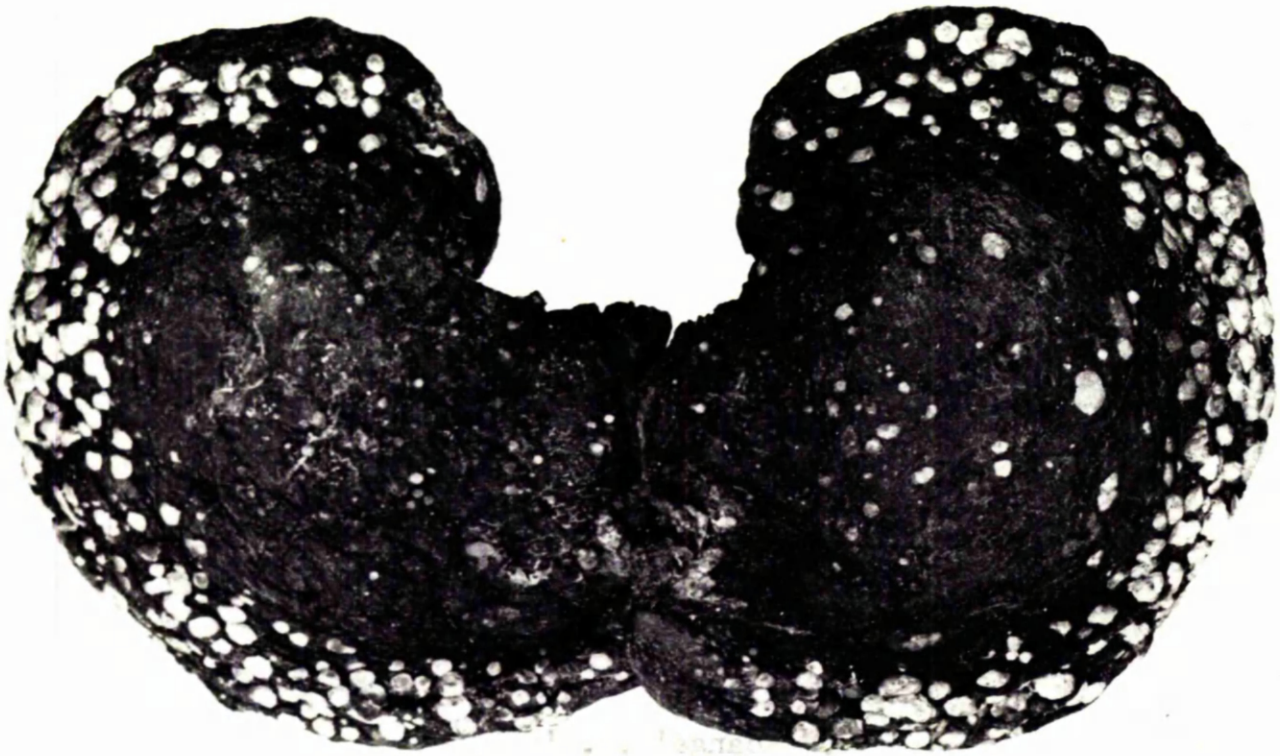

Frontispiece : 'Voa' . . 'Acanga' . . 'painted fruit'.

The dehisced fruit of Voacanga schweinfurthii Stapf (x 1¾).

STUDIES ON DRUGS FROM THE PLANT FAMILY APOCYNACEAE

THE GENUS *VOACANGA*

Studies on Drugs from the Plant Family Apocynaceae

The Genus *Voacanga*

by

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C O N T E N T S

PAGE

P A R T I I I

P A R T I V

P A R T V

PAGE

P A R T V I I I

P A R T I X

P A R T X

PART I

INTRODUCTION

From the expedient and empirical remedies of early man, through the ages when rite and symbol were as important as the medicament administered, to the present day with its highly developed chemical and pharmacological techniques, plants have featured in medicine.

Working from the assumption that many plants which have never been subjected to any systematic study may contain principles of possible value in medicine, phytochemistry has become a major theme in research throughout the world. The rediscovery of the ancient Indian drug Rauwolfia has rekindled interest and stimulated a vast research into phytochemistry. During the past decade numerous plants from the same family (Apocynaceae) have been investigated; over two hundred different alkaloids have been isolated from this source[1], including several of pharmacological interest from the African shrub Voacanga africana Stapf. Other species from the genus Voacanga have also been studied and the present work, which adds V. schweinfurthii Stapf to the list, is primarily concerned with the comparison of this species with V. africana.

To date no comprehensive survey has appeared on the genus Voacanga and there follows in Part II a survey of the taxonomy, the chemistry and microscopy of the barks, and the pharmacology of the known alkaloids from this genus.

The genus was first described in 1811[2] and since then over forty species have been named and described but much confusion has

existed over their classification. Several classifications have been made but some of these have dealt only with the species belonging to a particular geographical region. The first comprehensive classification was by Schumann[3] in 1895, which was amended by Stapf[4] in 1902. The most recent classification, which deals most particularly with the African species, was made by Pichon[5] in 1947. Since they were first described in 1894, the two species V.schweinfurthii[6] and V.africana[7] have always appeared as separate species being placed in the same section or subsection of the genus.

The stem bark of V.africana has been extensively studied and shown to contain at least eleven alkaloids[8-17]. These alkaloids were classified[16] according to their ultra-violet spectra into two main types, derivatives of 5-methoxy indole and of 2-acyl indole. One alkaloid, vobtusine, did not fit either of these types, although it was an indole derivative. Some of the alkaloids were also isolated from barks of other species of Voacanga, namely V.thouarsii var. obtusa[9,10], V.dregei[18,19], and V.bracteata[20]. From V.chalotiana[21] was isolated the indole alkaloid, voachalotine, whose ultra-violet spectrum differed from those previously described. No investigation of the constituents of the bark of V.schweinfurthii has been made.

Voacamine[8,11,22-30] has been extensively studied pharmacologically and though some reports were somewhat unfavourable, it appears that this alkaloid has considerable cardiotonic activity and a hypo-

tensive effect due to an action on the autonomic nervous system. Voacamine, in the form of the sulphate, has been used[11] with considerable success in the treatment of chronic cardiac insufficiencies. In common with the other Voacanga alkaloids, it was found to have a much lower toxicity than the cardiac glycosides. Other alkaloids, namely voacorine[13,27,29-32], voachalotine[29,30] and voacaminine[11] showed similar pharmacological properties. The depressant action of voacangine[33,34] on the central nervous system has been described. Other minor activities ascribed to some of the alkaloids were an anti-inflammatory action[33,34], analgesic action [27,35-37], and a local anaesthetic action[36]. Thus although the exact rôle of the Voacanga alkaloids in medicine is as yet unknown, it is clear that they are at least of potential use in therapeutics.

The general structure of Apocynaceous barks has been described by Metcalfe and Chalk[38] and the histology of some barks from certain Apocynaceous genera (Aspidosperma[39-44], Rauwolfia[45-52]) has been given but no detailed report has appeared on the microscopical structure of barks from Voacanga species.

In view of the interest in the potential therapeutic use of the Voacanga alkaloids, the first intention of this work was to examine the alkaloidal composition of the stem bark of V. schweinfurthii, in the hope of isolating some new alkaloid with valuable pharmacological properties. Further, a detailed anatomical study of the plant materials used was undertaken, so that subsequent batches of material could be

authenticated, either in the whole or powdered state. Comparisons were made chemically, anatomically, and taxonomically of this species with the closely related species V.africana. Finally, the chemo-taxonomy of the genus as a whole was considered.

P A R T I I

A REVIEW OF THE GENUS VOACANGA

Folklore

Several species of Voacanga were included in a paper by Braun[53] on the vernacular names and native uses of plants of German East Africa. The names were sometimes applied to more than one species, not always of the same genus, and various tribes often applied the names differently. To the Shambala, 'mwambe' indicated V.lutescens Stapf, Conopharyngia holstii Stapf or Olinia usambarensis Gilg. and 'muambe', V.thouarsii Roem. and Schult. or, again, C.holstii. 'Muambe' was also the name given to Hibiscus micranthus L. by the Swahili, and to V.obtusa K.Schum. by the Bondei[54]. V.obtusa was known by several other names; to the Shambala it was 'kilimboti', to the Yao, 'kiringoti', and to the Swahili, 'kirongasi'. Finally, V.angustifolia K.Schum. was known to the Shambala as 'mbozamakili'.

Several uses were ascribed to these plants, though few were medicinal. The latices of V.lutescens, V.obtusa and V.thouarsii were boiled with latex of Ficus species in the production of bird-lime. The long fibres present in the bark of V.obtusa were used to make hunting-nets and the wood to make knife-sheaths. The concentrated plant sap was used to cement knife-blades in the handles and also in the repairing of baskets. The only direct medicinal use described was that of V.angustifolia, root scrapings of which, when mixed with copra, were applied to promote the healing of wounds and

to alleviate itch.

Rindl and Groenwoud[55] reported that three plants were known by natives of South Africa as the 'quinine tree' ('koorsboom'); Rauwolfia natalensis Sond., Conopharyngia ventricosa Stapf and Voacanga dregei E.Mey. The 'quinine tree' was claimed to have an antifebrile action, to be of use as a blood purifier, and effective in the treatment of measles, urticaria and other rashes.

According to Dalziel[56], the fruit of V.obtusa was used by the Liberians who scattered it over the ground on their rice farms when the grain was ripening, to scare away the wild hog. Also, in Southern Nigeria, the fibres of the bark of V.africana were mixed with fibres of other sources in the weaving of fancy mats. In West Africa, the latex of the same plant had been used as a rubber adulterant and as a filling for carious teeth.

Taxonomy of the genus Voacanga

An examination of the flora of Madagascar at the beginning of the nineteenth century led to the naming, by Thouars, of several new genera, one of which was Voacanga. The year, according to Index Kewensis[57], was 1806; De Wildeman and Durand[58] quoted 1804, but attempts to trace the original article uncovered one of 1811 by Thouars[2] on new genera of Madagascar. Since the page number and title are the same as those given in Index Kewensis, this may in fact be the original. Thouars gave a brief description of the genus and referred it to the family Apocineae.

The plant described was a large-leaved tree, bearing characteristic flowers with a calyx of five involute sepals surrounding a contorted, funnel-shaped corolla which, in turn, enclosed five sessile anthers in the mouth of the tube, and two short-styled carpels on a fleshy receptacle. However the large, round fruits with warty, spotted walls seem to have been the most striking feature, as from these was derived the name of the new genus: 'Voa' fructus, 'Acanga' pintade; the 'painted fruit'.

This particular plant was again mentioned by Römer and Schultes[59] in 1819, who, in memory of the founder of the genus, added the specific name, V.thouarsii. However it was not until 1837 that the first detailed description of a member of the genus

was made by Meyer[60], when he described, among many South African plants, a tree of about thirty to forty feet high found in the region of Port Natal, naming it V.dregei.

In 1842 a new genus, Piptolaena, was described by Harvey[61]. He stated, however, that his material for examination was limited to one withered flower and it was clear that he was not fully convinced of the significance of his results. He identified his material with V.dregei, as described by Meyer, and having no specimen of the fruit, he used Meyer's description of the fruit of V.dregei in his description of Piptolaena. The presence of a deciduous calyx lined internally by several rows of fleshy scales, and certain properties of the stigma, convinced him that these specimens did not belong to the genus Voacanga but represented a new genus or, alternatively, a second species of the genus Orchipeda, established by Blume[62] in 1826 from work on the flora of the Dutch Indies, the first species described being named O.foetida.

In 1876, Bentham and Hooker[63] referred the genus Voacanga, admittedly with hesitation, to Tabernaemontana but the confused situation was clarified by a review of the genus by Rolfe[64], in 1883. Due to the lack of detail in the original description by Thouars and the scanty herbarium material available, the actual existence of the genus Voacanga was in doubt but with the adequate new materials from Madagascar, Rolfe was convinced he had samples of the plant originally described by Thouars, and he approved Meyer's description and nomination of V.dregei. He agreed

that Meyer's plant was the same as Harvey's Piptolaena dregei, but as he retained it in the genus Voacanga, the latter name took precedence. He stated further that V.dregei was identical with the two species Annularia natalense and Cyclostigma natalense which had been described by Hochst in 1841 and 1844. Another point of agreement with Harvey was that he realised the closeness of these plants to Orchipeda, and claimed these genera to be identical. Thus O.foetida and O.grandifolia became V.foetida and V.grandifolia but two other species, O.sumatrana and O.gracilipes, were not thought to belong to the genus. Pootia grandifolia Miq. was also included in the genus as V.grandifolia.

With the genus now more firmly established, new species were described. In 1884, Rolfe[65] named a species from the Philipine Islands, V.cumingiana, and in 1894 three other species were named. Stapf[6] described a plant found in Central Africa near the Yuru River by Schweinfurth and it was thus named V.schweinfurthii, and another, found at the Bagrao River and at Old Calabar, V.bracteata. In the same year Stapf[7] also reported on the Apocynaceous flora collected by the Sierra Leone Boundary Commission, and named a widely distributed species, V.africana.

