THE STRUCTURE OF THE ROOT AND STEM OF *RAUWOLFIA CAFFRA* SOND.

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DURING the past twenty years the root of *Rauwolfia serpentina* Benth. has been the subject of extensive chemical and pharmacological investigation[1,2]. The isolation of the alkaloid, reserpine and its use in psychiatric and hypertensive conditions has stimulated the study of other plants of the same genus. Of the African *Rauwolfia* species, reserpine has been isolated from *R. vomitoria* Afz[3]., *R. caffra* Sond[4]. and *R. cumminsii* Stapf.[5] and extracts of *R. mombasiana* Stapf. have been shown to have hypotensive properties[6]. *R. vomitoria* is used commercially and has now largely replaced *R. serpentina* as a source of reserpine; its histological structure has been investigated[7,8] and a number of non-African species have been similarly studied[9,10,11]. As *R. caffra* is a potential African commercial source of reserpine and is of similar geographical distribution to *R. vomitoria*, it was thought desirable to undertake a histological study of the root and stem; the results are recorded below.

R. caffra, one of the largest species of the genus, is a tree 15 to 25 m. high[12] with a trunk up to about 120 cm. in diameter. It is of widespread, but not abundant, occurrence in the secondary fringe forest of the high rainfall areas of Central, East and South Africa[13,14]. The plant has many vernacular names [13,15,16] and native tribes have employed it for a wide range of ailments varying from skin rashes to intestinal disorders[13,15].

A number of species of *Rauwolfia*, originally considered to be distinct[17], are now generally regarded as synonymous with *R. caffra*. These include *R. natalensis* Sond.[18], *R. ochrosioides* K. Schum.[12], *R. inebrians* K. Schum., *R. obliquinervis* Stapf., *R. goetzei* Stapf.[19], and *R. welwitschii* Stapf.[20] Pichon in his classification[21] of the genus, has grouped them under Section Afrovolfia.

In 1901 Juritz[22] demonstrated the presence of a bitter crystalline alkaloid in the bark of *R. natalensis*. Rindl and Groenewoud[23] (1932) could not confirm this finding but obtained amorphous alkaloids which they did not characterise and Koepfli[14] (1932) isolated a crystalline alkaloid rauwolfine, which had hypotensive activity. Recently Schüler and Warren[4] have isolated reserpine and ajmaline from the root bark and a small quantity of intractable alkaloidal material from the stem bark.

PLANT MATERIAL

The following material was used in this investigation:

1. *R. caffra* roots, stem wood and stem bark collected by D. B. Fanshawe, Esq., on the banks of the Ndola River, Northern Rhodesia.
2. *R. caffra* root bark from large roots, obtained by D. B. Fanshawe, Esq.

3. *R. caffra* roots supplied by John Ronaldson Ltd., London.
4. *R. caffra* roots supplied by B. O. G. Schüler, Esq., Natal University.

Macroscopy

The roots occur as cylindrical or flattened, occasionally branched pieces of varying lengths and up to about 10 cm. in diameter. The bark of larger roots separates from the wood and when dry, occurs as strips about 2 to 5 mm. thick. Externally, the irregularly furrowed, buff or light brown cork is frequently rubbed away revealing the buff cortical tissue which on careful examination shows glistening points of calcium oxalate. Often pieces of bark have broken away to reveal the reddish-brown longitudinally striated outer layer of the wood. Small roots may possess rootlet scars or stumps and rootlets with a marked longitudinal furrowing of the cork. The inner surface of the yellowish-brown root bark with its reddish-brown and greyish-brown patches, is marked by irregular longitudinal ridges. The surface exhibits glistening points of calcium oxalate.

Smoothed transverse surfaces of the roots show a narrow granular bark usually 0·5 to 3 mm. thick, but swelling considerably on soaking in water, and an inner buff, or yellowish finely-radiate, porous wood possessing a few distinct growth rings.

The dried root is almost odourless; the outer corky layer and the wood almost tasteless, and the outer cortex and phloem very bitter. The fracture of the bark is short and that of the wood splintery; small roots are brittle but larger roots are tough (Fig. 1: A).

The dark brown stem bark occurs as irregular pieces up to about 15 mm. in thickness. Externally it shows buff patches where cork has rubbed off, deep fissures being apparent between the patches. The smoothed transverse surface is characterised by a narrow buff cork layer external to a wide granular phloem (Fig. 6).

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. The ranges of measurements have been obtained from as wide a variety of specimens as possible, but the limits may not prove to be absolute.

Root

The appearance of the transverse section of the root shows considerable variation due, mainly, to the degree of development of stone cell layers within the secondary phloem. This development varies from a few isolated groups of stone cells in some small roots to about six interrupted concentric bands in some of the larger roots (Fig. 1: B-E).

The radially arranged cork cells are lignified and suberised showing no alternation of lignified and non-lignified zones. For the cork cells, R = 16 to **40** to **80** to 140 μ, T = 32 to **72** to **120** to 180 μ and L = 24 to **40** to **60** to 108 μ. As the soft cork cells rub off easily, most specimens

show relatively little cork, although samples with up to 26 radial rows have been encountered. In surface view, the cork cells appear polygonal (Fig. 2: A, C).

