*J. Pharm. Pharmac.*, 1966, **18**, *Suppl.*, 133S–141S Received May 2, 1966

# Comparative anatomy of the leaves of *Voacanga schweinfurthii* Stapf and *Voacanga africana* Stapf

F. NEWCOMBE AND B. A. PATEL

There is a marked similarity in the anatomical structures of the leaves of the closely related species *Voacanga schweinfurthii* and *V. africana* which are here described and compared. The evidence suggests that these two species should be considered as a single species.

IN a previous report (Fish & Newcombe, 1966), the taxonomic relationship of the species *Voacanga schweinfurthii* and *Voacanga africana* was discussed. The close similarity of the plants attributed to these species was emphasised with respect to their general morphology and their detailed anatomy, and the chemical constituents of their stem barks. The comparative study has been extended to cover the detailed anatomical structures of the leaves of the two species.

## MATERIALS AND METHODS

Two samples of leaves of *V. schweinfurthii* were obtained from Dr. D. B. Fanshawe, Division of Forest Ecology, Kitwe, Zambia; two samples of leaves of *V. africana* were supplied by Dr. M. B. Patel, University of Ife, Nigeria, and a further two supplied by A. G. Kenyon, Tropical Products Institute, London, were also collected in Nigeria. These species were differentiated on the size of the calyx and corolla tube (Pichon, 1947).

Sections were cut with a Reichert freezing microtome. Macerations were prepared using 20% w/v potassium hydroxide or Schultz's solution.

NOTE: The macroscopical and microscopical structures of the leaves of *V. schweinfurthii* and *V. africana* are so similar that a single account is given of their distribution and appearance. Illustrations from prepared sections and macerates are of *V. schweinfurthii*. Similarly, although a full range of measurements was made of the dimensions of the various structures of *both* species, these show such close correlation that only those of one species, *V. schweinfurthii*, are recorded.

## Macroscopical characters

The leaves are simple, varying in size from about 6 cm long and 3 cm broad to 17 cm long and 9 cm broad. The shape varies from ovate-lanceolate to lanceolate; the apex is most commonly acute or acuminate but occasionally obtuse; the margin is entire and slightly wavy; the base is symmetrical, cuneate, tapering abruptly to a short or very short petiole. The lamina is greenish-brown to grey on the upper surface which is glabrous except for occasional trichomes on the midrib near the base; the lower surface is greyish-green or pale greyish-brown and varies from glabrous, in older leaves, to smoothly puberulous, in younger ones, the trichomes being most numerous on the midrib and main veins but also

From the School of Pharmacy, University of Bradford, Bradford 7.

