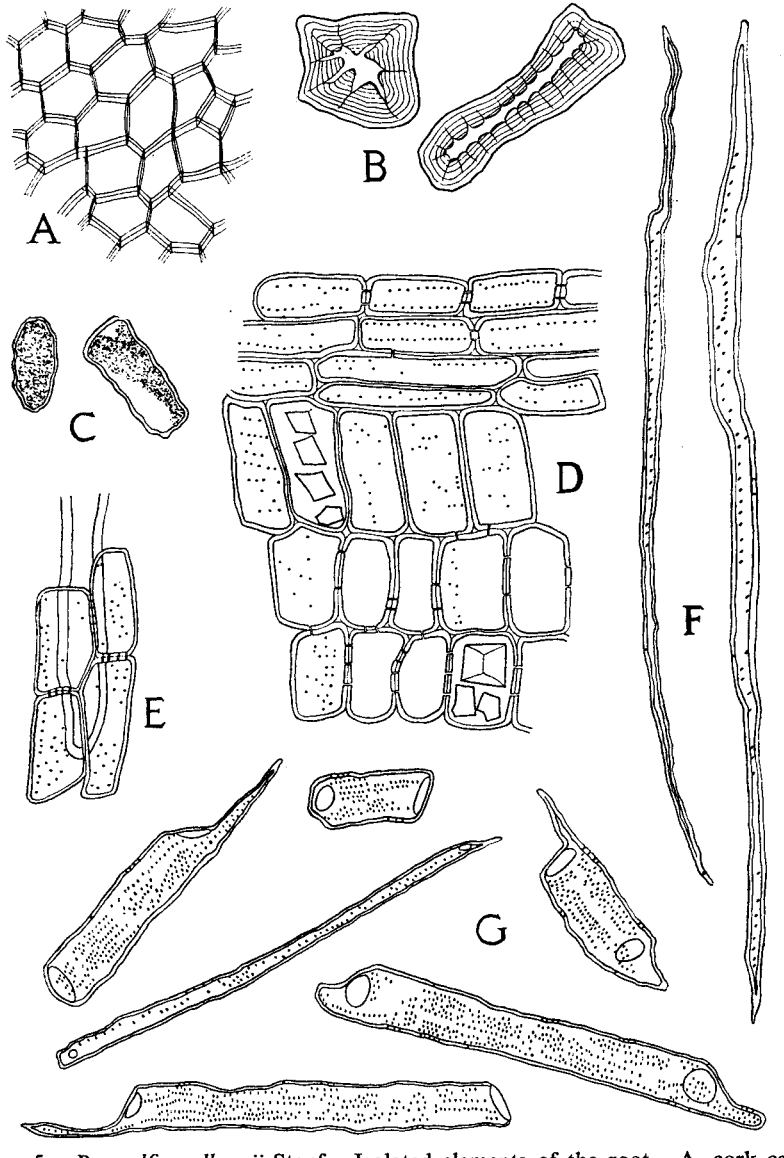

FIG. 5. *Rauwolfia volkensii* Stapf. Isolated elements of the root. A, cork cells, × 200; B, sclereids, × 200; C, secretion cells, × 200; D, lignified medullary ray cells, × 200; E, xylem parenchyma × 200; F, xylem fibres × 100; G, vessel segments, × 100.

96 μ. In tangential longitudinal sections intercellular spaces are apparent especially between the rounded procumbent cells (Fig. 4, B).

Starch grains, 3 to 7 to 11 to 30 μ in diameter, similar to those in the bark, occur in the parenchyma of the xylem and in the medullary rays. Occasional cells contain material staining with iodine and Sudan III and more infrequently calcium oxalate prisms are found in the ray cells.