The phellogen, consists of 3 to 4 rows of regularly arranged, radially flattened cells with thin cellulosic walls and the phelloderm is a zone of

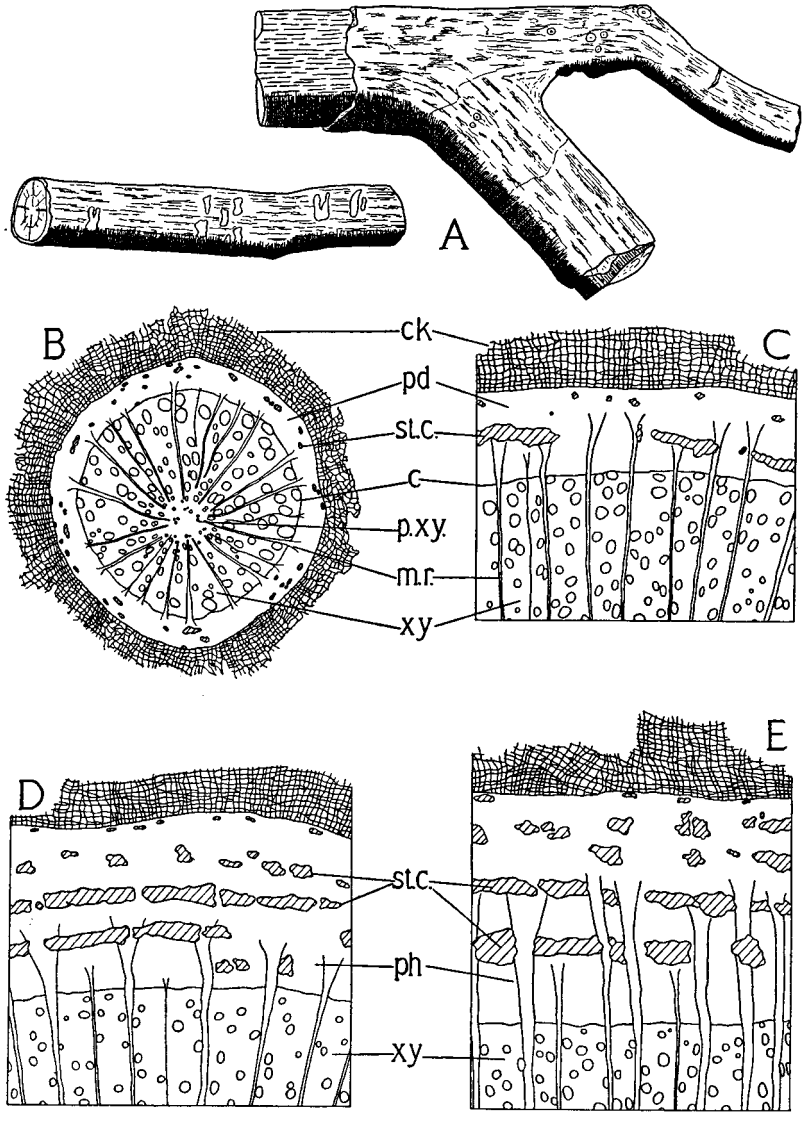


FIG. 1. *Rauwolfia caffra* Sond. Root. *A*, root segments × 1, *B-E*, general diagrams of transverse sections of roots, all × 15. *B*, 2·5 mm. diameter; *C*, 14 mm. diameter; *D*, 5 cm. diameter; *E*, 11 cm. diameter. *c*, cambium; *ck*, cork; *m.r.*, medullary ray; *pd*, phelloderm; *ph*, phloem; *p.xy.*, protoxylem; *st.c.*, stone cell group; *xy*, xylem.