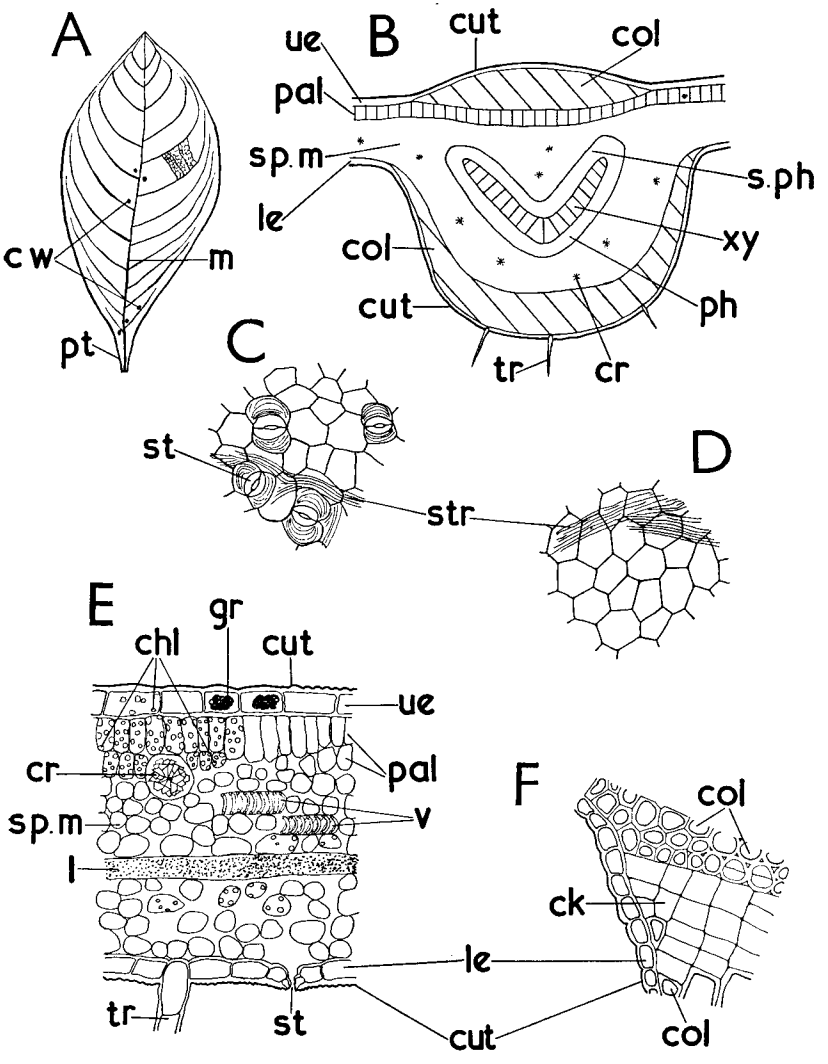


FIG. 1. *Voacanga schweinfurthii* leaf. A, lower surface of leaf. B, transverse section of midrib. C, lower epidermis. D, upper epidermis. E, transverse section of lamina. F, transverse section of cork wart. Magnification: A $\times\frac{1}{3}$, B $\times 20$, C to F $\times 200$. *chl*, chloroplast; *ck*, cork; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *cw*, cork wart; *gr*, granular content; *l*, latex tube; *le*, lower epidermis; *m*, midrib; *pal*, palisade layer; *ph*, phloem; *pt*, petiole; *s.ph*, supernumerary phloem; *sp.m*, spongy mesophyll; *st*, stoma; *str*, striations; *tr*, trichome; *ue*, uper epidermis; *v*, xylem vessel; *xy*, xylem.

being fairly frequent on the interneural areas near the base. Light brown cork warts occur on both surfaces, generally near the base, but most commonly on the lower surface, measuring up to about 2 mm in diameter. The venation is pinnate, about 9 to 19 pairs of lateral veins

leaving the midrib at about 90°, near the base, to 60°, towards the apex, those nearer the apex anastomosing; the midrib and veins are prominent on the under surface and slightly depressed on the upper. The leaves have no characteristic odour, a slightly bitter taste and a brittle and papery texture (Fig. 1, A).

## Microscopical characters

### LAMINA

The *upper epidermis* consists of a layer of polygonal cells, becoming elongated over the main veins, with straight anticlinal walls, the outer tangential walls being thicker than the inner walls; the cells frequently contain a few chloroplasts or yellowish-brown granular material (Fig. 1, B, D and E). The cells measure about H 16 to **21** to **27** to 33 $\mu$, Lev L 20 to **31** to **35** to 60 $\mu$ and Lev B 16 to **20** to **32** to 40 $\mu$. A very few, oval, paracytic *stomata* are present, slightly raised above the epidermis, measuring about 24 $\mu$ long and 16 $\mu$ broad. The epidermis is covered by a thin *cuticle* exhibiting long, irregular striations which continue over several contiguous cells, being most marked in the areas adjacent to the stomata (Fig. 1, D). Occasional *cork warts* are present consisting of a subcylindrical mass of cells arranged in tabular rows of 3 to 10 cells per row, there being from 10 to 28 rows per wart when seen in either transverse or longitudinal section; small warts are covered by an intact epidermis but larger warts protrude beyond the epidermis, frequently with one or two rows of broken collenchymatous cells on their outer side and a few layers of collenchyma on their inner side. The cell walls are slightly thickened and though always lignified, the intensity of staining with phloroglucinol and hydrochloric acid is very variable; the cells measure about H 10 to 40 $\mu$, Lev L and Lev B 20 to 40 $\mu$ (Fig. 1, A and F). *Trichomes* are absent.

