

RESEARCH PAPERS

THE PHARMACOGNOSY OF *RAUWOLFIA PERAKENSIS*

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EXTENSIVE investigations for an effective hypotensive agent have been made in the past, usually with little success. More recently attention has been directed towards medicinal plants not hitherto considered from that aspect. Preparations of *Veratrum viride* have been found successful in clinical trials^{1,2,3}, and the latest drug to be so tested is *Rauwolfia serpentina*, Benth. *R. serpentina* has been the subject of a systematic pharmacognostical description by Wallis and Rohatgi⁴. It is the subject of a monograph in the British Pharmaceutical Codex and in the recently published Indian Pharmaceutical Codex.

Wallis and Rohatgi⁴ and Youngken⁵ state that *R. serpentina* besides being found in India is also native to Malaya. Ridley⁶ and Corner⁷, however, record the presence in Malaya of two *Rauwolfia* species only, *R. perakensis*, King and Gamble, and *R. sumatrana*, Jack. Corner⁷ and Burkill⁸ state that *R. perakensis* may be only a variety of *R. serpentina*. However, Pichon⁹ subdivides the genus *Rauwolfia* and places *R. serpentina* and *R. perakensis* in the groups *Ophioxylon* and *Dissolena* respectively. The Department of Agriculture, Federation of Malaya¹⁰, has stated that *R. serpentina* has not been found growing in Malaya. In view of the conflicting statements in the literature as to the distribution and identity of the two species, it was thought that a pharmacognostical description of *R. perakensis* would be of value in characterising it and possibly differentiating it from *R. serpentina*.

R. perakensis is not an article of commerce. Its use in native medicine for inflamed eyes and suppurating eyelids is recorded by Bartlett¹¹ and that of the fruits as an ingredient in poisons by Gimlette¹². After numerous enquiries regarding supplies of *Rauwolfia*, the Department of Agriculture, Federation of Malaya, is now engaged with this department in determining the optimum conditions for the cultivation and collection of the roots of *R. perakensis*.

HABIT

The plant is a shrub 1 to 3 metres tall with thin, lanceolate, acuminate leaves, 7 to 25 cm. long and 2 to 8 cm. broad, in whorls of three. The small and slender flowers occur in corymbose cymes at the ends of the shoots. They are 18 mm. long, with a pink corolla tube swollen round the stamens and have short, ovate, blunt, white petals. The fruit consists of 2 short, oblong, pointed indehiscent pods about 12 mm. long with a thin pulp and containing one seed each. The fruit changes on ripening from pink to reddish and finally to purplish-black with a grey bloom. *R. perakensis*



FIG. 1a. *Rauwolfia perakensis* K & G. Young plants.

grows wild in the Northern States of Malaya in open spaces by villages, rivers, sea-shores and in the hills. *R. perakensis* occurs also in Siam⁶.

MATERIAL

The samples examined were collected by the Department of Agriculture from wild plants growing in the State of Kelantan and authenticated by the Senior Agronomist, Federation of Malaya. Plants grown from the same stock were authenticated by the Director of the Botanic Gardens, Singapore. Vein-islet determinations were made on specimens of *R. perakensis* and *R. serpentina*. The respective values were 6 to 10 and 17 to 23.

MACROSCOPICAL CHARACTERS

The description given is that of air-dried samples. The roots are about 4 to 25 mm. in diameter, the larger pieces measuring up to about 4 cm. They are cylindrical and tapering or slightly tortuous, tough with a short

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fracture, the larger pieces being unbreakable by hand. There is no branching but some rootlets are present. Externally the root shows a light brown cork which is irregularly and longitudinally furrowed with occasional transverse cracks. The cork does not exfoliate, but is very soft and easily rubbed off, exposing a dark brown inner bark. The smoothed