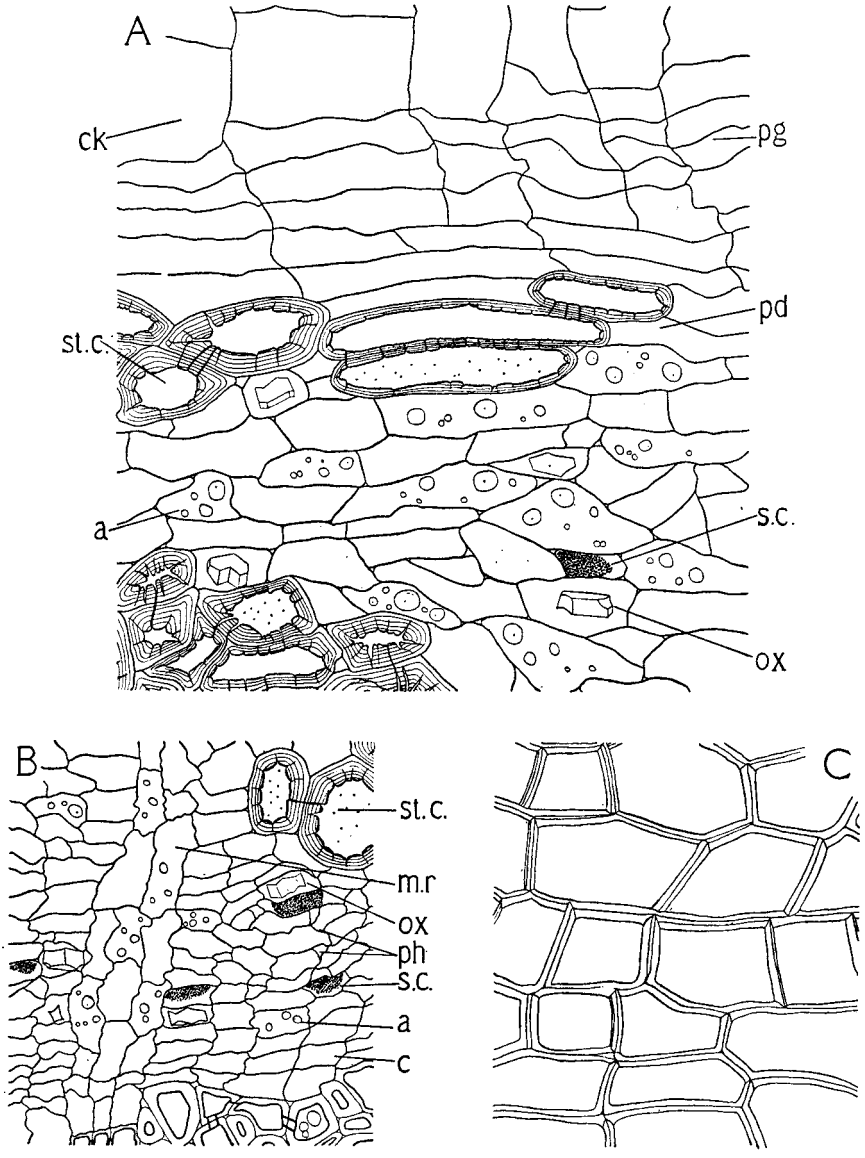


FIG. 2. *Rauwolfia caffra* Sond. Root. *A*, transverse section of the outer tissues, root diameter 33 mm. *B*, transverse section of inner phloem. *C*, cork cells in surface view. All × 200. *a*, starch grain; *c*, cambium; *ck*, cork; *m.r*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *pg*, phellogen; *ph*, phloem elements (companion cell and sieve tube); *s.c.*, secretion cell; *st.c.*, stone cell.

about 5 to 25 rows of cells dependent on the size of the root. The cells adjacent to the phellogen are usually regularly arranged in radial rows whilst the inner ones are more oval in shape with intercellular spaces and showing evidence of sliding growth. R = 16 to **24** to **40** to 72 μ,

T = 40 to **64** to **100** to 184 μ and L = 16 to **40** to **56** to 80 μ. The walls of most of the phelloderm cells are thickened with cellulose, but, with the exception of small roots, some are lignified, forming either isolated or groups of stone cells. Individual stone cells are frequently tangentially elongated and most are relatively thin walled with marked pitting and striation of the walls. R = 20 to **32** to **52** to 108 μ, T = 20 to **48** to **92** to 268 μ and L = 20 to **40** to **64** to 108 μ (Fig. 2: A; 3: A). Starch and scattered twinned prisms of calcium oxalate occur in the phelloderm. The starch consists chiefly of single, rounded grains 1 to **3** to **6** to 27 μ in diameter, with occasional 2 to 4 compound grains which may split into individual plano-convex or angular grains. The hilum is usually apparent as a central point or star-shaped cleft and many grains, with the exception of the larger partially gelatinised ones, show a Maltese cross effect when examined in polarised light (Fig. 3: E).

Internal to the phelloderm is the wide zone of secondary phloem, which, in the larger roots consists of an inner functional zone and an outer non-functioning secondary phloem characterised by up to about 5 interrupted bands of stone cells. The phloem consists of sieve tubes, companion cells, phloem parenchyma, secretion cells, medullary ray cells and groups of stone cells (Fig. 2: B). The heterogeneous rays consist of groups of small cells often with wavy walls, 3 to 5 cells wide and up to 28 cells in height with upper and lower uniseriate extensions consisting of 1 to 4 larger cells (Fig. 3: C). For the smaller cells R = 16 to **28** to **48** to 104 μ, T = 12 to **24** to **48** to 96 μ and L = 12 to **24** to **40** to 64 μ, and for the larger cells R = 12 to **20** to **40** to 64 μ, T = 24 to **36** to **56** to 132 μ and L = 36 to **48** to **68** to 104 μ. Occasional ray cells adjacent to the stone cell groups may be lignified to form radially elongated sclereids (Fig. 3: B).

The irregular stone cell groups in the outer phloem are up to about 8 cells in radial thickness and 30 cells in depth. Individual cells vary greatly from isodiametric to irregularly elongated fibre-like structures, R = 16 to **32** to **64** to 108 μ, T = 20 to **48** to **80** to 140 μ and L = 20 to **40** to **80** to 456 μ. Stone cells isolated by maceration using chromic-nitric acid reagent measured 44 to **60** to **108** to 576 μ in length and 20 to **36** to **52** to 108 μ in breadth. All the stone cells have funnel-shaped and occasionally branched pits and stratified walls. Calcium oxalate crystals completely fill the lumina of some cells (Fig. 3: B; 5: A, B).