It is relevant to this work to consider the two species V.schweinfurthii and V.africana in some detail. V.schweinfurthii[6] was described as a bush with slender branches bearing oblanceolate or oblong leaves whose laminae were gradually drawn out into a petiole or contracted abruptly to give a sessile form. The apex was acute or subacuminate and

the surface was glabrous. The inflorescence was in the form of a pseudo-umbel bearing bunches of four to six flowers with thin peduncles and deciduous oblong bracts. The calyx was tubular-campanulate with round to ovate lobes. The yellow corolla had tubes and lobes of equal lengths, the lobes being obovate, abrupt and shortly acuminate. The fruit was oval. The dimensions given were that the bush was 5 m. high with leaves 15.3 to 20.3 cm. long and 4.5 to 6.3 cm. broad and the pedicel 1.3 to 2.5 cm. long. Both the calyx, and corolla tubes measured 1 to 1.5 cm., the corolla lobes being nearly 2 cm. long.

The description of V.africana[7] was very similar. It was also a bush or low tree. The leaves were equally variable since they were described as obovate to oblanceolate, or occasionally oblong to ovate. They were more or less petiolate, or sessile and semi-amplexicaul. The apex of the leaf varied from shortly acuminate to either acute or obtuse, and the surface was glabrous or somewhat hairy on the under surface, particularly on the midrib and main veins. The inflorescence was in twice-branched corymbs with varying lengths of peduncle. In the calyx, the tube was the same length as the obtuse, somewhat reflexed lobes. The corolla was white, the tube widening in the middle, and the lobes obovate or oblong. The dimensions given for these structures were that the bush was 3 to 5 m. high, leaves 6 to 25 cm. long and 4.5 to 12 cm. broad, the petioles 1 to 1.5 cm. long, the peduncles 5 to 15 cm. and the pedicels 1 to 2 cm. long, the calyx was 8 to 10 mm. long, and the

corolla tube was 1 cm. and the lobes 1.5 cm. long.

Other details of the flower and fruit were given which were not present in the description of V.schweinfurthii. The anthers were 5 mm. long, sagittate and almost sessile. The two free carpels contained numerous ovules; usually only one carpel developed to give a single fruit which was a berry with a thick pericarp. The fruit was 4 to 5 cm. long and the seeds 7.5 mm. long.

It would be very difficult to distinguish the two species from these descriptions apart from the difference in colour of the corolla and possibly the size of the flowers which in V.schweinfurthii appear to be somewhat larger than those of V.africana. The sizes of other characters were very similar, although the range of values in V.africana tended to be wider, probably due to the greater number of plants examined.

Included in Stapf's list of sources of V.africana was one of the plants from Angola collected by Welwitsch[66] (No. 5978) in 1856. It was described under the name V.angolensis, but the title V.africana was also present in italics. Stapf appeared to have accepted this as genuine V.africana though it was a somewhat smaller bush than that described by him, and the flowers were sulphur-yellow instead of white. Also mentioned were two other of Welwitsch's plants, one (No. 5979) with flowers larger than those of any specimen of V.africana Stapf had seen, and another (No. 5980) with the same large flowers but with leaves having more num-

erous veins. Stapf concluded that these were but varieties of V.africana, yet the characters outlined above indicate that the materials may have been V.schweinfurthii. However V.angolensis later appeared as a separate species in Index Kewensis[67].

In an article on the flora of German East Africa, Schumann[68] listed four species of Voacanga: V.schweinfurthii and three new species, V.boehmii, V.angustifolia and V.dichotoma, although the latter two were doubtful species.

Elsewhere Schumann[3] outlined a classification of the genus. It was included in a list of six genera under the subdivisions Plumieroideae, Plumierae and Tabernaemontaninae of the family Apocynaceae. The genus Voacanga was divided into two sections, 1. Orchipeda, and 2. Streptostephanus.

In plants of the first section the corolla tube was straight. The section was further subdivided according to whether the anthers were enclosed in the corolla or projecting beyond it. The first subdivision contained V.foetida and a new species, V.papuana, both of which were originally described under the genus Orchipeda. No mention was made of V.grandifolia, also a former member of this genus. The other subdivision contained a single species, V.thouarsii.

In the second section, where the corolla tube was twisted, the characters of the anthers were again used as a method of subdivision. In the subdivision with projecting anthers, there was one group where

the corolla was very large and had a fringed edge, and a second with a smaller corolla and no fringed edge. In the first group was a new species, V.obtusa, collected by Schweinfurth in the same district as the original V.schweinfurthii, which belonged to the second group.

In the subdivision where the anthers were enclosed or only slightly projecting, two groups were again described, the first with a glabrous corolla, and the tips of the calyx lobes recurved, the second having a corolla which was softly pubescent on the back surface, and whose calyx was quite straight. The first group contained two new species, V.diplochlamys and V.glabra, both from the Cameroons. In the other group were V.boehmii and a new species, V.puberula.

Two surprising omissions from this list were V.dregei, which Schumann mentioned only to say that he had no knowledge of it, and V.africana, the widespread species. However it was interesting to note that the volume of Index Kewensis[69] covering the year 1895 listed V.glabra as being identical to V.africana. Thus, accepting this point, we find that V.africana and V.schweinfurthii were placed in the same section of the genus, but in different subdivisions. Later, in 1897, Schumann[70] described a plant he named as a variety of V.schweinfurthii, V.schweinfurthii var. parviflora.

In 1899 an article was published by De Wildeman and Durand[58] describing two plants found in the region of Leopoldville in the Belgian Congo by Dewèvre and Laurent. These plants were considered to be

V. schweinfurthii, the description being very similar to the original by Stapf but being given in greater detail and confirming the dimensions of calyx and corolla.

In 1902 a comprehensive article on the genus was published by Stapf[4] describing each member in detail, and outlining a further method of classification (Table 1). In this classification V. schweinfurthii and V. africana were differentiated by several points. Firstly the inflorescence of V. africana was a many-flowered corymb, and that of V. schweinfurthii a pseudo-umbel with only four to six flowers. Further, the calyx of V. schweinfurthii was about twice the length of that of V. africana, and the corolla yellow, not white. V. angolensis was differentiated from V. schweinfurthii only in being somewhat pubescent on the young branches and leaves.

Stapf also stated that the plant figured in the article by De Wildeman and Durand was in fact a pubescent form of V. africana, although no mention was made in the accompanying description of any such pubescence. The explanation of this apparent contradiction would seem to be that the article was the result of the examination of two different plants, and the description, which was certainly in agreement with that of genuine V. schweinfurthii, fitted exactly one of the plants (that of Dewèvre), whereas the illustration was of the other (that of Laurent) which had pubescent small branches and leaves, that is a pubescent form of V. africana. It may be of significance that this particular

Table 1 : Classification of the genus Voacanga, according to Stapf.

Flowers large (limb 2 in. or more in diam.), white, in few-flowered, racemiform or umbelliform, very robust inflorescences; calyx wide-tubular; lobes short, early circumscissile; disc cupular, exceeding the ovary.

Calyx 7-8 lin. long; corolla-tube as long as the calyx; leaves frequently subacute or obtuse (but not broadly rounded) at the apex ..... 1. V.obtusa.
Calyx 5½-6 (rarely to 7) lin. long; corolla-tube shortly exserted from the calyx; leaves usually broadly rounded at the apex ..... 2. V.thouarsii.

Flowers large (limb 2 in. in diam.) and yellow, or middle-sized to small and variously coloured, in few to many-flowered, racemiform to corymbose inflorescences, never so robust as in the preceding species; calyx more or less campanulate, lobes as long as the tube or nearly so, not or very tardily circumscissile at the base; disc annular, rarely obscure.

Bracts caducous; corolla-limb rotate; lobes always longer than the tube.

Inflorescences shortly racemiform or umbelliform, few-flowered; calyx 7-9 lin. long; corolla-limb 2 in. in diam. or almost so.

Young branches, leaves and inflorescences (except the flowers) more or less pubescent, at least when young ..... 3. V.angolensis.
Quite glabrous ..... 4. V.schweinfurthii.

Inflorescences corymbose, many-flowered; calyx 6-3 lin. long; corolla-limb 2 to less than 1 in. in diam.

Corolla-tube twisted, 6-4 lin. long.

Calyx 5-6 lin. long; corolla deep yellow (where the colour is known).

Leaves sessile or subsessile, broad ovate or elliptic ..... 5. V.spectabilis.
Leaves distinctly petioled, more or less lanceolate to ovate ..... 6. V.puberula.

Table 1 - continued.

Calyx $3\frac{1}{2}$-$4\frac{1}{2}$ lin. long; corolla not deep yellow.

Corolla greenish-yellow; leaves sessile, rarely subsessile..... 7. V.lutescens.
Corolla white; leaves petioled (petiole up to 6 lin. long), rarely subsessile ..... 8. V.africana.

Corolla-tube straight, $\frac{3}{4}$ lin. long. 9. V.chalotiana

Bracts persistent, more or less crowded at the base of the inflorescence; corolla-limb rotate or reflexed; lobes shorter than the tube.

Corolla-lobes almost as long as the tube, not darker than the tube; limb rotate.

Calyx 7-8 lin. long, sparingly glandular inside; lobes oblong; corolla-tube 7-8 lin. long .....10. V.psilocalyx.
Calyx 4-5 lin. long, multiglandular inside; lobes rotundate; corolla-tube 5-$5\frac{1}{2}$ lin. long .....11. V.zenkeri.

Corolla-lobes half as long as the tube or less, flat and dark when dry; limb tightly reflexed.

Leaves (at least below and when young) and inflorescences finely pubescent; calyx 5-6 lin. long, with a zone of glands above the base; lobes reflexed, rather longer than the tube; corolla greenish .....12. V.diplochlamys.
Leaves and inflorescences glabrous; calyx 4-$4\frac{1}{2}$ lin. long, with a ring of glands at the very base, lobes usually porrect, as long as or shorter than the tube; corolla-limb violet-brown .....13. V.bracteata.

character was the only difference between V.schweinfurthii and V.angolensis. It would appear that Stapf was still not quite convinced that Dewèvre's plant was V.schweinfurthii as the only definite source given by him for this species was the original one of Schweinfurth.

Under the description of V.africana, Stapf included V.glabra and V.schweinfurthii var. parviflora, both first described by Schumann, and another plant from the Cameroons, at first thought to be V.schweinfurthii, was now also included as V.africana, although the corolla tube was almost 1.3 cm. long, that is somewhat longer than Stapf expected in V.africana, but just acceptable according to his classification.

It was thus quite clear that although Stapf described a system wherein V.schweinfurthii and V.africana were distinctly separated, a great deal of confusion still existed.

The article also added several new species - V.spectabilis, V.lutescens, V.psilocalyx, V.zenkeri and V.caudiflora. V.boehmii, V.dichotoma and V.densiflora were listed as doubtful species, the latter in fact bearing no description. Also, a plant at first named as a separate species, V.klainii, was listed under V.puberula. Finally, Stapf advocated that the Malayan species having the corolla tube much prolonged beyond the stamens should be replaced in the genus Orchipeda, rather than being considered as a section of Voacanga as suggested by Rolfe and Schumann.

In 1908 and 1909 another two species from the Philipines were

named, V.plumeriaefolia[71] and V.globosa[72], and yet another in 1912, V.megacarpa[73]. Then in 1913[74] and 1914[75] Wernham described five new Nigerian species; V.magnifolia; V.obanensis, somewhat similar to V.zenkeri but with larger leaves, longer petioles and long corolla tube and lobes; V.talbotii, similar to V.diplochlamys but even less hairy and with smaller flowers; V.eketensis, similar to V.puberula but much less hairy; and V.glaberrima, described as a very distinct species, closest to V.caudiflora but with much larger and broader leaves, and broader corolla lobes.

Between 1914 and 1928 only five new species were named. One was from the Belgian Congo, V.bequaerti[76]; one from New Guinea, V.versteegii[77]; one from Borneo, V.havilandii[78], and two from the Philipines, V.dolichocalyx and V.latifolia[79].

Another classification of the members of the genus from West Africa was published by Hutchinson and Dalziel[80] in 1931. They reduced the total number of species to six, placing many of those more recently described under earlier specific names. Under V.africana were listed four of Wernham's Nigerian species, V.eketensis, V.glaberrima, V.magnifolia and V.talbotii; and another two of his species, V.obanensis and V.psilocalyx, were included under V.zenkeri. The other four species included in the classification were V.obtusa, V.caudiflora, V.diplochlamys and V.bracteata. There was no mention of V.schweinfurthii.

In 1935, one of the Orchipeda species, O.gracilipes, thought by

Rolfe[64] not to be eligible for inclusion in the genus Voacanga, was renamed V.gracilipes by Markgraf[81].

The most recent survey of the genus was in 1947 by Pichon[5], and although the title of the article was 'Les Voacanga d'Afrique', he in fact discussed the whole genus. He based his classification quite differently from previous workers, claiming that the factors they relied upon, for example the presence or absence of a petiole, the type of inflorescence, the size of the bracts, the colour of the corolla and length of the corolla lobes, were too variable. He found that the total length of the calyx and particularly the length of the tube of the expanded corolla, were specially suitable quantitative standards. Also used were the form of the leaf base and the presence or absence of an indumentum at the base of the stamens, although he felt that these two factors had little real systematic value.