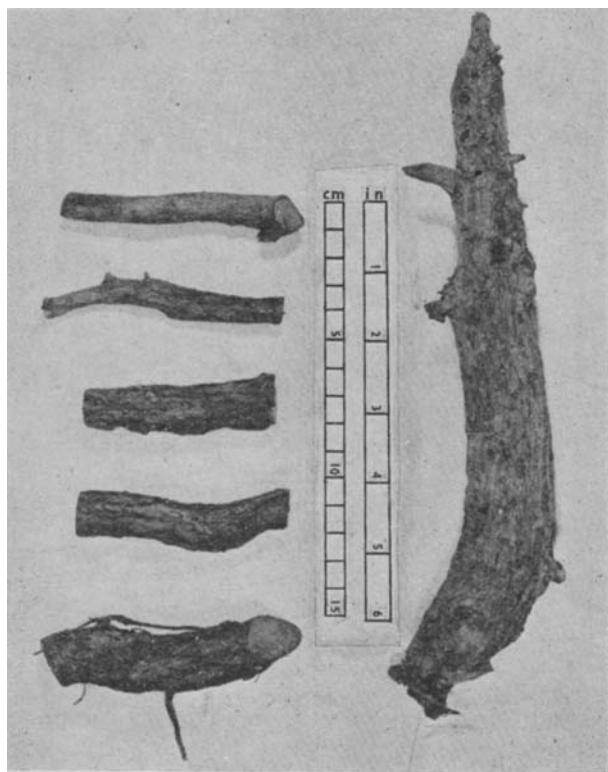


FIG. 1b. *Rauwolfia perakensis* K & G. Roots.

transverse section shows a thick cork, a narrow cortex and a finely radiate pale yellowish wood which occupies about nine-tenths of the diameter. Growth rings are well-defined in the wood, up to 4 occurring in many samples. The stele is often eccentrically placed and the cork and wood are lignified. Starch is present in abundance in the cortex and wood. The odour is slight and the taste bitter. The transverse section is immediately stained dark-reddish brown with concentrated nitric acid. With concentrated hydrochloric acid a bluish-green colour develops after about 2 minutes and gradually deepens. *R. serpentina* gives the same colour reactions.

Pieces of rhizome closely resemble the root. They are distinguished by the small central pith, about 1.2 mm. in diameter, which is usually split giving the appearance of a central cavity.

HISTOLOGY

Root. The cork consists of 3 to 5 alternate bands of suberised narrow and broad cork cells. The latter are also lignified. The zones of narrow-celled cork consist of layers of 2 to 10 cells which measure approximately