In radial and tangential longitudinal sections of the secondary phloem long rows of calcium oxalate crystals are evident (Fig. 3: C). These crystals consist of monoclinic prisms often twinned on one of the hemi-pyramid faces and exhibit, in polarised light, a bicolouration effect. Length of prisms, 18 to **24** to **32** to 50 μ; breadth, 8 to **10** to **16** to 28 μ. The occasional angular masses of calcium oxalate which can be seen probably arise from fracture of the prisms during sectioning (Fig. 3: F).

Starch grains occur freely in the outer non-functional phloem, but are less frequent in the inner functional phloem. In size and shape they resemble those of the phelloderm.

Secretory cells occur occasionally in the phelloderm and scattered

throughout the outer and inner phloem. The amorphous contents of these cells stain with iodine solution, Sudan III and Tincture of Alkanna. No latex vessels have been observed although occasional short vertical

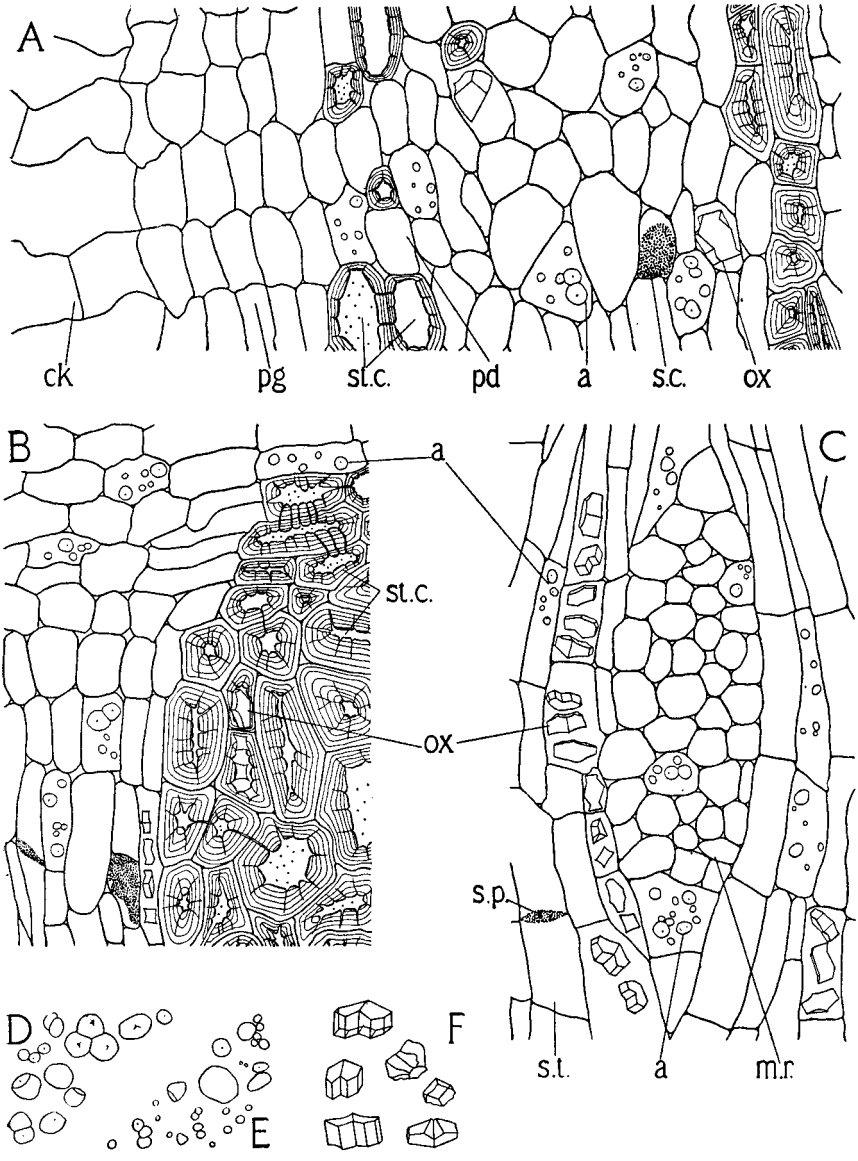


FIG. 3. *Rauwolfia caffra* Sond. Root. *A-C*, longitudinal sections of the bark, root diameter 33 mm. *A*, radial section of outer tissues. *B*, radial section of inner phloem. *C*, tangential section of inner phloem. *D*, starch grains from the wood. *E*, starch grains from the bark. *F*, calcium oxalate crystals from the root bark. All × 200. *a*, starch; *ck*, cork; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *pg*, phellogen; *s.c.*, secretion cell; *s.t.*, sieve tube; *s.p.*, sieve plate; *st.c.*, stone cell.