Stem

In general, the tissue distribution and cell dimensions of the stem resemble those of the root. The soft cork layer, which is not extensive consists of up to about 30 rows of cells showing the alternation of large celled and small celled zones as observed in the root. Internal to the phelloderm and cortex, which is a narrow layer of up to 10 radial rows of

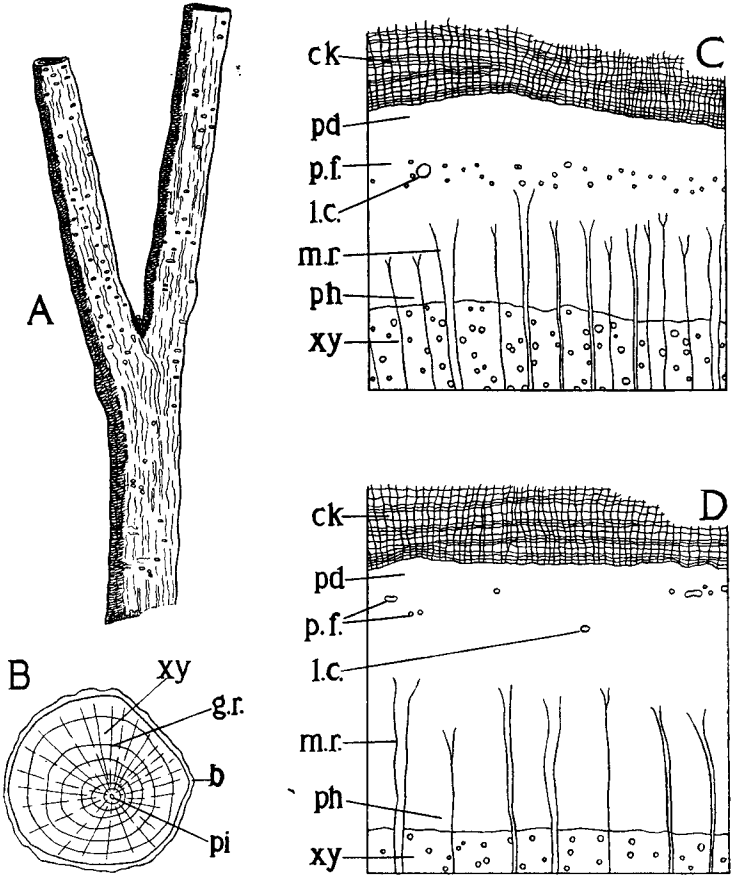

FIG. 6. *Rauwolfia volkensii* Stapf. Stem. A, external appearance, × 1/3; B, smoothed transverse surface of stem, × 1; C, transverse section, stem diameter 16 mm., × 25; D, transverse section, stem diameter 29 mm., × 25. b, bark; ck, cork; g.r., growth ring; l.c., latex canal; m.r., medullary ray; pd, phelloderm; p.f., unlignified fibre; ph, phloem; pi, pith; xy, xylem.

cells, is a zone of highly refractive unlignified fibres. In specimens of small diameter the fibres form an almost continuous layer and appear uniform in shape and measure 11 to **15** to **33** to 48 μ diameter (Fig. 7, B). In larger older specimens the fibres are more widely scattered and, after isolation by alkaline maceration, many show pronounced swellings, 30 to **44** to **96** to 163 μ in diameter (Fig. 8, D); hence their appearance in transverse section is variable (Fig. 7, C). The length of the fibres exceeds 13 mm.

Occasional latex canals containing granular matter which stains with iodine and Sudan III occur in the outer phloem usually associated with the fibres. In transverse section the latex canals appear rounded or oval, R = 26 to 53 μ; T = 37 to 113 μ. Fragments isolated by alkaline maceration measured 26 to **37** to **64** to 128 μ in width and exceeded 13 mm. in length. Secretion cells are scattered throughout the phloem tissue.

Sclereids rarely occur in the stem except in the region of the stem base, where groups of about 60 cells occur arranged in 1 or 2 rows in the secondary phloem.

The secondary phloem is traversed by the medullary rays and resembles the secondary phloem of the root. The medullary rays are usually 2 to 5 small cells in width and 11 to 23 small cells high with upper and lower uniseriate extensions 1 to 4 cells high (Fig. 7, E). Calcium oxalate prisms and starch grains resemble those present in the root bark.

The stem wood is similar to the root wood. Calcium oxalate prisms are occasionally present in the medullary ray and xylem parenchyma cells.

The large celled parenchymatous pith is characterised by peripheral groups of small-celled perimedullary phloem tissue, latex canals and occasional sclereids and non-lignified fibres (Fig. 8, A). A few starch grains and calcium oxalate prisms also occur in the pith.