The *mesophyll* is well differentiated. The *palisade* consists of two layers of cells; the layer adjacent to the epidermis being of subcylindrical, thin-walled cells measuring about H 13 to **23** to **35** to 39 $\mu$, Lev L and Lev B 7 to **10** to **12** to 17 $\mu$; the cells of the inner layer are somewhat more rounded, measuring about H 16 to **23** to **29** to 33 $\mu$ (Fig. 1, B and E). The palisade is continuous over the lateral veins and both layers contain numerous chloroplasts, about 7 to 10 $\mu$ in diameter, but no starch. The *spongy mesophyll* consists of about 8 to 12 layers of thin-walled cells, frequently isodiametric but often elongated axially and tangentially, particularly near the lower epidermis, measuring about H 10 to **12** to **17** to 19 $\mu$, Lev L and Lev B 10 to **18** to **34** to 36 $\mu$; the cells are fairly closely packed in places but with numerous large air spaces near the palisade and contain some chloroplasts but no starch (Fig. 1, B and E). Scattered in both the palisade and spongy mesophyll are occasional idioblasts containing cluster crystals of *calcium oxalate*, measuring about 19 to **30** to **41** to 49 $\mu$ in diameter (Fig. 1, B and E). Adjacent to the veinlets occurring in the mesophyll are occasional, long, unlignified, cortical *fibres* and occasional, simple *latex tubes*; the veinlets contain *xylem vessels* showing spiral and annular thickening (Fig. 1, B and E).

The *lower epidermis* consists of a layer of polygonal cells elongated over the main veins, with almost straight anticlinal walls; the outer tangential walls are somewhat thickened and covered by a thin *cuticle* having marked, long, irregular striations, particularly prominent near the stomata (Fig. 1, C); the cells have similar contents to those of the upper epidermis and measure about H 6 to **10** to **16** to 19 $\mu$, Lev L and Lev B 16 to **19** to **27** to 33 $\mu$. Numerous, oval, paracytic *stomata* are present, slightly raised above the epidermis, measuring about 24 $\mu$ long and 16 $\mu$ broad (Fig. 1, C and E). On the interneural regions of the epidermis, there occur occasionally, though sometimes fairly frequently, multicellular, uniseriate, covering *trichomes* with slightly thickened, smooth or very faintly longitudinally striated walls and acute or sub-acute apices; these are of two types, the commoner consisting of 15 to 25 almost isodiametric cells and measuring about 300 to 700 $\mu$ in length, the second type consisting of 5 to 12 elongated cells, one or more of which is typically collapsed, measuring about 80 to 700 $\mu$ in length. Similar trichomes occur with greater frequency over the veins and midrib (Fig. 2, C) and are accompanied by unicellular, thin-walled, covering trichomes of up to about 30 $\mu$ in length, with more or less blunted apices, and well-marked, rounded *papillae* (Fig. 2, B). Glandular trichomes are absent.

MIDRIB

The midrib shows a typical dicotyledonous structure (Fig. 1, B).

The *upper epidermis* is similar to that of the interneural zone, except that the cells are elongated parallel to the midrib, measuring about H 10 to **13** to **16** to 20 $\mu$, Lev L 10 to **16** to **29** to 36 $\mu$ and Lev B 7 to **10** to **16** to 20 $\mu$ (Fig. 2, A). The epidermis is covered by a fairly thick *cuticle*; *stomata* are absent; *trichomes* are generally absent but when present occur near the base and are of both multicellular, covering types described previously (Fig. 2, C).

The *cortex* contains two prominent zones of very thick-walled, rounded or slightly elongated, collenchymatous cells with few, small, intercellular air-spaces, adjacent to each epidermis, each zone being composed of about 8 to 10 rows of cells near the base, reducing to 2 to 3 rows at the apex. Beneath the upper hypodermal zone of collenchyma is a layer composed of 2 rows of subcylindrical to rounded, thin-walled cells continuous with the palisade of the lamina; this layer is prominent due to the presence in the cells of numerous chloroplasts. The remainder of the cortex is of fairly closely packed, thin-walled parenchyma in which occur elongated, unbranched *latex tubes*, measuring up to 20 $\mu$ in diameter, numerous in the region of the pericycle and scattered sparsely towards the outer cortex, with greyish, granular content which stains with Calco Oil Blue, and occasional pericyclic *fibres*, mostly occurring singly but occasionally being in groups of 2 to 4, which are markedly elongated longitudinally with uniformly thickened, unlignified walls and acute or somewhat blunt, unbranched ends; they are about 20 $\mu$ thick with lengths of about 2·4 to 3·2 mm. Occasional cluster crystals of *calcium oxalate* are also present (Fig. 1, B; Fig. 2, A; Fig. 3, A).

The *meristele* is arcuate with a central xylem surrounded by phloem (Fig. 1, B). The *phloem* abaxial to the xylem consists of narrow, thin-walled parenchyma; well-marked supernumerary phloem also occurs adaxial to the xylem (Fig. 1, B; Fig. 2, A). Occasional *latex tubes* occur towards the cortical side of the phloem.