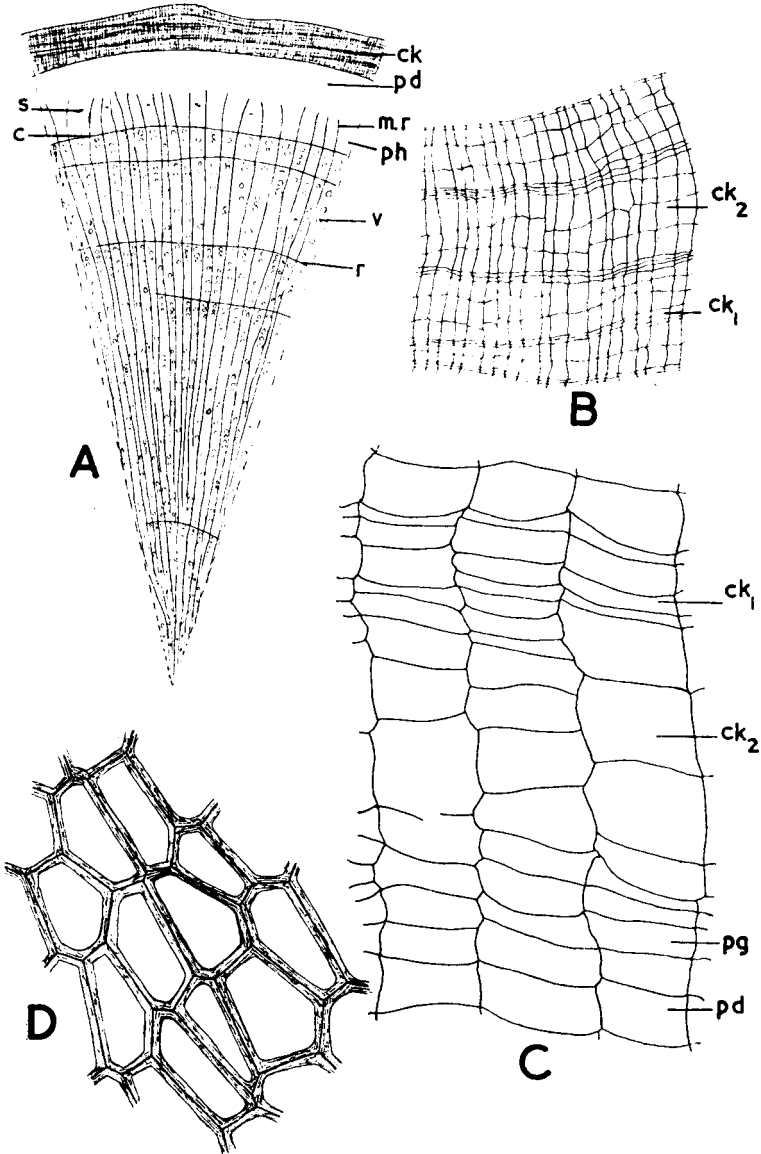


FIG. 2. *Rauwolfia perakensis* K & G. Root. A, diagrammatic transverse section $\times 10$; B, Transverse section of cork $\times 50$; C, section of B enlarged to show details $\times 200$; D, cork cells in surface view $\times 200$.

c, cambium; ck, cork; ck₁, narrow cork cells; ck₂, broad cork cells; m.r, medullary ray; pd, pelloderm; pg, phellogen; ph, phloem, r, growth ring; s, sclerenchyma; v, vessel.

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R 7 to 13 to 17 μ , L 20 to 40 to 66 μ and T 35 to 50 to 80 μ (where R, L and T represent measurements made in the radial, longitudinal and tangential directions respectively). The alternating bands of broad cells contain layers of 3 to 10 cells measuring about R 30 to 66 to 145 μ , L 20 to 40 to 66 μ and T 35 to 50 to 80 μ . The cork cambium consists of a single layer of thin-walled, rectangular cells, internal to which lies the phelloderm