This classification (Table 2) automatically reduced the total number of species. He applauded Hutchinson and Dalziel's move in grouping the Nigerian species and claimed it was now necessary to go further and demote V.zenkeri, V.lutescens, V.puberula, V.angolensis, V.obtusa and V.dregei from the status of distinct species. Although he had not examined them himself, he expected, from their descriptions, that V.diplochlamys and V.bequaerti would have to be dealt with similarly. One species, V.dichotoma, was excluded altogether.

He disagreed with Stapf[4] that those species classed under the name Orchipeda should be a separate genus, as they had only one character

Table 2 : Classification of the genus Voacanga, according to Pichon.

Sect. 1. Streptostephanus K.Schum.

Calyx tardily circumscissile, with 40-150 glandular scales. Corolla-tube 5.5-18 mm. long, stamens above the middle, rotated to the right in the staminal region; indumentum formed below the stamens, at least on the staminal veins. Disc free or shortly adnate at the base. Carpels free or nearly free.

Bracts persistent. Calyx lobes straight at the tip.

Largest leaves 6 cm. long. Calyx 4-6 mm. long. Corolla-tube 7.7-9.5 mm. long ..... V.micrantha.

Largest leaves 9-21 cm. long. Calyx 7-17 mm. long. Corolla-tube 9-18 mm. long.

Calyx 7-10.5 mm. long. Corolla-tube 9-11 mm. long ..... V.bracteata.
Calyx 11.5-17 mm. long. Corolla-tube 14-18 mm. long ..... V.psilocalyx.

Bracts normally caducous. Calyx lobes more or less spread out or recurved at the tip.

Calyx 5.5-10 mm. long. Corolla-tube 5.5-9 mm. long ..... V.africana.

Leaves drawn out at the base.

Vegetative parts glabrous ..... var. glabra.
Branches and young petioles pubescent ..... var. typica.

Leaves auriculate and amplexicaul at the base.

Vegetative parts glabrous ..... var. auriculata.
Branches and young petioles pubescent ..... var. lutescens.

Calyx 9.5-16 mm. long. Corolla-tube 12.5-17 mm. long ..... V.schweinfurthii.

Vegetative parts glabrous ..... var. typica.
Branches and young petioles pubescent ..... var. puberula.

Table 2 - continued.

Sect. 2. Chalotia nov.

Calyx tardily circumscissile at the base, with 20-35 glandular scales. Corolla-tube 4.5-5.5 mm. long, stamens above the middle, straight, indumentum formed below the stamens. Disc wholly adnate to the ovary, thus scarcely distinct. Carpels partly joined at the base. ..... V.chalotiana.

Sect. 3. Orchipeda (Bl.) K.Sch.

Calyx very early circumscissile (from the bud), with 200-750 glandular scales. Corolla-tube 7.5-60 mm. long, stamens arising at various points, straight or rotated to the right in the staminal region; no indumentum developed below the stamens. Disc free or shortly adnate at the base. Carpels free or nearly free.

Brevitubae - Corolla-tube 7.5-22 mm. long, stamens above the middle ..... V.thouarsii.

Disc shorter than the ovary ..... var. dregei.
Disc longer than the ovary and hiding it completely ..... var. obtusa.

Also, V.grandifolia, V.papuana, V.irosinensis, V.versteegii and V.havilandii.

Longitubae - Corolla-tube 30-60 mm. long, stamens below the middle.

V.globosa, V.dolichocalyx, V.megacarpa, V.gracilipes, V.latifolia and V.plumeriaefolia.

which was quite distinct from the other species, that is prolongation of the corolla tube beyond the stamens; they were not even a distinct geographical group. Thus he followed Schumann's[3] example of dividing the genus into sections, adding one new one, Chalotia, to the original two, Streptostephanus and Orchipeda. He also transferred V.obtusa from Streptostephanus to Orchipeda. Chalotia contained only one species, V.chalotiana from the Congo. Orchipeda had twelve species, one from Africa and the others from Australasia, and was divided into equal groups according to the length of the corolla tube. The subsection Brevitubae included V.thouarsii, V.grandifolia, V.papuana and V.irosinensis Elmer and other two species, not studied by Pichon, V.versteegii and V.havilandii. Further, V.thouarsii was subdivided into the varieties dregei and obtusa. The other subsection, Longitubae, contained V.globosa, V.dolichocalyx, V.megacarpa, V.gracilipes, V.latifolia and V.plumeriaefolia, although Pichon himself had only studied the first of these. The two species with which this work is primarily concerned were both placed in the section Streptostephanus which was subdivided initially according to whether the bracts were persistent or not. Three species were named with persistent bracts, V.micrantha, a species from Gabon described for the first time by Pichon, V.bracteata and V.psilocalyx. V.bracteata was taken to include the former species V.zenkeri and V.obanensis. Only two species were described with deciduous bracts, V.africana and V.schweinfurthii, which are discussed in

detail below. Three other species, V.caudiflora, V.densiflora and V.boehmii, although not examined, were also placed in the section Streptostephanus.

The species V.africana and V.schweinfurthii were separated on two points only, the sizes of calyx and of corolla tube. The range of the length of calyx for V.africana was 5.5 to 10 mm., and for V.schweinfurthii, 9.5 to 16 mm. The size of the corolla tube in V.africana was 5.5 to 9 mm. long, and in V.schweinfurthii, 12.5 to 17 mm. It is thus seen that as a very slight overlap of the values of calyx length exists between the two species, it is only on one specific point, the length of the corolla tube, that they can be reliably differentiated.

Included under these two species were several plants previously considered as species in their own right. This gave rise to the introduction of several varieties within these species.

V.africana was divided according to whether the lamina was somewhat decurrent at the base, or auriculate and semi-amplexicaul. Each subdivision was then further subdivided according to whether the vegetative parts were quite glabrous or had some pubescence, particularly on branches and petioles. The glabrous and pubescent types with decurrent bases were var. glabra and var. typica respectively; and with auriculate bases, var. auriculata and var. lutescens respectively. Included under V.africana var. glabra were the former species V.glabra, V.schweinfurthii var. parviflora, V.magnifolia and V.talbotii; under var. typica was

V.amplexicaulis; under var. lutescens was V.lutescens.

Only two varieties of V.schweinfurthii were described, the first with glabrous vegetative parts, var. typica, which included the former species V.glaberrima and V.bequaerti, and the second with pubescence on branches and petioles, var. puberula, which included V.diplochlamys, V.puberula, V.angolensis, V.spectabilis, V.klainii and V.eketensis. The inclusion of V.diplochlamys as a variety of V.schweinfurthii was attacked, however, by Brenan[82], who considered it quite distinct from this species.

No mention was made in Pichon's article of V.lemosii, another African species described in 1944 by Philipson[83], and since 1947 one more species, from Borneo, has been described by Markgraf[84], V.borneensis.

From this survey it is thus seen that from 1894 until the present, V.africana and V.schweinfurthii have been continuously recognised as two quite distinct species of the genus Voacanga, in spite of the great amount of confusion and controversy and the several methods of classification which have existed. Nevertheless, according to the most recent report[5], the difference between them is very small, and at different times various particular plants have been attributed to both species.

Chemistry of the Voacanga alkaloids

Occurrence of alkaloids in the genus Voacanga.

The first investigation of the chemical constituents of a plant of the genus Voacanga was reported by Greshoff[85] in 1890, as part of a systematic investigation of the medicinal and poisonous plants of Java. He examined V.foetida and reported the presence of an alkaloid, with a sharply bitter taste, in the bark (0.15 per cent) and pericarp (0.25 per cent) but absent from the seeds and latex. The only property given was its ready solubility in ether. Greshoff also mentioned the presence of a brownish alkaloidal substance, with a blue fluorescence, which made isolation of the first alkaloid difficult. This interfering substance was also reported present in the closely related plant, Ochrosia acuminata.

More precise investigations of the chemistry of the genus were not carried out until 1955 when two research groups reported almost simultaneously. Quevauviller et al,[86] showed that total extracts of the barks of V.africana Stapf and V.thouarsii Roem. and Schult. var. obtusa (K. Schum.) Pichon had marked hypotensive properties, and a series of papers followed on the isolation and characterisation of individual alkaloids from this extract. La Barre and Gillo[8] reported on the cardiotonic action of two alkaloids isolated from V.africana.

Janot and Goutarel[9] isolated voacamine and vobtusine from the

roots and trunk bark of V.africana. The alkaloids were extracted with acetified alcohol, precipitated by concentration, basified with ammonia and extracted with ether, then separated by adsorption chromatography on alumina.

The two alkaloids isolated similarly, by La Barre and Gillo[8], from the root of V.africana were named voacangine and voacanginine. Voacanginine was subsequently stated to be identical to voacamine[12,22], the latter name being retained by precedence[23].

Janot and Goutarel[10] later confirmed the presence in their material of voacangine. The isolation of another alkaloid was also reported, this base having all the characteristics of voacamine except that its melting-point was much higher. A similar occurrence had been noted with the Vinca alkaloid, vincamine[87]. The presence of this fourth alkaloid was confirmed by La Barre et al.[11], who named it voacaminine.

In the following year both teams isolated a fifth alkaloid. Goutarel and Janot[12] isolated voacorine and La Barre and Gillo[13], voacaline. The close similarity of these two alkaloids was noted and several workers considered them identical[31,88]. Also, Percheron[88] doubted the existence of voacaminine and suggested it might be a mixture of voacamine and voacorine. However this suggestion does not seem to have been accepted generally[89].

Since 1956, V.africana has been further examined by various workers and several other alkaloids have been described. Using the counter-

current technique, Renner[14] isolated from the trunk bark voacamidine (an isomer of voacamine) and voacristine. In 1958, Stauffacher and Seebeck[15] eluted the alkaloidal fractions voacangine, voacamine, voacamine/vobtusine, and, finally, voacorine from an alumina chromatogram of the crude base of V.africana. Using counter-current distribution between chloroform and phosphate-citrate buffer, at pH 2.9, followed by further chromatography on alumina, they also obtained an alkaloid which they named voacangarine. However, Renner and Prins[90] claimed that this alkaloid was identical to their voacristine.

Also in 1958, Rao[16] described the presence of two further alkaloids, voacafrine and voacafricine, in the trunk bark of V.africana. Renner[17] separated the alkaloids vobasine and voacryptine.

Thus V.africana appears to contain at least eleven alkaloids, namely, voacamine, vobtusine, voacangine, voacaminine, voacorine, voacamidine, voacristine, voacafrine, voacafricine, vobasine and voacryptine. Although most of the reports do not give details of yields of individual alkaloids, it would seem reasonable to say, from the readiness of their isolation, that the major alkaloids of this plant are voacamine, vobtusine, voacangine and voacorine.

V.thouarsii var. obtusa was also examined by Janot et al.[9,10,86] and found to contain several of the alkaloids isolated from V.africana, namely voacamine, vobtusine and voacangine.

V.dregei, which according to Pichon[5] is not a separate species

but a variety of V. thouarsii, was examined in 1932 by Rindl and Groenwoud[55] who could isolate no crystalline material from the bark. However, in 1958, Schuler et al.[18] found vobtusine as the sole alkaloid of the root bark, and, accompanied by greater quantities of voacangine, in the stem bark. The two alkaloids were separated on the basis of the insolubility of vobtusine in ethanol. Shortly after this, Neuss and Cone[19] reported the presence of a new alkaloid, dregamine, in the trunk bark of this plant but they were unable to find vobtusine or voacangine. Such complete conflict of observations throws doubt on the authenticity of the materials examined.

In 1957, Janot et al.[20] described the isolation of voacamine, vobtusine, voacangine and voacorine from V. bracteata.

The only other species of Voacanga in which alkaloids have been reported is V. chalotiana Pierre ex Stapf. In 1960, Pecher et al.[21] isolated a new alkaloid, voachalotine, from the trunk and root bark of this tree. The presence of one other alkaloid in this genus has been reported, that of papuanine. This substance was named in a review article of Apocynaceous alkaloids by Raffauf and Flagler[1] which gave its melting-point and empirical formula and referred it to the genus Voacanga but not to any particular species. From the name it is likely to be from V. papuana K. Schum.

Occurrence of Voacanga alkaloids in other genera.

Voacamine and voacangine have been reported in two species of Stemmadenia[91]; S.donellsmithii (Rose) Woodson and S.galleotiana (A.Rich.) Miers. and three species of Tabernaemontana[92]; T.oppositifolia, T.psychotrifolia and T.australis. The presence of these two alkaloids has also been claimed in the genus Ervatamia[1], however Gorman et al.[92] reported their absence from E.coronaria and E.divaricata. Voacangine has also been reported in the genus Rejoua[1] and it has been isolated from the root of Tabernanthe iboga[93].

Dregamine was reported present in small amounts in Ervatamia coronaria[92], and vobtusine was isolated from various species of Callichilia; C.subsessilis[94], C.barteri and C.stenosepala[95]. Finally, vobasine was one of three alkaloids isolated from Peschiera affinis (Meull.-Arg.) Miers.[37].

It is interesting to note, from the chemo-taxonomic point of view, that all these genera are very closely related taxonomically within the family Apocynaceae.

Structure of the Voacanga alkaloids.

The Voacanga alkaloids may be classified according to their ultra-violet spectra, as first suggested by Rao[16]. Alkaloids of the largest group have maxima in the regions of 225, 287 and 295 mμ and are derivatives of 5-methoxy indole. Alkaloids of the other main

group have maxima in the regions of 240 and 315 mμ and are 2-acyl indole derivatives. Two alkaloids, vobtusine and voachalotine, have ultra-violet spectra quite different from these types and from each other.