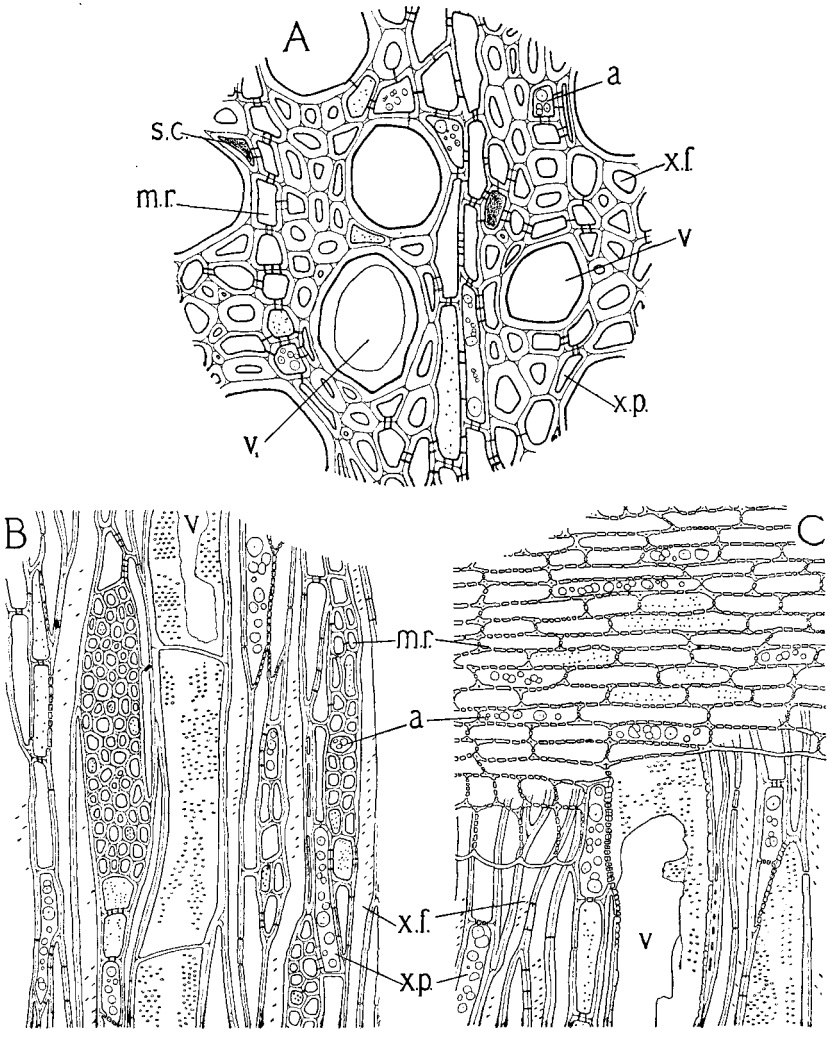


FIG. 4. *Rauwolfia caffra* Sond. Root. Secondary Wood. *A*, transverse section × 200. *B*, tangential longitudinal section, × 100. *C*, radial longitudinal section, × 100. *a*, starch; *m.r.*, medullary ray; *s.c.*, secretion cell; *v*, vessel; v_1, vessel showing portion of end plate; *x.f.*, xylem fibre; *x.p.*, xylem parenchyma.

rows of secretion cells occur. Individual cells can be isolated from alkali macerations (Fig. 5: D).

The primary xylem is indicated by four to six small groups of vessels near the centre of the root and the completely lignified secondary xylem is composed of medullary rays, vessels, fibres and wood parenchyma (Fig. 4: A, B, C). In transverse sections the rounded or somewhat radially elongated vessels occur singly or occasionally in pairs. The relatively thin vessel walls bear numerous alternately arranged bordered

pits, and isolated vessel segments show transverse and oblique perforation plates. Occasional vessels are occluded by brown amorphous material. R = 44 to **99** to **151** to 195 μ, T = 20 to **79** to **127** to 167 μ and for isolated segments, length = 137 to **412** to **715** to 962 μ, breadth = 68 to **104** to **152** to 224 μ. (Fig. 4; 5: G).

The numerous xylem fibres which support the vessels possess thick lignified walls with spirally arranged slit-like pits (Fig. 5: H). The length of the fibres is about 850 to **1290** to **1950** to 2340 μ, and breadth 20 to **28** to **36** to 56 μ. The ends of the fibres are frequently contorted, occasionally bifurcated and may show scalloping corresponding to the positions of adjacent medullary ray cells.

The apotracheal wood parenchyma occurs in short uniseriate rows connecting the vessels and medullary rays. In longitudinal sections the cells are arranged in vertical rows. The walls bear simple or half bordered pits, dependent on the nature of the adjacent cell structure. R = 10 to **28** to **40** to 88 μ, T = 12 to **28** to **40** to 64 μ and L = 56 to **100** to **140** to 240 μ.

The heterogeneous medullary rays resemble those of the bark being 3 to 5 cells wide, up to 20 small cells in height with uniseriate upper and lower extensions of about 1 to 4 large cells. For the small cells R = 28 to **72** to **120** to 248 μ, T = 10 to **12** to **20** to 40 μ and L = 10 to **18** to **28** to 56 μ, and for the larger cells R = 12 to **20** to **40** to 64 μ, T = 24 to **36** to **56** to 132 μ and L = 36 to **48** to **68** to 104 μ. In tangential sections, the small cells have a rounded appearance and intercellular spaces are apparent (Fig. 4: B).

Starch grains, 2 to **6** to **15** to 33 μ in diameter, similar to those in the bark, fill the wood parenchyma and medullary ray cells (Fig. 3: D). Occasional cells contain material staining with iodine and Sudan III. Some medullary ray cells of a few root specimens contained calcium oxalate prisms.