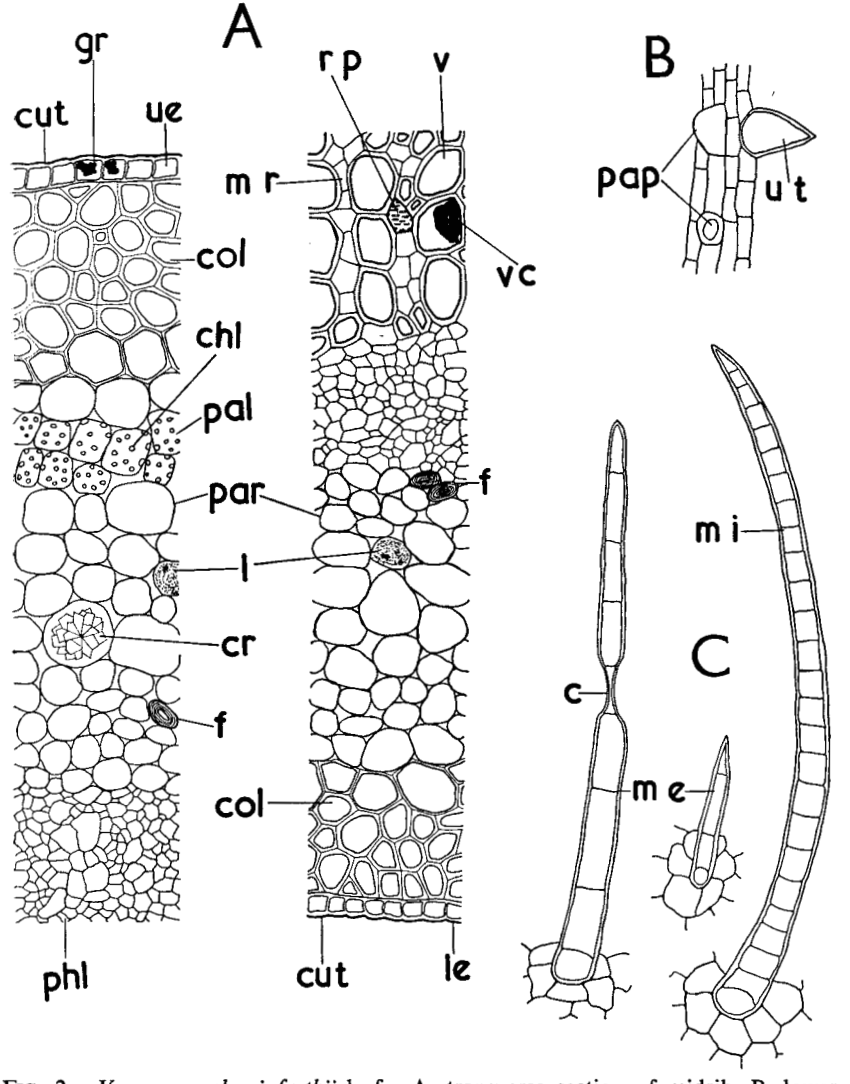


FIG. 2. *Voacanga schweinfurthii* leaf. A, transverse section of midrib. B, lower epidermis of midrib. C, trichomes of midrib. Magnification ×200. *c*, collapsed cell; *chl*, chloroplast; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *f*, fibre; *gr*, granular content; *l*, latex tube; *le*, lower epidermis; *me*, multicellular trichome with elongated cells; *mi*, multicellular trichome with isodiametric cells; *mr*, medullary ray; *pal*, palisade layer; *pap*, papilla; *par*, parenchyma; *phl*, phloem; *rp*, reticulate xylem parenchyma; *ue*, upper epidermis; *ut*, unicellular trichome; *v*, xylem vessel; *vc*, xylem vessel content.

The *xylem* consists of radially arranged vessels and tracheids exhibiting annular, spiral and reticulate, lignified thickenings, measuring about 10 to 30 $\mu$ in diameter, the larger vessels occasionally having some yellowish-brown, amorphous content; and *medullary rays*, one or, less commonly, two cells wide, composed of lignified, reticulate parenchyma, markedly smaller than the conducting elements (Fig. 2, A; Fig. 3, A).

The *lower epidermis* is composed of cells similar to those of the upper epidermis, measuring about H 10 to **11** to **16** to 20 $\mu$, Lev L 16 to **23** to **38** to 40 $\mu$ and Lev B 7 to **10** to **14** to 23 $\mu$; the *cuticle* is fairly thick; stomata are absent; *papillae* and both unicellular and multicellular covering *trichomes*, as described previously, occur frequently, though in greatly varying amounts, being most numerous near the base and rare towards the apex (Fig. 2, B and C).