The Powdered Root

The principle 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; many cells often containing starch grains and sometimes calcium oxalate crystals or yellowish granular material.

3. Rounded, ovoid or plano-convex starch grains 1 to **3** to **11** to 30 μ in diameter; occasional 2 to 4 compound grains.

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

5. Very occasional isodiametric, elongated or irregularly shaped lignified sclereids either singly or in small groups.

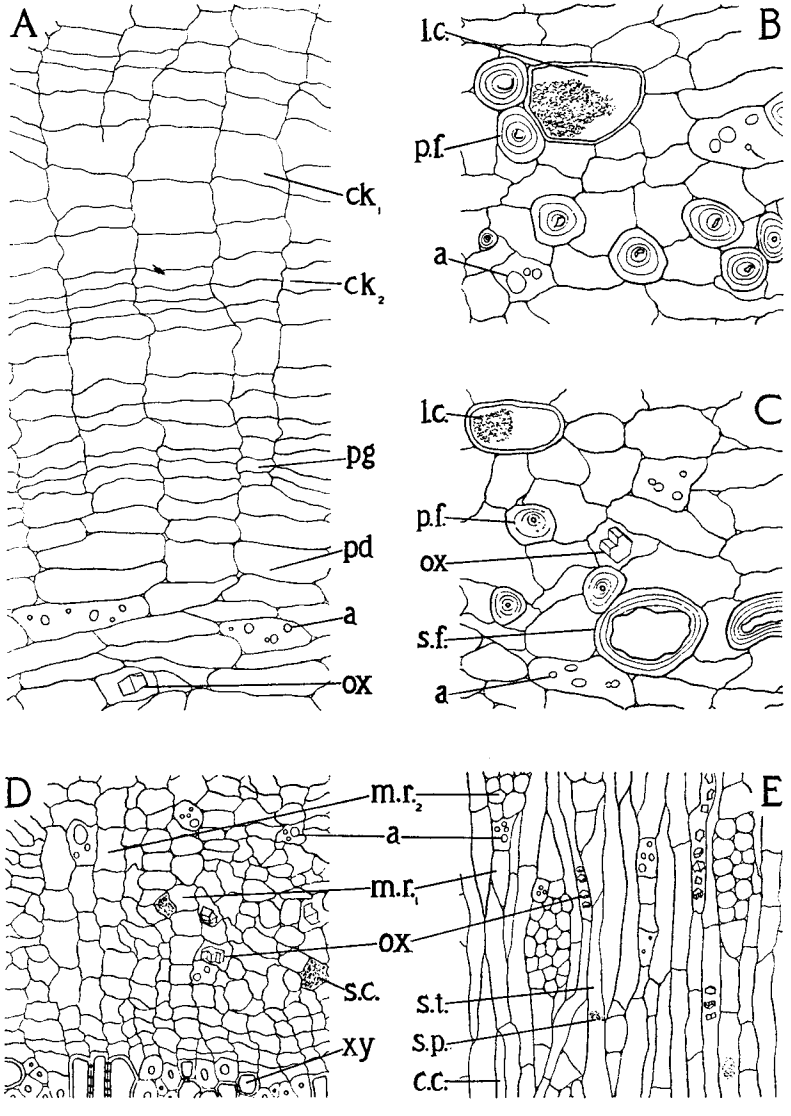

FIG. 7. *Rauwolfia volkensii* Stapf. Stem bark. A, transverse section of outer tissues, stem diameter 29 mm., × 200; B, transverse section in region of outer phloem, stem diameter 8 mm., × 200; C, transverse section in region of outer phloem, stem diameter 16 mm., × 200; D, transverse section of inner phloem, root diameter 16 mm., × 200; E, tangential longitudinal section of inner phloem, stem diameter 16 mm., × 100. a, starch; c.c., companion cell; ck_1, large lignified cork cells; ck_2, small unlignified cork cells; l.c., latex canal; $m.r_1$, uniseriate upright medullary ray cells; $m.r_2$, multiseriate procumbent medullary ray cells; ox, calcium oxalate crystal; pd, phelloderm; p.f., unlignified fibre; pg, phellogen; s.c., secretion cell; s.f., swollen fibre; s.p., sieve plate; s.t., sieve tube; xy, xylem.