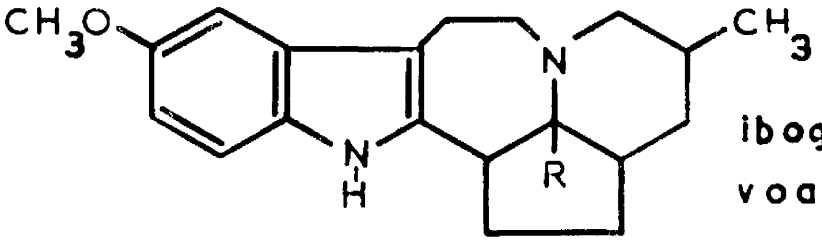
5-methoxy indoles.

1. Voacangine. Although it was La Barre and Gillo[8] who first described this alkaloid, they merely commented on the similarity of its ultra-violet spectrum to that of voacamine (voacanginine). Janot and Goutarel[10] stated it was clearly a 5-methoxy indole derivative and suggested the formula $C_{22}H_{28}O_3N_2$, with two methoxyl groups, one of which was probably in the form of a methyl ester. This was borne out by the infra-red spectrum which indicated an -NH- group, a tri-substituted benzene derivative and a -COOR group.

Saponification of voacangine[96] yielded an acid, voacangic acid, and decarboxylation of this yielded a base identical in physical characteristics to ibogaine, an alkaloid of Tabernanthe iboga. Voacangine was thus shown to be a carbomethoxy ibogaine. Later voacangine was also found to be present in T.iboga[93]. Although the absolute configuration of ibogaine was not at that time known, a structure had been suggested, and a possible position of the carbomethoxyl group in voacangine was also postulated (Fig. 1).

In 1957, several papers were published on the related structures of ibogaine and voacangine. Firstly, Taylor[97] showed that the formulae

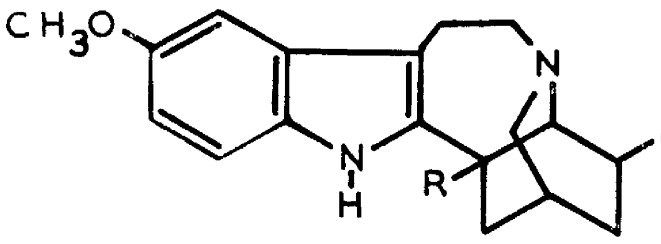
suggested for these substances were untenable as a Kuhn-Roth determination produced propionic acid, showing that the C-alkyl function



ibogaine, R=H,
voacangine, R=$COOCH_3$.

Fig. 1

postulated as C-methyl must, in fact, be C-ethyl. This was confirmed by others[98,99]. Studies of the results of selenium dehydrogenation indicated the structure shown (Fig. 2a) for ibogaine. Taylor also stated that in voacangine the most probable position of the carbomethoxyl group was at position ten. This was confirmed by Percheron et al.[100] who prepared the reduction product voacangol, by the action



a. ibogaine, R=H,
b. voacangine, R=$COOCH_3$.

Fig. 2

of lithium aluminium hydride on voacangine, and on heating this product at 210° formaldehyde was liberated to give ibogaine. It seemed this could only take place by tautomerism of voacangol and the formation of a hydrogen bond between the alcohol group and the nitrogen of the indole nucleus. Thus voacangine had the formula shown in Fig. 2b. This structure was further confirmed by a detailed report on the structures of several Iboga alkaloids, including voacangine, by Bartlett et al.[101].

2. Voacristine. This alkaloid, first isolated by Renner[14], was accorded two possible formulae, $C_{45}H_{58}O_8N_4$ and $C_{23}H_{30}O_4N_2$. Later, from new analytical figures, Renner and Prins[90] decided the alkaloid was a mono-indole derivative having the formula $C_{22}H_{28}O_4N_2$, with two methoxyl groups and one $-C.CH_3$ group. They also stated that from a comparison of their physical constants, voacristine was identical to voacangarine, reported by Stauffacher and Seebeck[15].

Stauffacher and Seebeck had described voacangarine as a 5-methoxy indole derivative similar to voacangine, whose ultra-violet spectrum was almost identical to that of voacangarine, and attributed to it the above formula. Because of this obvious structural similarity, similar degradative studies were carried out on voacangarine to those employed on voacangine, namely saponification followed by decarboxylation, resulting in decarbomethoxy voacangarine. The nature of the product was confirmed by its infra-red spectrum, which differed from

that of voacangarine in the absence of a carbonyl ester band; and the equivalent weight, determined by potentiometric titration, which indicated the loss of a carbomethoxyl group.

The infra-red spectra of both voacangarine and decarbomethoxy voacangarine showed the presence of a hydroxyl group, so decarbomethoxy voacangarine was treated with p-toluene sulphonyl chloride in pyridine solution, to form the tosylate. This proved to be quaternary in nature, similar in type to the tosylate of iso-rubijervine[102]. Thus the inference was made that, as in iso-rubijervine, the hydroxyl group of decarbomethoxy voacangarine was sufficiently close to a tertiary nitrogen to form a linkage. Thus if voacangarine were hydroxy voacangine, the hydroxyl group would seem to be on the terminal carbon of the ethyl side-chain. Evidence to support this was obtained by reduction of decarbomethoxy voacangarine tosylate with sodium in ethanol, producing a mixture of four isomers, one of which was characterised as ibogaine. The position of the carbomethoxyl group in voacangarine was established by treatment with lithium aluminium hydride, which converted the group to a primary alcohol, followed by dry hydrogen chloride in acetone, which linked the carbon of the side-group to the nitrogen of the indole nucleus in an ~~acetonyl~~ isopropylidene compound.

These conclusions agreed with the observation of Renner and Prins[90] that decarbomethylation of voacristine produced iboxygaine, an hydroxy ibogaine[103]. However, although they agreed with Stauffacher

and Seebeck that the hydroxyl group present was on the ethyl side-chain of the parent voacangine, Renner and Prins, having shown the presence

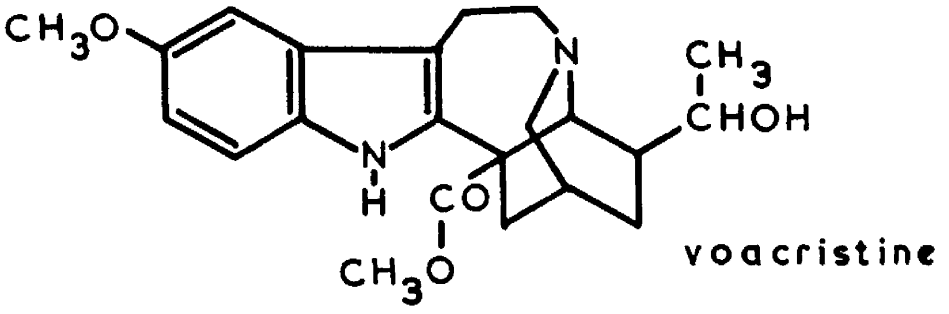


Fig. 3

of one -C.CH_3 group in the molecule, concluded it was on the penultimate, rather than the terminal carbon atom. Percheron[88] supported this indirectly by establishing the presence of a terminal methyl group, by Kuhn-Roth oxidation, in iboxygaine. The structure of voacristine was thus that shown in Fig. 3.

3. Voacryptine. Renner[17,104] accorded this alkaloid the formula $C_{22}H_{26}O_4N_2$, with two methoxyl groups and one -C.CH_3 group. The infra-red spectrum indicated an ester and a ketone group, Renner concluding that voacryptine had the structure of an oxovoacangine. As the carbonyl group present was capable of forming an oxime, he concluded it was either an aliphatic or alicyclic keto-group. Kuhn-Roth oxidation

yielded only acetic acid, thus establishing the presence of a side methyl group. His conclusion was that the ethyl side-chain of voacangine was replaced by $-COCH_3$ in voacryptine, which established the position of the carbonyl group. Confirmation of this was the production of ibogaine from voacryptine by simultaneous decarboxymethylation and reduction. Also, mild reduction of voacryptine produced dihydrovoacryptine, and acetylation of this derivative produced an acetate identical to the acetate prepared by direct acetylation of authentic voacristine. Thus the hydroxyl group of voacristine and the carbonyl group of voacryptine were in the same position. Voacryptine thus had the formula shown in Fig. 4.

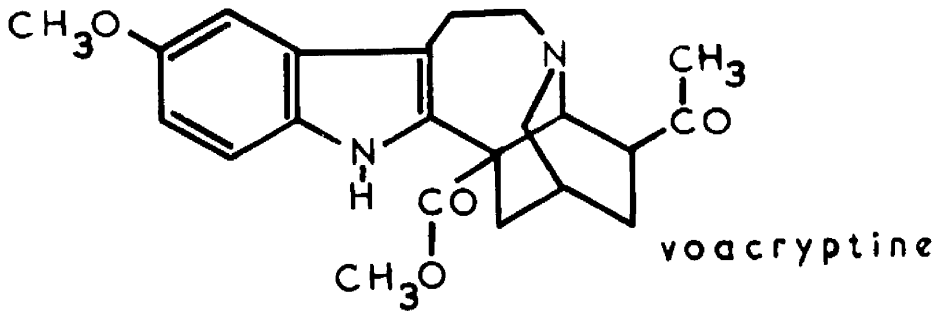


Fig. 4

4. Voacamine. In the initial description of voacamine, Janot and Goutarel[9] proposed, from analytical data, the formula $C_{42}H_{52}O_5N_4$, with three methoxyl groups and, from the infra-red spectrum, two ester groups.

Thus they considered the base to be a double molecule, that is a di-indole derivative. They admitted[10] it might be a single indole derivative with the formula $C_{22}H_{26}O_3N_2$, but from the presence of two ester bands and the percentage of methoxyl groups present, they felt a double molecule was more likely.

Later analyses[105] showed that the formula $C_{45}H_{56}O_6N_4$ was more appropriate than that previously suggested. The presence of three methoxyl groups was confirmed, also of one $-N.CH_3$ group and four labile hydrogen atoms. Thus voacamine had a double indole structure, one indole nucleus being 5-methoxy substituted, the other having two methoxyl groups corresponding to two methyl ester groups (from the infra-red spectrum).

The close similarity of part of the voacamine molecule to voacangine was noted. It was shown that decarboxymethylation, as previously carried out with voacangine to yield ibogaine, caused the loss of two methoxyl groups to yield a di-acid, from which one carboxyl group was readily eliminated by the action of acid, the other probably forming a lactone. The ultra-violet spectrum was unchanged showing that the remaining methoxyl group was in the five position on an indole nucleus. Also, one of the basic tertiary nitrogen atoms had a pK value very close to that of voacangine. This information, plus the production, on potassium fusion, of 3-methyl-5-ethyl pyridine (which contained the above mentioned tertiary nitrogen), confirmed that part of the

molecule of voacamine was identical to voacangine. Pyrolysis of voacamine in vacuo produced, stoichometrically, one molecule of carbon dioxide and one molecule of trimethylamine. This indicated that the second basic nitrogen was tertiary also.

The report may be summarised as follows:-

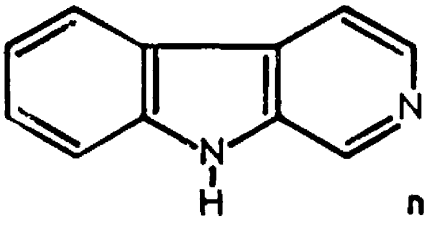
(i) the two non-basic nitrogen atoms were in indole groups,

(ii) the two basic nitrogen atoms were both tertiary, one weakly basic, existing in a piperidine group (in a similar position to the tertiary nitrogen in voacangine), and the other more strongly basic and bearing a methyl group.

(iii) of the six oxygen atoms, one was from an alcohol function, one from a methoxyl group (from the 5-methoxy indole nucleus) and the other four from two methyl ester groups. One of these ester groups was from the voacangine portion of the molecule.

Percheron[88] agreed with the findings just described. His major additional contribution to the results already published was identification of the products of selenium dehydrogenation. These consisted mainly of two fractions, the first neutral in character, being a mixture of substances containing the 5-methoxy indole nucleus, and the second which was strongly basic, being a mixture of 3-ethyl-4- methyl pyridine (c.f. the results of potassium fusion) and nor$\pm$harman (Fig. 5). An $-N.CH_3$ group was known to be present and it had been established that the methyl group was not attached to the nitrogen of an indole

group and the other nitrogen of the voacangine portion of the molecule was already tri-substituted, so it had to be on the nitrogen of the nor-harman portion. Thus voacamine was stated to consist basically of voacangine linked to N-methyl tetrahydro nor-harman.



nor-harman

Fig. 5

Percheron postulated that these two indole derivatives were connected by two linkages. He pointed out that the ultra-violet spectrum of voacamine was that of a 5-methoxy indole derivative and also that the log ϵ values of the maxima indicated the summation of two 5-methoxy indole chromophores. Therefore the nor-harman moiety of the molecule had a spectrum corresponding to 5-methoxy indole. This could be explained by assuming an ether linkage between C_5 of the nor-harman portion and the ethyl side-chain of the voacangine portion. This was confirmed by a Kuhn-Roth oxidation which yielded only acetic acid, showing that a methyl side-chain was now present but no ethyl side-chain. The position of the second link was surmised from the production of 3-ethyl 4-methyl pyridine on selenium dehydrogenation, the methyl substitution arising from cleavage of the link.