## PETIOLE

The *petiole* is subcylindrical with two, slight, longitudinal, lateral ridges and is about 5 to 10 mm long and 2 to 6 mm broad. The structure (Fig. 3, B) is very similar to that of the midrib; the epidermis is of elongated, polygonal cells with very occasional, paracytic *stomata*, numerous *papillae*, frequent unicellular and multicellular covering *trichomes* and covered by by a fairly thick *cuticle*; occasional *cork warts* are present; the hypodermal collenchyma is arranged in a continuous ring; no well-differentiated chlorenchymatous region is present but the cortical parenchyma contains some chloroplasts; a few cluster crystals of *calcium oxalate* are scattered in the cortex; occasional *latex tubes* occur, being most numerous towards the pericycle; occasional pericyclic *fibres* occur; the central arcuate *meristele* is composed of the same type and distribution of cells as in the midrib. Additional structures present are one or more small, round, vascular bundles towards each lateral ridge, with a central *xylem* area of annular and spiral and, very occasionally, reticulate vessels and reticulate, lignified parenchyma surrounded by phloem consisting of narrow sieve tubes with associated parenchyma and occasional *latex tubes* and *fibres* on the periphery (Fig. 3, C).

## NUMERICAL VALUES

Determinations of palisade ratio and stomatal index (lower surface) were made. No statistically significant difference between the two species could be shown for these parameters (Table 1).

Using 10 leaves of each species, determinations were made of vein-islet number, veinlet termination number, absolute vein-islet number and absolute veinlet termination number according to the method of Gupta & Kundu (1965) (Table 1). There was no significant difference between the values for absolute vein-islet number. Some difference was suggested by the other parameters but because of the small number of measurements made, further work would be necessary to confirm this. Snedecor's F test and Student's *t*-test were used to assess the measurements made.