Stem

The general tissue distribution and cell dimensions of the stem bark resemble those of the root bark (Fig. 6: C). The soft cork layer is generally more extensive than that of the root, stem bark with up to 65 radial rows of cork cells having been examined. The phelloderm and cortex consist of about 30 radial rows of cells resembling the corresponding tissues in the root bark. The pericycle is not a well defined zone, but is indicated by scattered highly refractive non-lignified fibres 8 to **12** to **24** to 40 μ in diameter occurring either isolated or in association with groups of stone cells. After maceration in alkali, the isolated fibres, particularly of older, thicker bark show marked swellings 28 to 92 μ in diameter (Fig. 7: E).

The cortex and secondary phloem are characterised by up to about 16 interrupted bands of stone cells, individual stone cell groups being up to 12 cells wide and 19 cells deep.

Occasional latex vessels, containing granular matter which stains with iodine, occur in the outer non-functional secondary phloem and

cortex. In transverse sections they appear circular or ellipsoidal and in macerated material occasional branching is evident. R = 44 to 140 μ, T = 76 to 200 μ and length up to 0·5 mm. (Fig. 7: B, F).

The medullary rays are usually 4 to 6 small cells in width and 9 to 23 small cells high. Many medullary rays in older, thicker bark are com-

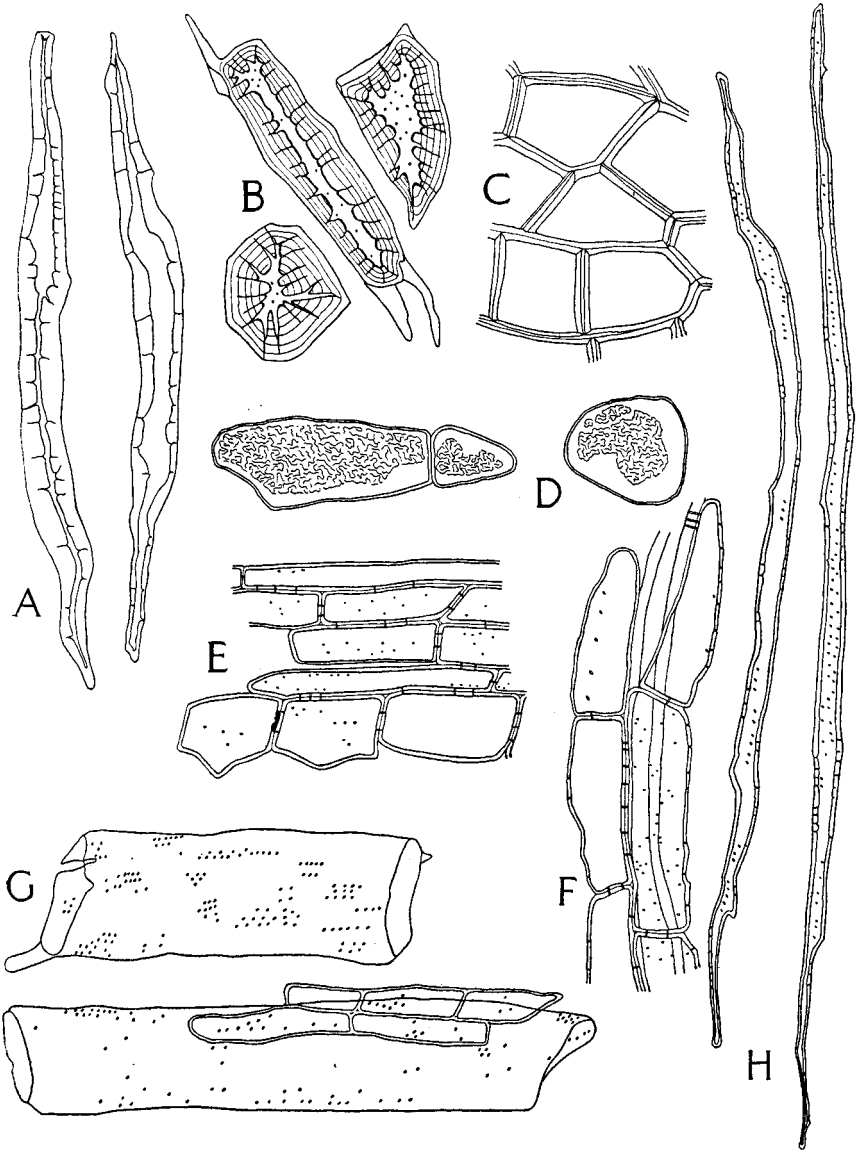


FIG. 5. *Rauwolfia caffra* Sond. Isolated elements of the root. *A*, elongated stone cells, × 100. *B*, stone cells, × 200. *C*, cork cells, × 200. *D*, secretion cells, × 200. *E*, lignified medullary ray cells, × 200. *F*, xylem parenchyma, × 200. *G*, portions of xylem vessels, × 100. *H*, xylem fibres, × 100.