In addition to the postulated di-indole structure, there remained unplaced 10 C, 17 H and 2 O, of which it was known there was one $-COOCH_3$ function (from decarboxymethylation) and one $-C.CH_3$ function (from spectral data). Percheron produced the working formula shown in Fig. 6, in agreement with all experimental results.

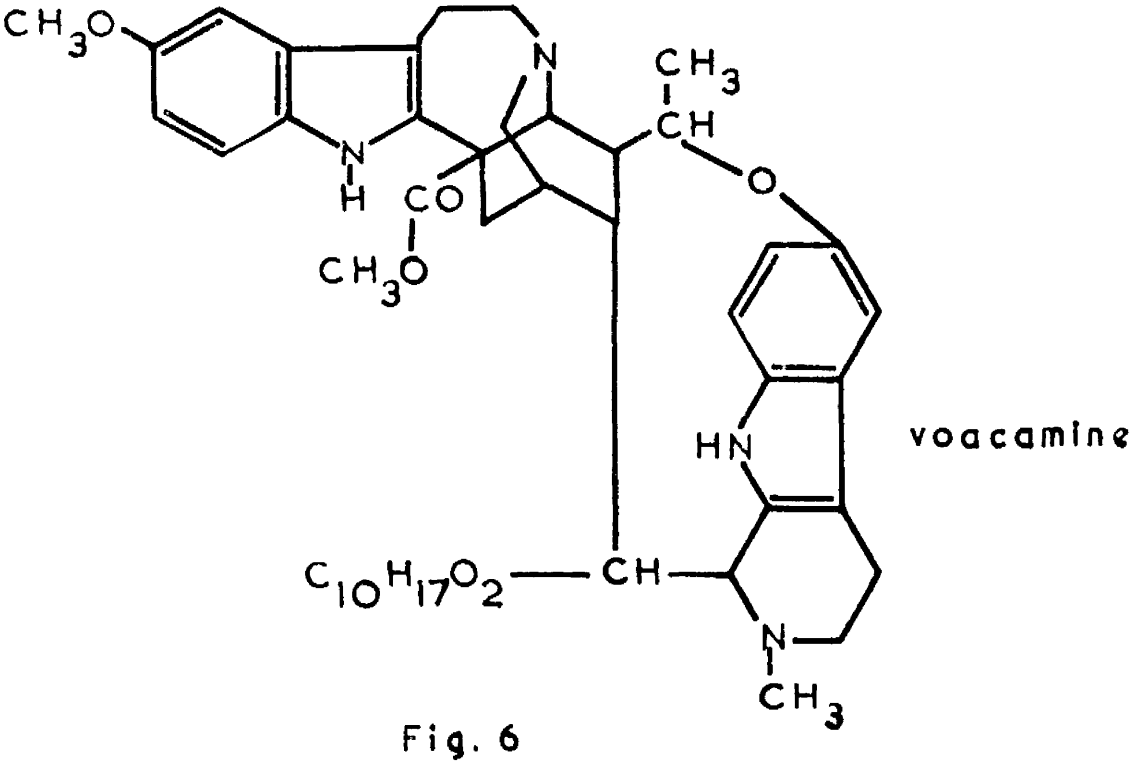


Fig. 6

Confirmation of the presence of voacangine within the voacamine molecule was presented by Winkler[106]. He showed that voacamine, voacangine and voacorine could be separated from one another efficiently by thin-layer chromatography on alumina, thus furnishing a useful method of following degradative studies of these alkaloids. Following the method of Bertho et al.[107], who studied the products of acid-

cleavage of geissospermine, Winkler heated voacamine with 3N hydrochloric acid which split the molecule into two basic portions which were separated on alumina. One of these products was isolated and shown to be voacangine, the other, with a shorter Rf value, was presumed to be the other part of the voacamine molecule but no details of its structure were given.

5. Voacorine. This alkaloid was described by Goutarel and Janot[12] who attributed to it the formula $C_{41}H_{50}O_6N_4$, with three methoxyl groups and four $-N.CH_3$ groups. Of the four nitrogen atoms present, two were in indole groups and the fact that potentiometry revealed only one dissociation constant, compared with two in voacamine, could be explained by the remaining two nitrogens having equal basicity. Voacorine differed from voacamine in having three additional $-N.CH_3$ groups and only a single band at 5.83 μ compared to strong bands in the region 5.8 to 6 μ in the case of voacamine.

In spite of these differences, voacamine and voacorine were very similar[20]. They were both di-indole derivatives, their ultra-violet spectra were almost superimposable and they behaved similarly on pyrolysis in vacuo, on saponification and on fusion with potassium. Of the two non-indole nitrogen atoms both were tertiary, one being part of a piperidine ring, the other bearing a methyl group. From revised analytical figures, the most probable formula was $C_{45}H_{54}O_7N_4$,

though the possibility of $C_{46}H_{56}O_7N_4$ could not be excluded.

Winkler[106,108] examined the products of acid-cleavage of voacorine. Chromatography showed four spots, besides some residual voacorine, of which two were much more strongly marked than the others. Of these two the one with the higher Rf value was voacristine but no details of the other were given. Winkler concluded that voacristine formed part of the voacorine molecule in a similar manner to the voacangine-voacamine relationship.

6. Voacaminine. This alkaloid[10] was found to be identical to voacamine in all its physical constants except its melting-point. Although at first accorded the formula $C_{22}H_{26}O_3N_2$, it was later[12,13] considered as a double molecule, like voacamine, with the formula $C_{44}H_{50}O_6N_4$ (which was also the formula given in the same paper[13] for voacamine). No further information has been published on the structure of this alkaloid.

7. Voacamidine. Voacamidine was considered by Renner[14] to be an isomer of voacamine, formula $C_{45}H_{56}O_6N_4$, with three methoxyl groups. It was capable of forming an acetyl derivative on treatment with pyridine and acetic anhydride, and saponification yielded an acid which was readily decarboxylated to yield a substance with only one methoxyl group. It was assumed the other two methoxyl groups were present as methyl esters which were hydrolysed by the alkali. Thus voacamidine,

on degradation, followed a similar pattern to voacamine.

2-acyl indoles.

1. Voacafrine. Rao[16] accorded this alkaloid the formula $C_{22}H_{26}O_4N_2$ and reported its ultra-violet and infra-red spectra, the former being quite different from those alkaloids already described. Renner[17] pointed out its similarity to that of 2-phenyl indole[109] and suggested that voacafrine and the other alkaloids of this group might be derived from this substance. However, Neuss and Cone[19] named the group as 2-acyl indole derivatives, which proved more accurate.

2. Voacafricine. This was reported by Rao[16] also, and again the only structural information given was a proposed formula, $C_{22}H_{24-26}O_4N_2$.

3. Vobasine. Renner[17] suggested the formula $C_{21}H_{24}O_3N_2$, with one methoxyl group, one $-N.CH_3$ group and one $-C.CH_3$ group. This was in agreement with the infra-red spectrum which also indicated the presence of a 1,2-di-substituted phenyl derivative and an ester group. He concluded that the methoxyl group was part of the methyl ester group. The presence of an $-N.CH_3$ group, and the re-arrangement on saponification, were two points in common between vobasine and the more strongly basic portion of the voacamine molecule and Renner suggested that monomeric vobasine might be a stepping-stone in the biogenesis of dimeric voacamine.

In studying the structure of vobasine further, Renner[110] first established the presence of an acyl group by formation of a 2,4-dinitrophenyl hydrazone whose ultra-violet and infra-red spectra indicated a strongly conjugated keto-imine. Controlled reduction of the alkaloid yielded an alcohol, the hydroxyl group being confirmed by acetylation, whose ultra-violet spectrum was that of a 2,3-dialkyl indole. The presence of two double bonds in vobasine was established by hydrogenation, one belonging to the carbonyl group α to the indole nucleus. It was found, further, that the $-C.CH_3$ side-group was converted to an ethyl group during hydrogenation, establishing the presence in the original alkaloid of an ethylidene side-chain. Renner hydrogenated this ethylidene group without affecting the carbonyl group and examination of the products, separated by counter-current distribution,

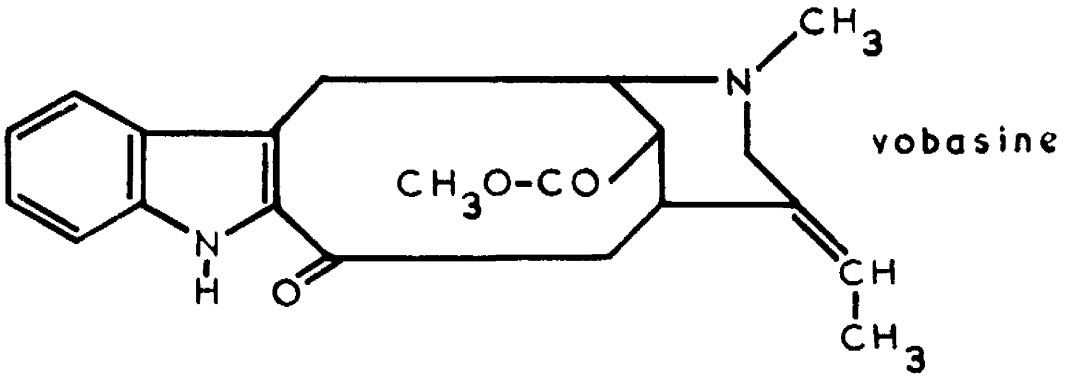


Fig. 7

showed them to be dregamine and tabernaemontanine, the latter being

an alkaloid present in Tabernaemontana coronaria[92,111]. It was thus established that vobasine, dregamine and tabernaemontanine were all 2-acyl indole derivatives, the last two being stereoisomers produced by hydrogenation of the ethylidene group of vobasine. The structure (Fig. 7) postulated for vobasine by Renner and Prins[112], was based primarily on the production of a 2-acyl-3-vinyl indole derivative on Hofmann degradation of vobasine methiodide. This structure was confirmed by Cava et al.[113] from the study of the nuclear magnetic resonance spectra of the alkaloid and its reduction products.

4. Dregamine. When first described, Neuss and Cone[19] proposed the formula $C_{21}H_{28}O_3N_2$ for dregamine, but since its structure (Fig. 8) has been determined from its relation to vobasine[110], it is now known to be $C_{21}H_{26}O_3N_2$.

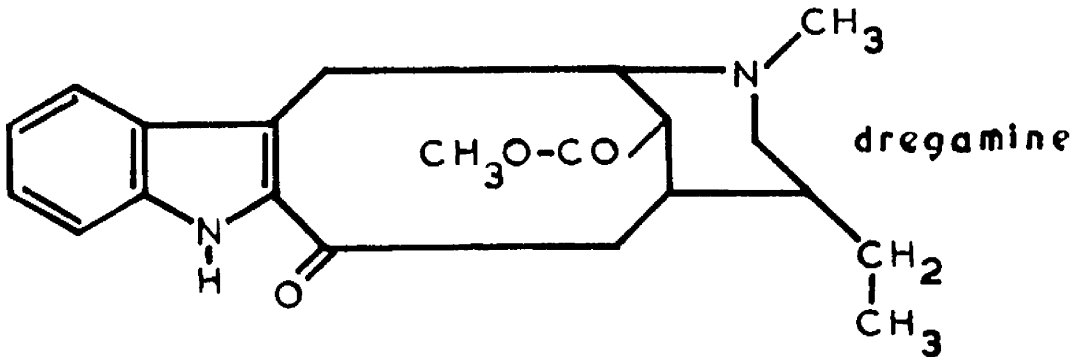


Fig. 8

Other alkaloids.

1. Vobtusine. The ultra-violet spectrum of vobtusine[9] with four maxima, placed it in a group of its own. Due to its tenacity for water it was found difficult to establish the formula of the alkaloid. Initially it was accorded the formula $C_{21}H_{26}O_3N_2$, with one methoxyl group, then it was considered a double molecule with the formula $C_{45}H_{54}O_8N_4$[12], and, later, Schuler et al.[18] assigned to it $C_{42}H_{50}O_7N_4$, incorporating one molecule of water. This was accepted by Goutarel et al.[94], although they expressed it without the molecule of water, that is $C_{42}H_{48}O_6N_4$.

From its spectral data[9,18], vobtusine was clearly aromatic in nature showing a linkage very similar to that of three unsaturated rings, probably with an -NH-CO- linkage and a conjugated ester with a double bond. It contained two methoxyl groups but no $-C.CH_3$ groups. There was some controversy as to the presence[94] or absence[18] of an $-N.CH_3$ group. It was thought probable[9] that the two non-indole nitrogen atoms had the same basicity, as only one dissociation constant was measurable. Selenium dehydrogenation yielded quinoline[18].

The only other information on the structure of vobtusine[94] described its similarity to callichiline, both alkaloids being present in Callichilia subsessilis. The ultra-violet spectrum of callichiline had three of the four maxima exhibited by the spectrum of vobtusine, and the infra-red spectra differed only in the details of the finger-

print region. From this evidence it was tentatively suggested that the monomeric callichiline ($C_{22}H_{24}O_3N_2$, with one methoxyl group but no $-N.CH_3$ group) might be part of the dimeric vobtusine molecule.