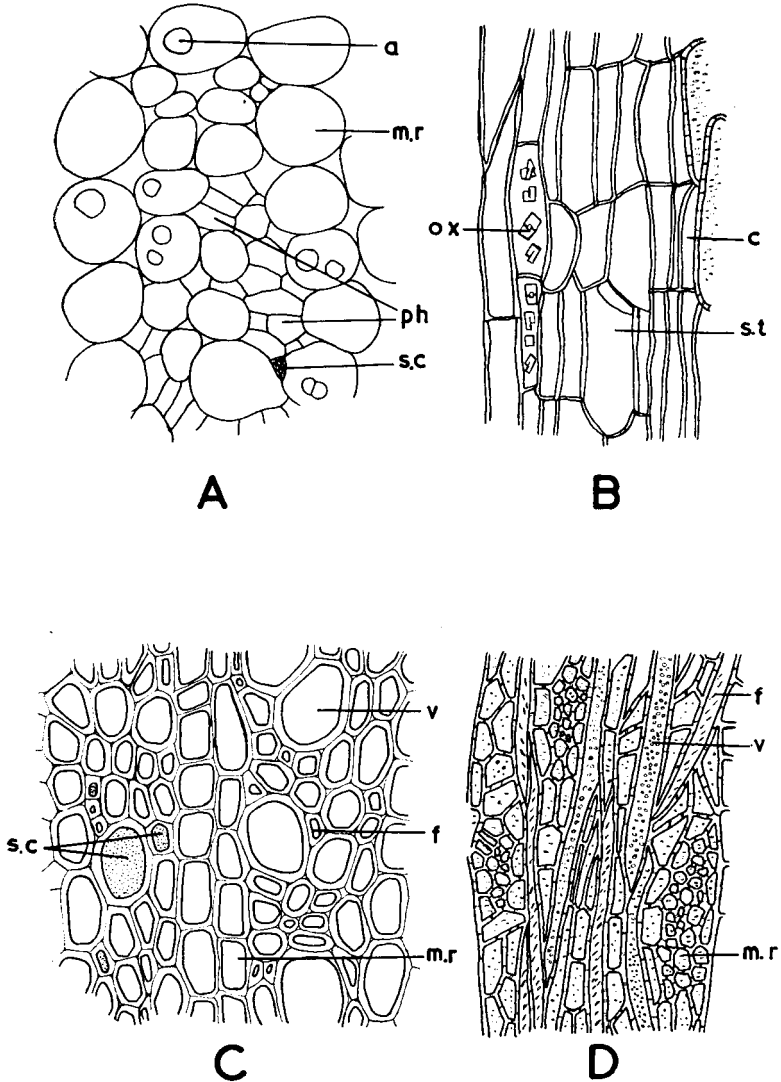


FIG 3. *Rauwolfia perakensis* K & G. Root. A, transverse section of phloem $\times 200$; B, radial longitudinal section of phloem and cambium $\times 200$; C, transverse section of xylem $\times 200$; D, tangential section of xylem $\times 100$. a, starch; c, cambium, f, xylem fibre; m.r, medullary ray; ox, calcium oxalate crystal; ph, phloem; s.c, secretion cell; s.t, sieve tube; v, vessel.

consisting of about 17 layers of cells which are irregularly arranged except for those near the cork cambium. These are rectangular in shape and radially arranged. The phelloderm cells contain numerous simple starch grains with some 2- to 3- and occasionally 4-compound grains. The individual grains measure 7 to 10 to 13 μ . Occasional scattered sclerencymatous fibres are present in the phelloderm and secondary phloem of older roots. They are thick-walled, often contorted, 415 to 750 μ long, 30 to 45 μ wide and they occur singly or more rarely in groups of two. Stone cells were rarely found and then only in more mature roots.

TABLE I
CELL MEASUREMENTS OF *R. perakensis* AND *R. serpentina*

| | <i>R. perakensis</i> | <i>R. serpentina</i> |
|------------------------------|---|--|
| 1. Broad cork | R 30-66-145 μ L 20-40-66 μ T 35-56-80 μ 7-10-13 μ | R 28-42-49 μ L 18-35-56 μ T 39-56-70 μ 4-10-20 μ |
| 2. Starch | 200 μ | Absent |
| 3. Stone cells | 415-750 μ | Absent |
| 4. Phloem fibres | 10-26 μ | 11-14 μ |
| 5. Calcium oxalate | | |
| <i>Xylem</i> | | |
| 6. Starch | 10-17 μ | 6-20-46 μ |
| 7. Medullary rays | 1-3 cells wide R 20-33-96 μ L 17-31-82-118 μ T 10-20-60-82 μ | 1-5 cells wide R 14-63-86 μ L 17-42-70 μ T 14-42-60 μ |
| Fibres | 750-1350-1420 μ long 33-70 μ wide | 432-576-774 μ long 36-54 μ wide |
| <i>Rhizome</i> | | |
| 9. Pericyclic fibres | 1.3 cm. long, enlargements up to 69 μ | 1.5 mm. long, enlargements up to 50 μ |
| 10. Phloem fibres | 430-600 μ | Absent |
| <i>Xylem</i> | | |
| Starch | 5-10 μ | 2-11-21 μ |
| Medullary ray | R 17-33-72 μ L 16-63-115 μ T 20-36-60 μ | R 7-32-53 μ L 14-46-92 μ T 17-21-49 μ |
| Fibres | 700-825-1350 μ | 193-560-753 μ |

They are often much contorted and sometimes branched. The medullary rays are radially arranged in the inner bark, 1 to 3 cells wide, the cells being ovoid in transverse section and filled with starch grains. The narrow groups of the phloem alternate with the medullary rays and consist of sieve tubes, companion cells and phloem parenchyma. Scattered, isolated, short secretion cells with yellowish granular contents occur in the phloem near the cambium. The contents stain brown with iodine. Numerous calcium oxalate crystals in the form of twin prisms and clusters measuring about 10 to 26 μ are present in longitudinal rows. The cambium consists of a ring of rectangular cells.