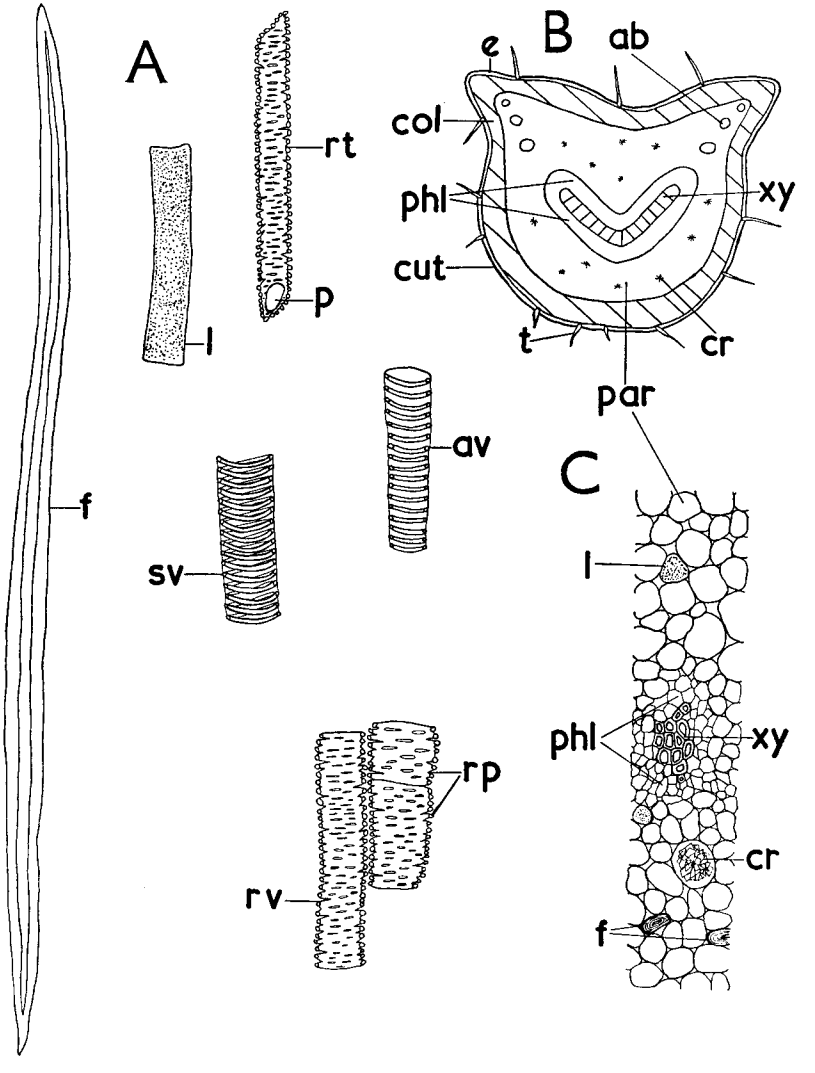


FIG. 3. *Voacanga schweinfurthii* leaf. A, isolated elements of the midrib. B, transverse section of the petiole. C, transverse section of accessory vascular bundle. Magnification: A and C ×200, B ×10. *ab*, accessory vascular bundle; *av*, annular vessel; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *e*, epidermis; *f*, fibre; *l*, latex tube; *p*, pore; *par*, parenchyma; *phl*, phloem; *rp*, reticulate xylem parenchyma; *rt*, reticulate tracheid; *rv*, reticulate vessel; *sv*, spiral vessel; *t*, trichome; *xy*, xylem.

## Discussion

Although *V. schweinfurthii* and *V. africana* have always been considered as separate species since their original descriptions (Stapf, 1894,a,b), they can be differentiated (Fish & Newcombe, 1966) on two morphological

characters only. These are the size of the calyx and corolla tube (Pichon, 1947). Both are greater in *V. schweinfurthii*, though there is some overlap of the values for calyx size. Confusion has arisen over the identification of particular plants in these species, several having been placed in one then transferred to the other, and it has been suggested that the differences between the plants of the species may be insufficient to warrant such discrete separation (Newcombe, 1964).

TABLE 1. NUMERICAL VALUES OF *V. schweinfurthii* AND *V. africana*

| Character | Species | Range | Variance Ratio (calc.) | V.R. P = 0·05 | *t* (calc.) | *t* P = 0·05 |
|---|---|---|---|---|---|---|
| Palisade Ratio | *V. schweinfurthii*<br>*V. africana* | 3·2– 4·4– 7·4– 9·0<br>3·5– 4·4– 7·1– 8·5 | 1·33 | 1·90 | 0·43 | 1·67 |
| Stomatal Index | *V. schweinfurthii*<br>*V. africana* | 7·2– 9·8–18·0–26·7<br>7·4–10·2–17·1–22·2 | 1·40 | 1·69 | 0·28 | 1·67 |
| Vein-islet number | *V. schweinfurthii*<br>*V. africana* | 6·7– 7·5–12·9–14·2<br>6·0– 7·0– 9·2– 9·5 | 5·92 | 3·18 | 2·27 | 2·10 |
| Veinlet termination number | *V. schweinfurthii*<br>*V. africana* | 14·0–16·5–25·3–27·8<br>18·0–19·3–23·7–26·3 | 3·90 | 3·18 | 0·37 | 2·10 |
| Absolute vein-islet number $\times 10^{-4}$ | *V. schweinfurthii*<br>*V. africana* | 1·1– 1·9– 6·7– 8·8<br>1·6– 4·1– 8·0– 8·8 | 1·49 | 3·18 | 1·77 | 2·10 |
| Absolute veinlet termin. number $\times 10^{-4}$ | *V. schweinfurthii*<br>*V. africana* | 2·1– 3·3–15·1–21·5<br>3·7– 9·4–18·8–19·4 | 1·90 | 3·18 | 2·31 | 2·10 |

The stem barks of the two species have a similar alkaloid content and are structurally indistinguishable (Fish & Newcombe, 1966). Investigation of the macroscopy of the leaves of both species shows that they are indistinguishable, both showing similar variations in size and shape; the type, distribution and sizes of the microscopical structures are also very similar. Leaves of co-generic species are likely to have similar structures but frequently may be differentiated by numerical values. The two most commonly used are palisade ratio and stomatal index, neither of which can be used to differentiate the leaves of *V. schweinfurthii* and *V. africana*.

Although there is a suggestion that the vein-islet number and the absolute veinlet termination number may be different, assessment of the taxonomic significance of these two last-mentioned parameters is difficult since few examples have been reported of absolute veinlet termination number and, regarding vein-islet number, not only may related species have similar vein-islet numbers, e.g. *Barosma betulina* and *B. crenulata*, but also different varieties of a single species may have different vein-islet numbers, e.g. *Barosma serratifolia var. latifolia* and *B. serratifolia* var. *longifolia* (Levin, 1929).

These results support the hypothesis that *V. schweinfurthii* and *V. africana* should be considered as a single species.

*Acknowledgement.* We wish to thank Dr. K. R. Fell for his interest and guidance in this work.

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