2. Voachalotine. From its ultra-violet spectrum[21], which is very similar to that of voacangine, it might be thought that this alkaloid should be placed in the 5-methoxy indole group. However, its structure has proved quite different from those yet described and thus is placed in a group of its own.

The formula $C_{22}H_{26}O_3N_2$ was attributed to the alkaloid which contained one methoxyl group, one $-N.CH_3$ group and one non-phenolic hydroxyl group. The ultra-violet spectrum indicated the presence of a carbomethoxyl group and the infra-red spectrum an ortho substituted benzene ring. The production of acetaldehyde on ozonolysis suggested the presence of an ethylidene side-chain and this was partly confirmed by the production of acetic acid (no propionic acid, c.f. voacangine) on Kuhn-Roth oxidation, indicating a methyl side-chain.

An unusual feature of voachalotine[114] was that the nitrogen of the $-N.CH_3$ group was the indole nitrogen. This was shown by the nuclear magnetic resonance spectrum, which gave a peak similar to that of known N-methyl indole derivatives. The infra-red spectrum of voachalotine itself was indefinite as to the presence or absence of an -NH- group, but in the prepared derivatives O-acetyl voachalotine,

O-O-diacetyl voachalotine and anhydrovoachalotine, it was clearly absent in contrast to its presence in voacangine. Final confirmation was the closeness between the ultra-violet spectra of voachalotine and the above derivatives to that of 1,2,3,4, tetrahydrocarbazole.

It was also suggested that the carbomethoxyl group might be in the same position relative to the indole nitrogen as in voacangine. This would explain the normal CO band at 5.75 μ in voachalotine, compared to the modified band at 5.84 μ in voacangine. The latter case may be explained by hydrogen bonding between the carbomethoxyl group and the hydrogen of the indole nitrogen. This obviously could not occur in voachalotine. This also explained why reduction of the carbomethoxyl group to the alcohol, followed by the addition of dry hydrogen chloride in acetone did not produce a cyclic ketal, as described for voacristine.

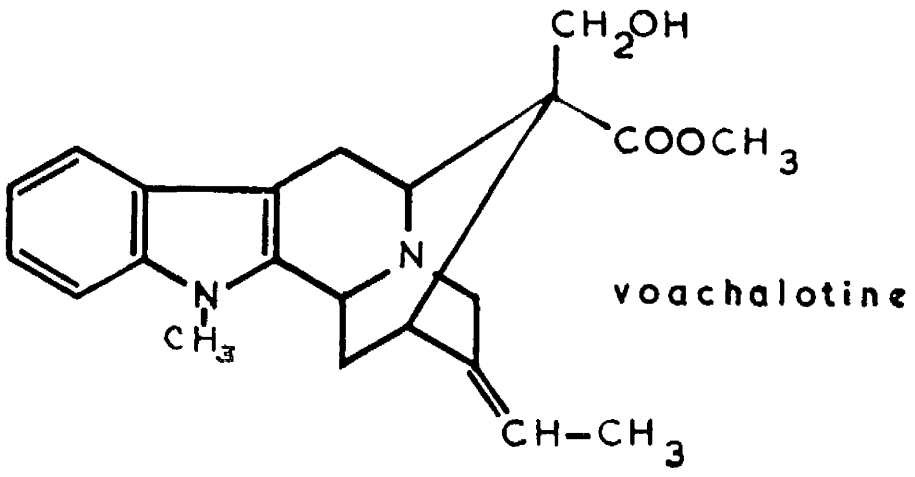


Fig. 9

Another report[115] commenced by adding several points to the characters of voachalotine already mentioned. The molecule was said

to be pentacyclic with a primary alcohol group and a basic tertiary amine function. It was then stated that the alkaloid had been shown to be the hydroxyl ester of vincamajine (one of the Vinca alkaloids whose structure had just been elucidated[116]), by the preparation of several derivatives and comparison of these with authentic samples. The structure of voachalotine is shown in Fig. 9 and it is seen to differ more from voacangine than was originally thought. It was also noted that the alkaloid was the N-methyl derivative of nor-macusine A, the tertiary base corresponding to a quaternary alkaloid, macusine A, isolated from Strychnos toxifera[117,118]. This structure agreed with that reported later by Janot et al.[119].

Pharmacology of the Voacanga alkaloids

Toxicity.

The toxicities of the alkaloids of Voacanga and their derivatives were examined primarily by determination of the L.D.100 on intravenous injection in the guinea-pig. Voacamine[8] was found to have an L.D.100 of 118 mg. per kg. and its derivatives, voacamine sulphate[22] and voacamine dimethylaminoethyl ester[30], 348 mg. per kg. and 130 mg. per kg. respectively. Intravenous injection in the mouse[25] of the sulphate and camphosulphonate of voacamine gave values of 21.5 mg. per kg. and 46.2 mg. per kg. respectively for the L.D.50. The dose of voacamine was readily excreted from the guinea-pig within two or three days[11] and no cumulative effects were observed in young dogs receiving 1 mg. daily for twenty-five days[11], or in rats receiving subcutaneous injections over longer periods[26]. Doses of up to and including the lethal dose failed to produce emesis in pigeons (c.f. digitoxin)[22]. Oral administration in rats[28] and mice[26] confirmed its low toxicity.

With regard to the toxicity of the other Voacanga alkaloids in the guinea-pig, the L.D.100 of voacangine[8] was found to be 100 mg. per kg., of voacorine[13] 313 mg. per kg. and of voachalotine[29], which was considerably more toxic than the other alkaloids, 58 mg. per kg. The most toxic Voacanga alkaloid, however, was vobasine[37] which caused death in the anaesthetised cat with as little as 10 mg. per kg.

It is thus seen that, with the possible exception of vobasine, the alkaloids of Voacanga are markedly less toxic than the cardiac glycosides, digoxin, for example, having an L.D.100 in the guinea-pig of 2.5 mg. per kg.[8]. Also, in contrast to the cardiac glycosides, death resulted from respiratory depression causing asphyxia, the heart continuing to beat for a short time after cessation of breathing[25,33,37].

Cardiotonic action.

The first investigation of the pharmacology of the Voacanga alkaloids[86] revealed that total alkaloidal extracts of the trunk bark and root bark of V.africana, and of the trunk bark of V.thouarsii var. obtusa, had marked cardiotonic effects when administered intravenously to chloralosed dogs. This action has since been confirmed by various workers[8,28,33].

Subsequently the cardiotonic activity of the individual alkaloids was described. Voacamine (voacanginine)[8,11,22-24,30], in doses of 0.1 mg. per kg., exhibited a positive inotropic effect on the isolated rabbit auricles. The equivalent dose of digitoxin required to produce a similar response was 0.03 mg. per kg., but voacamine being much less toxic than the glycoside, had a therapeutic index of about ten times that of digitoxin. The water-soluble sulphate of voacamine showed similar quantitative effects to digitoxin and possessed a therapeutic index of about eighty times that of the glycoside. Voacamine campho-

sulphonate[25,26] was shown to have four times the cardiotonic activity of the sulphate on the isolated rabbit auricles, it was also less toxic. Voacamine dimethylaminoethyl ester[29,30] also had a cardiotonic effect on the isolated rabbit auricles, quantitatively similar to that of voacamine sulphate but differing qualitatively in being more resistant to repeated washing. This was presumably due to a union of the substance with cardiac muscle, demonstrated by inactivation with protein (horse serum).

The effect of voacamine sulphate on the electrocardiogram of the chloralosed cat[11] was similar to that of digitalis, although requiring much larger doses, commencing with inversion of the T wave (100 mg.), then with larger amounts (300 to 600 mg.) bradycardia developed, and with very high doses (700 to 800 mg.) disturbances in the auriculo-ventricular conduction were observed, followed by nodal arrhythmnias accompanied by extrasystolic beats, with doses of 900 to 1000 mg.

Voacamine camphosulphonate[26] showed a clear cardiotonic action in the anaesthetised (urethane) rabbit but was ineffective in the chloralosed dog.

Voacangine[8,22] was reported to have a tonic effect on the isolated rabbit auricles similar, qualitatively and quantitatively, to voacamine.

Voacorine (voacaline)[13,27,29-32] also had a tonic effect on the isolated rabbit auricles similar to that of voacamine, though was more persistent, but on the isolated rabbit heart it showed a greater effect

than voacamine.

Like voacamine camphosulphonate, voacorine showed a cardiotonic action in the anaesthetised (urethane) rabbit but the positive inotropic effect was accompanied by a negative chronotropic effect. Also like the voacamine derivative, voacorine produced no cardiotonic effect in the chloralosed dog.

Voachalotine[29,30] showed a cardiotonic effect on the isolated rabbit aurioles, generally somewhat more marked than that of voacamine, which persisted even after extensive washing due to its affinity for cardiac muscle, demonstrated by its inactivation by horse serum. It also had a cardiotonic effect in the anaesthetised (barbiturate) cat.

Cardiotonic properties were also accorded to voacaminine[11].

It thus seems well-established that these alkaloids have a significant tonic action on the isolated rabbit auricles and a cardiotonic action in the rabbit and cat, but not in the dog. However, in spite of these results some workers have doubted the efficacy of these substances as cardiotonic drugs. On the isolated auricles of the rabbit and guinea-pig, Maffii et al.[28] found the inotropic action of voacamine sulphate to be slight and inconstant, partly due to the instability of the alkaloidal salt solution. On the frog heart, small doses of voacamine produced some inconstant cardiotonic effect but higher doses produced a constant negative effect leading eventually to cessation of the heart. It was found that voacangine had little or

no cardiotonic action, and this was confirmed by Blanpin et al.[36]. Maffii et al. emphasised their inability to demonstrate any positive inotropic action in the whole animal.

Vogel and Uebel[33,34] were also less impressed with the slight inotropic action of the Voacanga alkaloids, in comparison to the cardiac glycosides, than with their neuroplegic action, primarily attributed to voacangine.

Effects on the autonomic nervous system.

Total alkaloidal extracts of Voacanga[86] were shown to antagonise the lowering of blood pressure observed with the injection of acetyl choline and with stimulation of the vagus nerve. The extracts were also shown to reduce the rise in blood pressure resulting from the injection of adrenaline and occlusion of the carotid arteries. Thus it would appear that the mixture of alkaloids exhibited both mild parasympatholytic and sympatholytic properties. Individual alkaloids were then examined.

Potentiation of the fall in blood pressure produced by peripheral stimulation of the vagus and injection of acetyl choline was seen with voacamine[26,27] and the hypotension produced by injection of the alkaloid itself was abolished by atropine. The alkaloid was also shown to exert a spasmogenic action by causing the contraction of the small intestine of both the rat and the dog, similar to the effect seen with

acetyl choline. Voacamine therefore exhibited some parasympathomimetic actions. The rise in blood pressure induced by administration of adrenaline or occlusion of the carotids was attenuated by voacamine. This could have been a sympatholytic action or simply the resolution of the two opposing effects.

Voacorine[27] had a similar action in diminishing the rise in blood pressure resulting from administration of adrenaline or carotid artery occlusion as was observed with voacamine but this substance was also able to antagonise the hypotensive effects after administration of acetyl choline or vagal stimulation, that is an atropine-like action. Voacorine stimulated smooth muscle, as did voacamine, but the effect was not blocked by atropine suggesting that the spasmogenic action was not of a parasympathetic origin.

On the intestine *in situ*, voacangine[36] produced a marked increase in peristalsis accompanied by increased gastric secretion. That this was a parasympathomimetic effect was shown by its inhibition by atropine. Potentiation of the raising of blood pressure by adrenaline and occlusion of the carotids, in the chloralosed dog, indicated voacangine also possessed sympathomimetic properties.

Although large doses of vobasine hydrochloride[37] produced lachrymation and mydriasis, it appeared to have little effect on the autonomic nervous system.

Effects on the central nervous system.

As already mentioned, lethal doses of the Voacanga alkaloids caused death by respiratory depression. This could result from either depression of the central nervous system or by a peripheral action in constricting the smooth muscle of the bronchioles.

A close examination of the central effects of the Voacanga alkaloids[120], and of ibogaine, revealed that only voacangine and ibogaine had any significant depressant properties, in that they were able to afford some protection against electrically-induced seizures. Voacangine was also able to potentiate barbiturate-induced hypnosis and to depress the analeptic effect of leptazol, amphetamine and strychnine. It was, however, claimed elsewhere[36] that voacangine had little inhibitory effect on central nervous stimulants and indeed potentiated the convulsant action of strychnine. Both voacangine and ibogaine lowered rectal temperature, voacangine being the more active.

Vobasine[37] was also found to have a weak central depressant action in mice, including a slight antipyretic action.

From the inability to influence artificially-induced convulsions and the comparatively slight effect of the alkaloids on respiration, it was thought[33] their action was more likely to be at some subcortical level rather than a direct effect on the cerebral cortex.

Vascular effects.