Transverse sections of the root show a tetrarch primary xylem. The wood is compact and strongly lignified with clearly differentiated growth rings. Abundant starch grains are present in the medullary rays and xylem parenchyma, the grains being simple or 2- to 4-compound, most showing a central cleft and measuring 10 to 17 μ individually. The medullary rays are straight and continuous with those of the phloem, 1 to 3 cells wide. The cells of the medullary ray measure approximately R 20 to

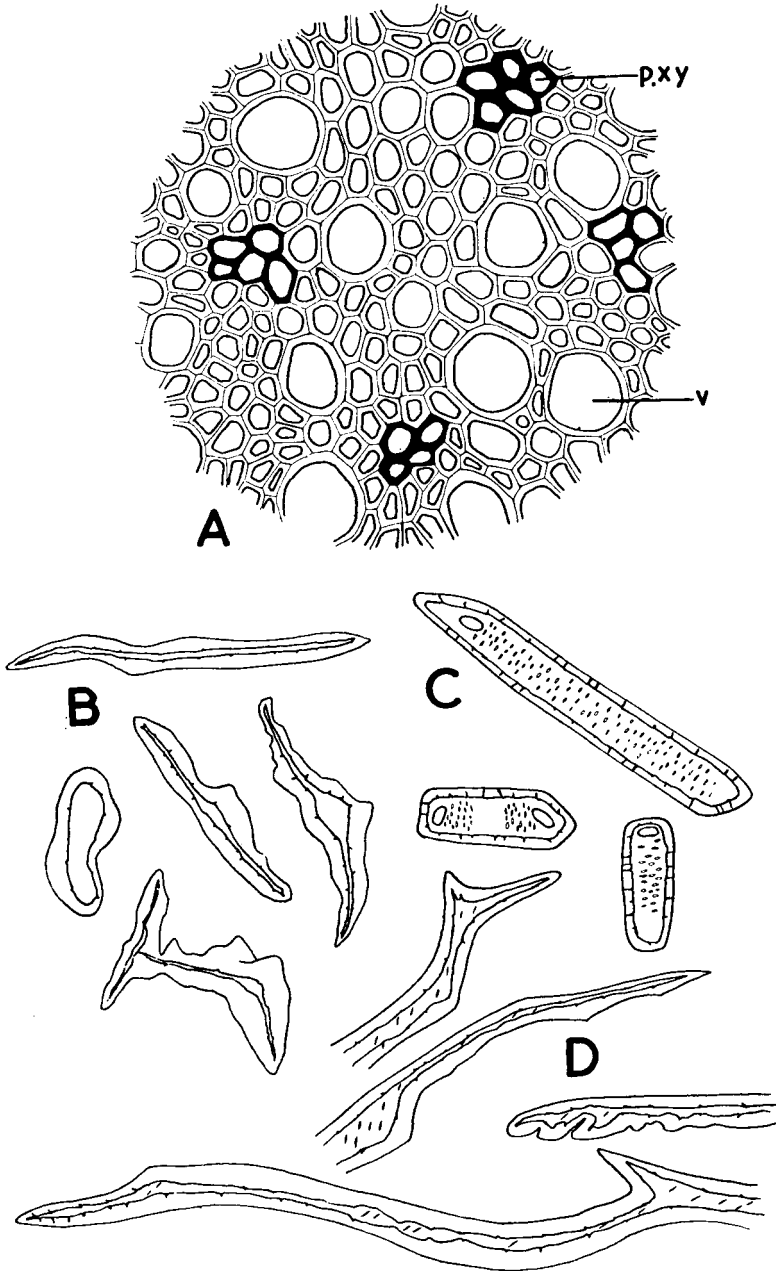


FIG. 4. *Rauwolfia perakensis*, K & G. Root. A, transverse section showing tetrarch primary xylem $\times 200$; B, Isolated schlerenchymatous elements $\times 100$; C, vessel element $\times 200$; D, Tips of xylem fibres $\times 200$.
p.xy, primary xylem, v, vessel.