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

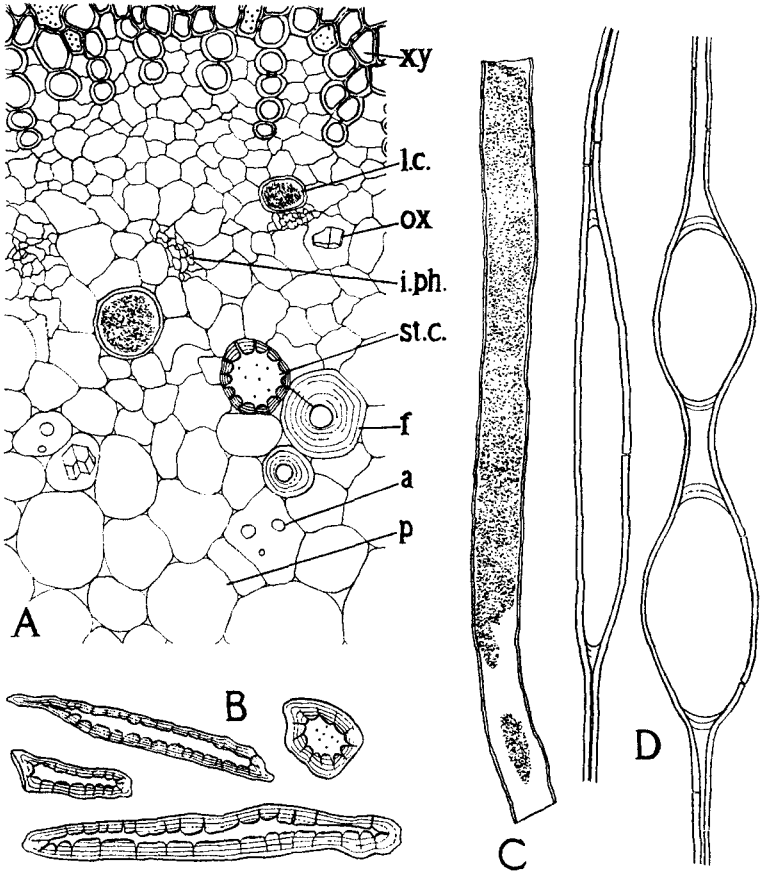

FIG. 8. *Rauwolfia volkensii* Stapf. Stem. A, transverse section of pith, stem diameter 16 mm., × 200; B, isolated sclereids of stem, × 100; C, isolated latex canal, × 100; D, isolated fibre segments, × 100. a, starch; f, fibre; i.ph., internal phloem; l.c., latex canal; ox, calcium oxalate crystal; p, large celled parenchyma; st.c., sclereid; xy, xylem.

DISCUSSION

The structure of the root and stem of *R. volkensii* exhibits the typical features of the family Apocynaceae and of the genus *Rauwolfia* (Metcalfe and Chalk, 1950). The stem structure is clearly differentiated from the root structure by the presence of unlignified fibres in the outer phloem (pericycle), latex vessels and a central pith. The absence of marked sclereid development and the small vessel diameters are related to the herbaceous habit of the plant.

The presence of well defined sclereid groups distinguishes the roots and stems of *R. vomitoria* (Evans, 1956), *R. caffra Sond.* (Court, Evans and Trease, 1957), *R. macrophylla* Stapf (Paris, Dillemann and Chaumelle, 1957) and *R. mombasiana* Stapf (Delourme-Houdé, 1944) from those of *R. volkensii*. The structure of *R. obscura* K. Schum, a species indigenous to the Congo, closely resembles the structure of *R. volkensii*; neither species shows marked sclereid development and the reported vessel diameters are identical (Paris and Dillemann, 1956). Until more information is available concerning *R. obscura* roots, differentiation will remain difficult.

Summarising, the adulteration or substitution of *R. vomitoria* roots with *R. volkensii* roots could be readily detected, but the precise identification of the adulterant or substitute by anatomical methods presents a complex problem, which it is hoped to investigate further when other East African species have been examined.

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