Total alkaloidal extracts of Voacanga[86] produced hypotension in the dog. This was probably due primarily to voacamine which was reported[26] to produce hypotension in the rabbit, dog and cat, despite peripheral vaso-constriction. However, Maffii et al.[28] claimed the alkaloid to have no effect on the blood pressure of these animals and La Barre and Gillo[22] noted a marked and sustained hypertensive effect in the cat. Voacorine[27] showed no clear effect on blood pressure, though again causing peripheral vaso-constriction. In contrast to voacamine[26], it caused a marked increase in coronary flow similar to that produced by the digitalis glycosides. Voachalotine[29] had a mild hypertensive effect in the cat, and voacangine[36] had a hypotensive effect in this animal. A similar reaction was produced by small doses of voacangine in the dog but higher doses reversed the effect causing marked hypertension.

It was concluded that the total vascular effect of the alkaloids was a resolution of several actions; thus with voacamine the general hypotensive effect was broughtabout in spite of the opposition of the potentially hypertensive effects of vasoconstriction and cardiotonicity, probably through the autonomic nervous system[26].

Miscellaneous effects.

Voacangine[33,34] showed an anti-inflammatory action similar to

that of reserpine and chlorpromazine. All three substances inhibited the formation of oedema in the paw of the rat but the duration of the action of voacangine was shorter than that of reserpine and also it increased the action of thyroxine on metabolism whereas reserpine inhibited it. It was concluded that the action of voacangine was more similar to that of chlorpromazine.

An analgesic effect was shown by voacamine sulphate[35] which afforded considerable protection to pain evoked by burning, in mice. A dose of 10 mg. per kg. had an action nearly equivalent to that of 1.25 mg. per kg. of morphine hydrochloride. In a second experiment, depending on the release of ascorbic acid from the suprarenal capsule in the rabbit on painful electrical stimulation of a paw, some alleviation was obtained by intravenous administration of high doses of the sulphate, but morphine was much more effective. Voacorine[27], voacangine[36] and vobasine[37] all had a slight analgesic action but not voacamine dimethylaminoethyl ester[29].

Voacangine hydrochloride[36] proved to have a local anaesthetic action on both the cornea of the rabbit and the skin of the guinea-pig, four times as effective as procaine in the latter case, but it also produced irritation. Voacamine and voacorine[27] showed no local anaesthetic action.

Voacamine[26] and vobasine[37] had no diuretic action.

Voacamine[26] did not affect blood coagulation (c.f. digitalis[121]).

Vobtusine[11,28] appeared inactive pharmacologically.

Therapeutic applications.

The value of administering mixtures of voacamine sulphate and strophanthin has been examined[122] and it was found that potentiation of the positive inotropic effect on the isolated rabbit auricle was achieved, and the effect achieved more rapidly. There was, simultaneously, a marked reduction in heart rate, even when the amount of strophanthin present was less than that normally producing bradycardia. A similar action was found in the chloralosed cat. It was concluded that it might be useful to treat those conditions where cardiac insufficiencies were accompanied by tachycardia with a mixture of cardiac alkaloid and cardiac glycoside.

The only Voacanga alkaloid which has been the subject of clinical trials is voacamine[11], as the sulphate. The drug was tested on twenty-one patients all suffering from chronic cardiac insufficiency of common origin, e.g. arteriosclerosis, arterial hypertension and rheumatism. The pattern of the reaction was the same in all cases. Administration was primarily by intravenous injection, though was sometimes followed by oral therapy. Amelioration of the symptoms was normally apparent within forty-eight hours, diuresis being markedly increased, with a corresponding reduction in oedema, and, if present, pulmonary stasis and dyspnoea disappeared. Venous pressure was usually reduced but

little effect was observed on cardiac frequency. However it was considered that much more experience was required before exact details of posology and therapeutic application could be derived.

Summary.

It would seem from the work of La Barre et al. and Quevauviller and Blanpin that the cardiotonic properties of at least voacamine sulphate are well established. Its mild toxicity, in comparison to the cardiac glycosides, is particularly important and this aspect appears not to be emphasised in those reports, particularly of Maffii et al., which find the cardiotonic properties less impressive. The clinical trials, although somewhat limited in extent, certainly appear very promising.

The neuroplegic action of voacangine, stressed by the more recent studies, may also prove to be of value.

In conclusion, it appears that the Voacanga alkaloids may prove to be of considerable therapeutic value but their exact role is not yet clear.

Microscopy of the stem bark of Apocynaceous plants

Little detailed information is available on the microscopical structure of the stem barks of plants of the family Apocynaceae, but their general structure was described by Metcalfe and Chalk[38].

The cork frequently consisted of alternate layers of thick and thin-walled cells giving a characteristic stratified appearance; and the cortex and phloem often contained sclereids, occurring either singly or in groups; but the most characteristic features were the presence of pericyclic fibres and laticiferous tissue. The fibres, referred to as 'white fibres', were described as having unlignified, striated walls with constricted and enlarged portions along their lengths, and occurring either singly or in groups. The laticiferous tissue was always present and might occur in the cortex, pericycle, phloem, or, occasionally, medullary rays.

The types of medullary rays occurring in Apocynaceous plants were given in some detail as they showed considerable variation. One group of six genera, including Voacanga, had rays which were markedly heterogeneous, seen in tangential longitudinal sections to consist of uniseriate 'wide and tall upright cells' and multiseriate small cells, there frequently being more than one multiseriate portion per ray.

Crystals were common in unlignified tissues and had been observed in cork cells.

Detailed anatomical studies have been made on the stem bark of several species of Aspidosperma[39-44]. The walls of the cork cells varied from being unlignified or only slightly lignified to being heavily lignified, but did not show any stratification. Sclereids occurred in the phelloderm, either in groups or in a more or less continuous band; in the cortex, either in groups or bands; and in the outer phloem, with the exception of one species, in groups. Only in A.ulei, were fibres reported in the cortical region and these occurred singly, embedded in groups of sclereids. These fibres had thick, unlignified or only slightly lignified walls, showing concentric striations in transverse section. Lignified fibres occurred, either singly or in groups, in the phloem of all species. Latex canals were present in the cortices of most barks, being isodiametric, tangentially or axially elongated, according to the species. The medullary rays were one to three cells wide and four to forty-five cells deep, the cells occasionally being lignified when associated with sclereid groups. No mention of a heterogeneous structure was made. In all species, prismatic crystals of calcium oxalate occurred in association with some of the sclereid groups and as a crystal sheath round the fibres.

Thus although these barks of the genus Aspidosperma fitted to some extent the general picture of Apocynaceous barks given by Metcalfe and Chalk, they were atypical in the absence of pericyclic fibres and

in the absence of laticiferous tissue from some species.

The histological structures of the roots, rhizomes and, in fewer cases, aerial stems of several species of Rauwolfia have been described[45-52], and the barks of the rhizomes and aerial stems proved to have the characters typical of Apocynaceous barks.

Although in some of the species examined the cork was in the form of a homogeneous band of cells, more often it consisted of alternate bands of small cells and larger cells, the former being unlignified and the latter lignified, giving a stratified appearance. Sclereids were present in all the species described, although the amount and distribution varied considerably. Most commonly they occurred in the phloem, particularly in the outer phloem, usually as fairly small groups but occasionally as bands, and they were frequently present in the cortex and phelloderm.

All the species examined contained highly refractive, unlignified or only very slightly lignified pericyclic fibres which were particularly abundant in thin bark, and laticiferous tissue, present usually in the cortex and outer phloem, occurring either as circular or oval latex cells or ducts, or as more highly developed tubes. Most of the species had heterogeneous medullary rays, appearing in tangential longitudinal section as oval groups of small cells with upper and lower uniseriate extensions of larger cells. Usually these extensions consisted of only one to four cells, but in two species,

R.ligustrina and R.vomitoria, they were sufficiently long to link up with other groups of small cells, as described by Metcalfe and Chalk for the genus Voacanga.

Prismatic crystals of calcium oxalate were present ir the parenchymatous tissue of all the species described, being most abundant in the phloem where they occurred in vertical rows of numerous crystals.

Thus these barks from plants of the genus Rauwolfia fitted very closely to the general description of Apocynaceous barks by Metcalfe and Chalk.

P A R T I I I

SOURCES OF PLANT MATERIALS

Voacanga schweinfurthii Stapf.

The material used in the examination of the chemistry and anatomy of this species was the stem bark gathered in forests near Kitwe, Northern Rhodesia. The samples were collected and authenticated, by reference to the herbarium of the Royal Botanic Gardens, Kew, by D. B. Fanshawe, Division of Forest Ecology, Kitwe.

The authentication was by comparison of the floral parts of the plants from which the bark was collected with those of the type specimen of the species, that is the plant collected by Schweinfurth. The herbarium at Kew contains no samples of stem bark of this species.

Repeated attempts were made to obtain further samples of this plant from several other sources but all were unsuccessful.

Voacanga africana Stapf.

The materials used in the examination of this species were from several sources. Samples of stem bark collected in French Guinea, in Nigeria and in the Congo were supplied by Professor M.-M. Janot, Faculté de Pharmacie, Paris, Dr. M. B. Patel, University of Ife, and Professor J. Pecher, Université Libre de Bruxelles, respectively.

All samples had been dried prior to their supply.

Details concerning the origin of the various samples of bark are given in Table 3.

Table 3 : Sources of plant materials.

Species	Batch No.	Source	Supplier	Receiving Date
Voacanga schweinfurthii Stapf	VS/1	N. Rhodesia	D. B. Fanshawe	February, 1958
	VS/2	N. Rhodesia	D. B. Fanshawe	April, 1959
	VS/3	N. Rhodesia	D. B. Fanshawe	August, 1962
Voacanga africana Stapf	VA/1	Fr. Guinea	M. M. Janot	February, 1960
	VA/2	Fr. Guinea	M. M. Janot	February, 1960
	VA/3	Nigeria	M. B. Patel	September, 1960
	VA/4	Congo	J. Pecher	November, 1961
	VA/5	Nigeria	M. B. Patel	April, 1962

P A R T I V

CHEMISTRY OF *VOACANGA SCHWEINFURTHII*

Preliminary Investigation

Alkaloids are most commonly extracted from plant materials by either an alcohol, under neutral or acid conditions, or an organic solvent, under alkaline conditions. Small-scale trial extractions were carried out on the powdered bark using as solvents (a) 90 per cent ethanol, (b) chloroform and (c) benzene. Neutral ethanol was used in the first extraction, but the two others were carried out in the presence of solution of ammonia. All three solvents proved efficient in extracting the Voacanga alkaloids but the ethanol extracted much more colouring material than the organic solvents and hence one of these appeared preferable. Benzene was chosen rather than chloroform for two reasons. Firstly, it is in general a more selective solvent than chloroform, and, secondly, chloroform readily forms emulsions when shaken with water and this can be a considerable hazard when large volumes of solvents are involved.

Extraction of the alkaloids of V. schweinfurthii by benzene percolation

2.4 kg. of stem bark were reduced to a No. 60 powder in an impact mill, basified by macerating overnight with dilute solution of ammonia, packed in a percolator, and extracted with benzene to exhaustion (negative to Meyer's reagent). To ensure that all the alkaloid had been removed from the marc, a small sample of it was extracted with dilute hydrochloric acid and this extract, after filtering, gave no reaction with Meyer's reagent. Attempts were then made to separate alkaloids from the dark coloured, concentrated extract, as follows.

The base was extracted from the concentrate by shaking with 5 per cent aqueous hydrochloric acid, precipitated by the addition of excess strong solution of ammonia, and then taken up in chloroform. This chloroformic solution was separated and dried over anhydrous sodium sulphate, then evaporated to dryness under reduced pressure, to yield the dark brown crude base. The residue was carefully dissolved in the minimum amount of hot, dry methanol and retained in an attempt to induce crystallisation of the alkaloids. Although the alcoholic solution was very strongly basic, as shown by Meyer's reagent, no crystals were produced. It appeared that the presence of colouring matter and other impurities were having a solubilising

effect on the alkaloids, and further attempts were made to remove some of these impurities.

Initially the effect of partition between various solvents was examined and it was noted that there was a considerable amount of non-basic material present which was insoluble in ether, whereas the alkaloidal fraction appeared ether-soluble. This observation led to the following procedure.

A second benzene percolation was carried out and the dried residue of crude base bulked with that from the first extraction. This material was then extracted continuously, in a Soxhlet apparatus, with ether. Although some base was readily removed by the ether, it was found that even prolonged extraction failed to exhaust the residue. The ether extract was evaporated to dryness and re-extracted continuously with light petroleum (B.Pt. 40 to 60°C) and while again some base was extracted readily, prolonged treatment failed to remove all the base.

On evaporating the light petroleum extract to dryness, the residue was fairly pale in colour, but attempts to crystallise base from it again failed. Further purification of the residue by solution in acid and re-precipitation as base yielded no improvement.

The conclusion at this stage was that some fractionation of the alkaloids by solubility may have been effected, separating the following fractions:

(i) ether-insoluble,

(ii) ether-soluble but light petroleum-insoluble, and

(iii) ether- and light petroleum-soluble.

However, the method was extremely inefficient and the resultant solutions still contained sufficient solubilising materials to prevent crystallisation.

Column chromatography of the crude base.