33 to 96 μ , L 17 to 31 to 82 to 118 μ and T 10 to 20 to 60 to 82 μ . The walls of the cells are simple pitted. Single, monoclinic calcium oxalate prisms are present in the medullary rays. They measure about 16 to 20 μ and are best seen in the radial longitudinal section. Alternating with the medullary rays are narrow rays of secondary xylem consisting of fibres, vessels and xylem parenchyma. The xylem fibres are thick-walled with oblique bordered pits and measure about 750 to 1350 to 1420 μ long and 15 to 33 μ wide. The ends of the fibres are often contorted and chisel-like.

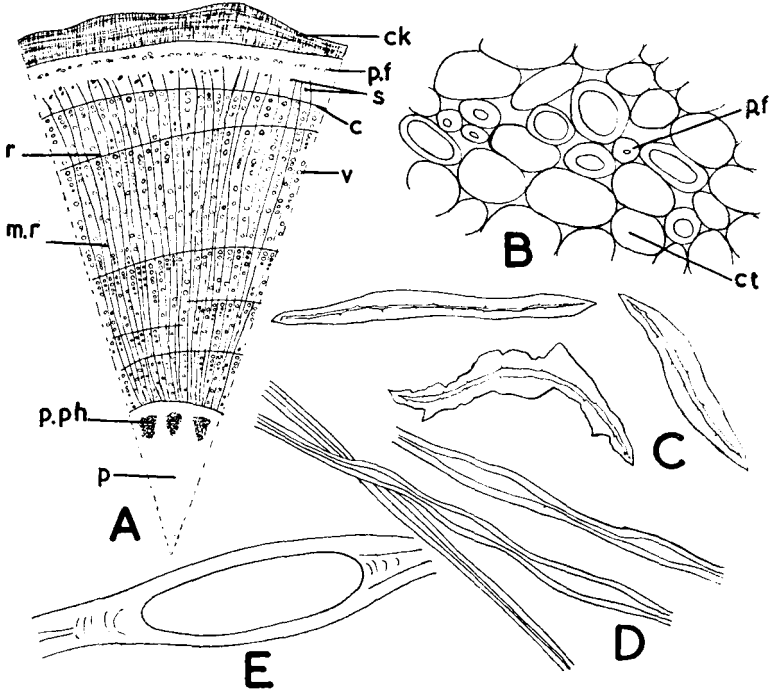


FIG. 5. *Rauwolfia perakensis*, K & G. Rhizome. A, diagrammatic transverse section $\times 10$; B, transverse section of cortex showing pericyclic fibres $\times 200$; C, sclerenchyma elements $\times 100$; D, pericyclic fibres from maceration $\times 50$; E, pericyclic fibre showing local enlargement $\times 200$.

The vessels are arranged mostly in short radial rows or singly and are about 150 to 225 to 450 μ in length and 33 to 70 μ in width. Occasional groups of the vessels and xylem parenchyma are filled with yellow to dark-brown contents some of which stain bright red with phloroglucinol and hydrochloric acid. Many of the vessels exhibit tyloses.

Rhizome. The rhizome is bicollateral. It is distinguished from the root in the transverse section by the presence of a ring of non-lignified pericyclic fibres and by the presence of a pith containing 16 bundles of perimedullary phloem. The cork consists of the two types of narrow and broad cells, in alternate bands. The narrow cells are more elongated radially than in the root, measuring R 10 to 15 to 23 μ , L 20 to 40 to 66 μ

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and T 35 to 50 to 80 μ . The broad cells measure R 56 to 75 to 145 μ , L 20 to 40 to 66 μ and T 35 to 50 to 80 μ . The pericyclic fibres have highly refractile thick walls, a narrow lumen and measure about 1.3 cm. long and 6 to 26 μ wide. Many of the fibres show a series of ovoid local enlargements with a maximum width of 69 μ . The sclerenchymatous fibres present in the phloem measure approximately 430 to 600 μ long and 15 to 40 μ wide. They are very thick-walled, the lumen rarely exceeding 6 μ in diameter. Secretion cells with yellowish granular contents staining brown with iodine and calcium oxalate prisms are present in the phloem.