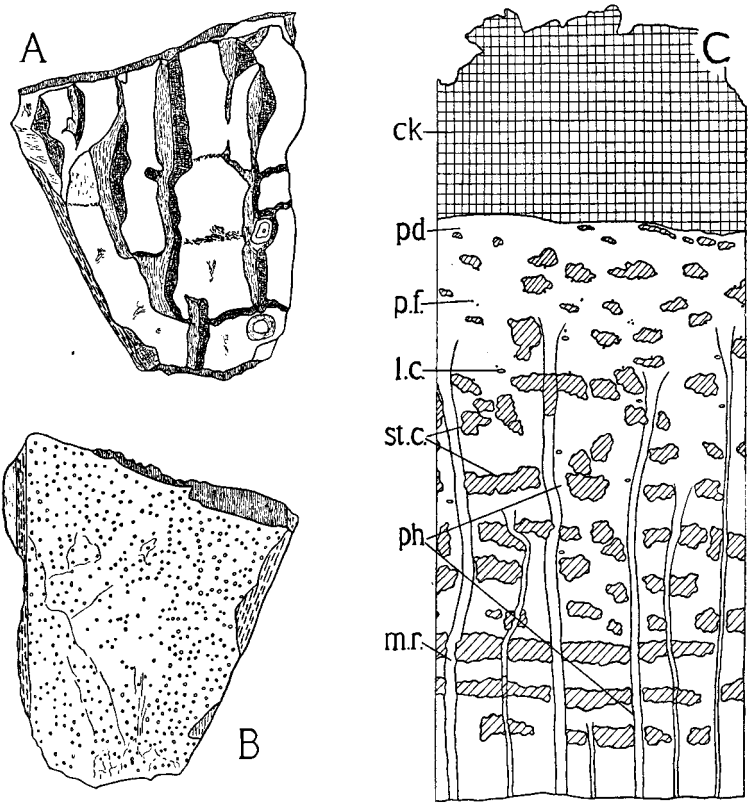


FIG. 6. *Rauwolfia caffra* Sond. Stem bark. *A*, outer surface. *B*, inner surface. Both × 1. *C*, transverse section, × 10. *ck*, cork; *l.c.*, latex canal; *m.r.*, medullary ray; *pd*, phelloderm; *p.f.*, pericyclic fibre; *ph*, phloem; *st.c.*, stone cell group.

pletely lignified forming groups of radially elongated sclereids. R = 40 to **52** to **88** to 136 μ; T = 24 to **32** to **40** to 68 μ and L = 20 to **24** to **32** to 48 μ.

The innermost layer of the phloem is the functional zone consisting of sieve tubes, companion cells, phloem parenchyma and medullary ray cells. Many cells, which often appear yellowish in chloral hydrate mounts, contain granular material staining with iodine solution and Sudan III. (Fig. 7: C, D). Calcium oxalate and starch resemble that present in the root bark.

The stem wood resembles the root wood although the fibres appear to be somewhat larger, length 627 to **1710** to **2310** to 2772 μ and breadth 20 to **28** to **40** to 56 μ. (Fig. 8).

Calcium oxalate which is only occasionally found in the root wood occurs more freely in the ray cells and xylem parenchyma of the stem wood and consists of the usual monoclinic and twinned prisms.

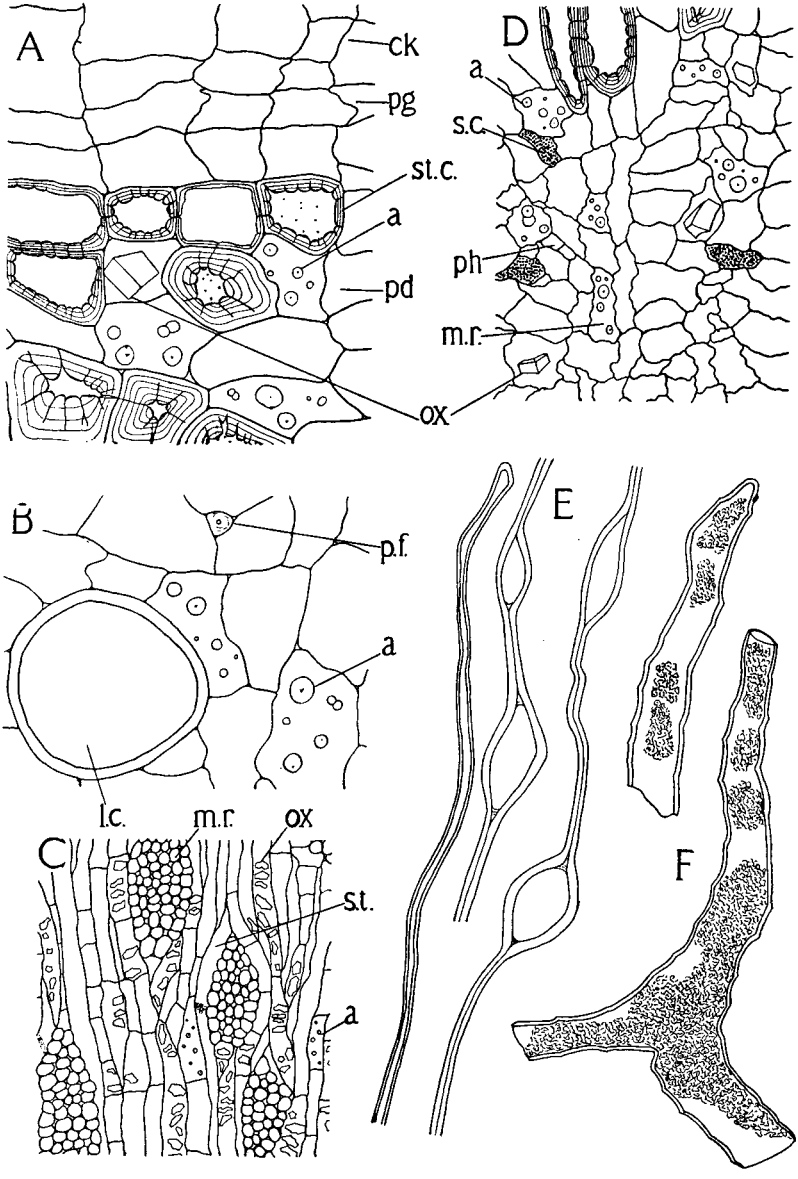
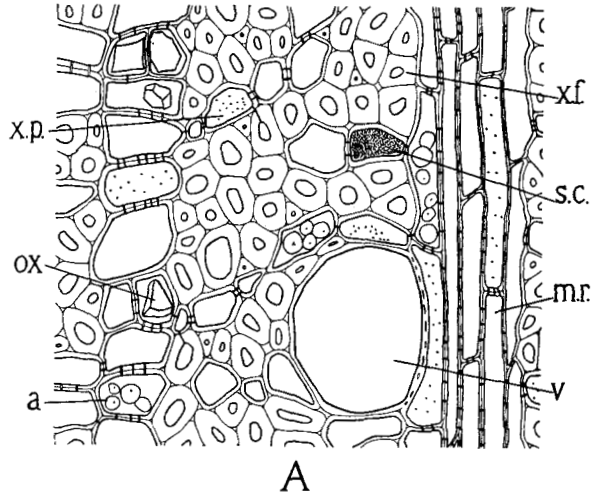