The medullary rays of the xylem are 1 to 3 cells wide and contain abundant starch grains and calcium oxalate crystals. The starch grains are smaller than in the root and measure 5 to 10 μ . The crystals are about 20 μ wide. The cells of the medullary ray measure R 17 to 33 to 72 μ , L 16 to 63 to 115 μ and T 20 to 36 to 50 μ . The xylem fibres are more abundant, shorter and narrower than in the root, measuring approximately 700 to 825 to 1350 μ in length, and 16 to 23 μ in width. The walls of the fibres are straighter than those of the root, but many still show the chisel-like ending. The vessels are longer, being 430 to 600 μ long and 30 to 60 μ wide. Many of the vessels show tyloses and are filled with the same granular material as in the root.

POWDERED *Rauwolfia perakensis*

The characteristic features of the powdered samples are:—

1. Numerous rounded and plano-convex single starch grains, 5 to 10 to 17 μ , with some 2- to 3- and occasionally 4-compound grains. The grains show a central hilum or cleft.
2. Monoclinic and twin prisms of calcium oxalate 16 to 26 μ wide.
3. Cells with yellow to brown granular masses.
4. Abundant lignified tissue, including fragments of xylem fibres up to 33 μ wide with oblique bordered pits and fragments of vessels 33 to 70 μ wide with bordered pits.
5. Contorted sclerenchymatous fibres up to 750 μ long and 45 μ wide with a very narrow lumen.
6. Contorted and sometimes branched stone cells not exceeding 200 μ in length.
7. Lignified tabular cork, polygonal in surface view consisting of narrow and broad cells.
8. Fragments of non-lignified highly refractile pericyclic fibres showing local enlargements.

EVALUATION

The total ash of the root was determined according to the method of the British Pharmacopœia and found to be 1.4 per cent. and the acid-insoluble ash to be 0.4 per cent. The total alkaloidal content was found to be 1.25 per cent. using the method of assay of the British Pharmaceutical Codex for *R. serpentina*. With the method of the Indian Pharmaceutical Codex the value obtained was 0.8 per cent.

DISCUSSION

Although Corner⁷ and Burkill⁸ have suggested that *R. perakensis* may be only a variety of *R. serpentina*, a comparison of the quantitative data and certain diagnostic structural elements reported by Wallis and Rohatgi⁴ for *R. serpentina* and those presented in this paper, reveal distinct differences which can hardly be accounted for on the basis of a mere variation. The essential quantitative data are given in Table I.

Consideration of this reveals that in *R. perakensis* the broad cork cells are up to 3 times longer radially. The xylem fibres are wider and twice as long and the medullary ray cells have significantly greater dimensions than those of *R. serpentina*. Calcium oxalate crystals are also bigger but the starch grains are smaller. The pericyclic fibres in the rhizome of *R. perakensis* are about 9 times longer than those of *R. serpentina*. Stone cells and phloem fibres are present in *R. perakensis* but not in *R. serpentina*. These differences would thus support the view of Pichon⁹ that the two species are quite distinct. This is further supported by the different veinlet numbers obtained with the two species.

The present work substantially confirms the findings of Youngken⁵, except that secretion cells not reported by him have been found. However the absence of stone cells and sclerenchymatous fibres as his criterion for differentiating *R. serpentina* is not completely valid. Young roots of *R. perakensis* of up to about 7 mm. in diameter do not possess any sclerenchymatous elements and consequently exhibit the same histological appearance as *R. serpentina*. A definite differentiation can then only be established on the basis of cell measurement.

SUMMARY

1. The macroscopical and histological characters of roots and rhizome of *Rauwolfia perakensis*, King and Gamble, have been described.
2. *Rauwolfia perakensis* has been shown to be distinguishable from *Rauwolfia serpentina* on the basis of cell measurements and also by the presence of sclerenchymatous elements.

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