FIG. 7. *Rauwolfia caffra* Sond. Stem bark. *A*, transverse section of outer tissues, × 200. *B*, transverse section in region of pericycle, × 200. *C*, tangential longitudinal section of inner phloem, × 75. *D*, transverse section of inner phloem, × 200. *E*, isolated pericyclic fibres, × 100. *F*, portions of isolated latex canals, × 100. *a*, starch; *ck*, cork; *l.c.*, latex canal; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *p.f.*, pericyclic fibre; *pg*, phellogen; *ph*, phloem elements; *s.c.*, secretion cell; *s.t.*, sieve tube; *st.c.*, stone cell.



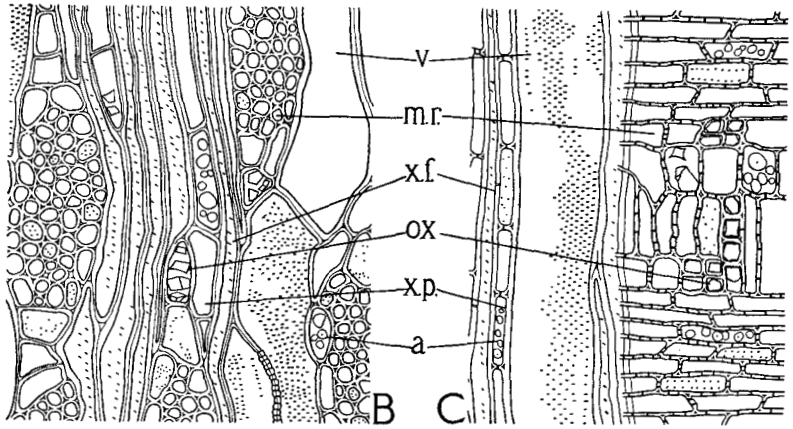


FIG. 8. *Rauwolfia caffra* Sond. Stem wood. *A*, transverse section, × 200. *B*, tangential longitudinal section, × 100. *C*, radial longitudinal section, × 100. *a*, starch; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *s.c.*, secretion cell; *v*, vessel; *x.f.*, xylem fibre; *x.p.*, xylem parenchyma.

THE POWDERED ROOT

The principal features of the powdered root are:

1. Thin-walled yellow, lignified cork cells appearing polygonal in surface view.

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

3. Rounded, ovoid, plano-convex and concavo-convex starch grains about 1 to **3** to **15** to 33 μ in diameter; occasional 2 to 4 compound grains.

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

5. Isodiametric, elongated or irregularly shaped lignified stone cells either singly or in groups. Occasional stone cells contain calcium oxalate crystals.

6. Large amounts 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.

Discussion

The structure of *R. caffra* root and stem is typical of the family Apocynaceae and of other members of the genus. The dried roots are readily distinguishable from *R. serpentina* in both the whole and powdered conditions due to the complete absence of stone cells in the latter. Its structure however closely resembles that of *R. vomitoria* root, the principal distinguishing feature being the absence of alternating lignified and unlignified cork cells in the transverse sections of *R. caffra*. As a consequence the cork of the whole root does not tend to flake off in small scales as with *R. vomitoria*. A comparison of the microscopical measurements of *R. caffra* with those recorded for *R. vomitoria*[7] indicates that the ranges of measurements are so similar in the two species to be useless for differentiation purposes. Further work will therefore be necessary to obtain a more suitable method for the differentiation of the powdered roots. It is hoped to attempt this after other African species have been studied in detail.

Summary

1. The gross morphology and histology of the stem and root of *R. caffra* Sond. have been described and illustrated.

2. The dimensions of the principal cell structures and contents are recorded.

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