Attempts to purify the crude base by column chromatography were made, and the first method applied was adsorption on alumina. Several columns were tried using pure and mixed solvents as eluants (benzene, ethanol, and acetone), but it was found impossible to separate the colouring matter from the bases, although some fractionation of the latter was achieved. The most successful results were obtained by loading the crude base in solution in benzene and eluting initially with benzene and subsequently with benzene containing increasing proportions of acetone. Examination of the eluate with Meyer's reagent suggested the presence of five different basic fractions. However, chromatographic analysis on paper indicated the presence of three bases in the first fraction and these persisted, along with a fourth base, in the second fraction. The last three fractions gave identical results, showing the presence of one base only, being one of those present in the other two fractions. Thus again the experi-

mental evidence pointed to the presence of several alkaloids but still no crystallisation could be induced, and all basic residues were dark in colour and varnish-like in texture.

The success of the paper system prompted attempts at partition column chromatography using buffered kieselguhr, and buffered cellulose. A series of kieselguhr columns carrying buffer solutions of varying pH values over the range from 7.3 to 2.3 was tried, eluting with ether saturated with the appropriate buffer solution. In each case nothing was held on the column, although increasing the acidity prolonged the time for complete elution. Several runs were then carried out using a column of buffered cellulose powder. The base was applied in the dry state, mixed with some unbuffered cellulose powder, to the top of the column. Elution was with ether, followed by various other organic solvents, but it was found that the total basic fraction came off in the ether. No fractionation of bases was obtained, although some colouring matter remained on the column.

In an attempt to completely free the extract of colouring matter, a technique using columns of formaldehyde-treated alginic acid[123] was employed. Previous experience with tinctures of cinchona had shown that the alkaloids could be held, by ion-exchange, on such columns, while colouring matter was eluted with ethanol. Subsequent elution with dilute mineral acid gave a clear solution of alkaloidal salts. Before use the powdered alginic acid was mixed with sufficient

solution of formaldehyde to form a slurry, which was heated for 16 to 18 hours at 80°C. The resultant paste was partially dried over a boiling-water bath, then granulated and sifted. The portion passing a No. 20 sieve, but retained by a No. 30 sieve, was used. The column was packed by adding the granules to the tube containing 90 per cent ethanol and then a little of the crude base, from the bark of V. schweinfurthii, dissolved in 90 per cent ethanol, was added. Much of the colour came straight through with the alcohol, and this fraction was found to be slightly basic. It was thought that this might have been due to overloading of the column, but subsequent experiment showed that some base always came with the alcohol. It was clear, however, that most of the alkaloid was still on the column and elution with several organic solvents and alcohols removed only a little further colouring matter; but a 5 per cent solution of acetic acid yielded a bright yellow eluate giving a strongly positive reaction with Meyer's reagent, and further elution with 5 per cent hydrochloric acid gave a separate basic fraction which was, however, rather dark in colour. The results appeared sufficiently promising to warrant further examination.

The alginate columns were unfortunately very readily overloaded, and eventually three were run in series, but still only a fraction of the total extract could be fractionated in this manner. The method proved extremely cumbersome, and the separated fractions on concentration were so strongly coloured, failing to yield any crystalline material, that it was condemned as being too inefficient.

Paper chromatography

Although up to this point no definite separation of alkaloids had been achieved, it was obvious that the material contained a mixture of bases and therefore the advantages of having an efficient method of examining the composition of the various fractions throughout the study were apparent. Paper chromatography seemed to be the most convenient method available, provided a suitable solvent system could be devised.

The first solvent system tried was one used successfully in many alkaloidal studies, namely a mixture of butanol, acetic acid and water, but it was found that all the material (both bases and colouring matter) ran with the solvent front. A series of small-scale tests was then set up, examining several solvent systems, running the crude alkaloid both as free base and as salt (hydrochloride), and investigating the effect of pH variation. The results, listed in Table 4, showed that in almost all cases the material behaved as in the first trial and no separation was accomplished. However development of the base with ether, on paper buffered to pH 4, showed under ultra-violet light that separation of the components had been achieved. Exposure of the paper to iodine vapour produced three distinct brown spots.

Repetition of the above system showed it to be quite satisfactory,

Table 4 : Paper ohromatographic trial systems.

State of solution examined	Solvent system	pH of buffered paper	Result
base	butanol/acetic acid/ water	unbuffered	All material moved with the solvent front
base	benzene/water	do.	
base	ether/water	do.	
base	light petroleum/water	do.	
base	carbon tetrachloride/ water	do.	
base	chloroform/water	do.	
base	pyridine/water	do.	
base	5 per cent solution of ammonia	do.	No movement
base	butanol/water	9	Moved with the solvent front
base	butanol/water	4	Moved just behind the solvent front
base	ether/water	9	Moved with the solvent front with some trailing
base	ether/water	4	Separation
salt	butanol/water	9	Moved with the solvent front
salt	butanol/water	4	Moved with the solvent front
salt	ether/water	9	Little movement
salt	ether/water	4	Little movement

but a wide variation in Rf values, caused by small changes in conditions, was noted. Practical details of the system were as follows. Whatman No. 1 paper was buffered to the required pH by soaking with M/5 potassium hydrogen phthalate buffer solution (B.P. 1958) and allowed to air-dry. The solutions to be examined were applied using a glass capillary, and the prepared paper allowed to saturate with water vapour prior to development with solvent ether, previously saturated with the buffer solution. Equilibration of the paper with water vapour was found to be essential in order to achieve any separation. The ascending technique gave slightly better separation than the descending method, and circular chromatography on discs (24 cm. diameter) proved most efficient for the accurate comparisons essential in the final examination of purified bases. In the ascending system, at room temperature, a solvent front movement of about 25 cm. was necessary and this required six to seven hours, but on discs a solvent front movement of about 10 cm. was satisfactory and this required only one and a half to two hours. It was found imperative to maintain an atmosphere of ether in the tank during running to minimise losses of the solvent from the paper by evaporation. Exposure of the air-dried, developed paper to iodine vapour in a closed chamber, proved an effective way of indicating the position of the alkaloids, which stained brown. However, one disadvantage was that the iodine staining quickly faded on exposure to the atmosphere, and a more satisfactory

method was that of spraying the papers with Dragendorff's reagent, which stained the alkaloids orange and the background yellow. This staining was more persistent, especially as the yellow background faded more quickly than the alkaloidal spots.

It was found that slight changes in pH affected the results and, as is detailed later, sometimes pH 4.0 and sometimes pH 4.7 was used. The lower pH was more suitable for examination of the weaker bases (with high Rf values), but the stronger bases (particularly vasicorine) were not separated as distinctly as they were at pH 4.7.

Extraction of the alkaloids of V.schweinfurthii by alcoholic percolation

It appeared that percolation with benzene removed impurities with properties very similar to those of the alkaloids and in subsequent attempts at purification, no suitable method was found of separating these impurities from the desired bases. It was decided to extract the bases under neutral conditions, following the method described by Percheron[88] for the isolation of the alkaloids of V.africana.

Sample VS/1.

Extraction of crude base.

2.4 kg. of powdered (No. 60) stem bark was extracted, by reserved percolation, with 70 per cent ethanol. 45 l. of percolate were collected before exhaustion (negative to Meyer's reagent) was achieved.

The first 15 l. of percolate, containing the bulk of the base, were concentrated at reduced pressure, at a temperature below 50°, to about 1 l. The concentration was difficult due to copious frothing of the solution, which worsened progressively with later fractions. The resultant thick, dark, aqueous suspension was shaken with ethyl acetate (1.25 l.) in which most of the solid dissolved. The two fractions were then separated and the aqueous fraction extracted with further quantities of ethyl acetate (2 x 500 ml., 1 x 200 ml.). A

considerable amount of solid material collected at the interface during these extractions and was filtered off; the dark brown, gummy residue being well-washed with solvent to remove as much adsorbed alkaloid as possible. The various ethyl acetate fractions were bulked, washed with distilled water (400 ml.), and on separating constituted Solution I. The water used for washing was returned to the partially extracted aqueous fraction.

To this aqueous fraction was added ethyl acetate (1 l.) before adjusting to pH 9, by the addition of solid sodium carbonate, portionately and with shaking. Much solid matter remained, and this was filtered off and ground with numerous small portions of sodium carbonate solution and ethyl acetate, until as much base was in solution as possible. This solution was added to the main bulk of aqueous fraction/ethyl acetate in a separating funnel. The aqueous portion was removed and further extracted with ethyl acetate (1 l., 800 ml., 400 ml., 5 x 200 ml.) until free of base. The bulked ethyl acetate solution was divided into two portions, to facilitate handling, and each half was washed with distilled water (400 ml.). These two ethyl acetate solutions together constituted Solution II.

Thus the total extract had been divided according to solubility in ethyl acetate at two pH values, and a further subdivision of each section was then obtained by extraction with an organic acid, followed by a mineral acid, yielding strong and weak base fractions respectively.

Solution I was extracted with 5 per cent acetic acid (4 x 500 ml., 1 x 200 ml.) until no more base was removed, each fraction being washed with the same ethyl acetate (500 ml.). The acid fractions were then bulked and an aliquot (20 ml.) portion titrated with 20 per cent ammonia solution until just alkaline. This allowed a fairly accurate estimate to be made of the quantity of ammonia solution required to basify the bulked fractions. In the presence of ethyl acetate (1 l.), the ammonia solution was added to the acid solution in portions (4 x 25 ml.) until alkaline, shaking well on each addition; a further 25 ml. being added to ensure the pH was well on the alkaline side. After shaking, the aqueous portion was separated and further extracted with ethyl acetate (2 x 500 ml., 1 x 200 ml.). As before, the combined extracts were washed with distilled water (400 ml.).

The washed ethyl acetate solution was dried over anhydrous sodium sulphate for 18 hours, strained through cotton wool and then taken to small volume under reduced pressure. The concentrate was transferred to a weighed flask and evaporated to dryness under reduced pressure. This yielded a residue of crude strong base (Residue IA, 3.66 g.).

Solution I, after acetic acid extraction, was further extracted with 5 per cent hydrochloric acid (2 x 500 ml., 6 x 200 ml.) until free from base. Each acid fraction was washed with the same portion of ethyl acetate (500 ml.), the bulked fractions basified with

20 per cent ammonia solution (after estimating an aliquot portion as before) in the presence of ethyl acetate (1 l.), and the extraction of the aqueous fraction with ethyl acetate continued (2 x 500 ml., 1 x 200 ml.) until the aqueous fraction was negative to Meyer's reagent.

The ethyl acetate solution was washed with distilled water (400 ml.), separated, and dried over anhydrous sodium sulphate. After filtration the solution was evaporated to dryness in a tared flask giving a residue of crude weak base (Residue IB, 0.73 g.).

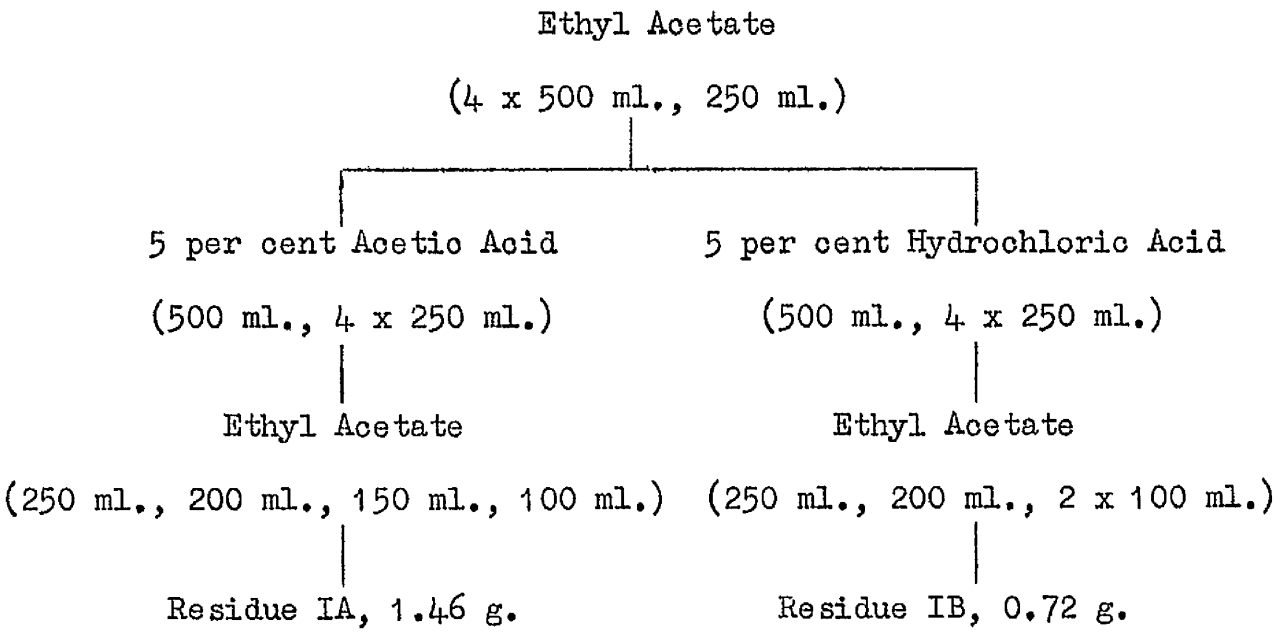
Each half of Solution II was subjected to the extraction procedure described for Solution I, using different volumes of solvent. Acetic acid solution (500 ml., 7 x 200 ml.) followed, after basifying, by ethyl acetate (500 ml., 3 x 200 ml.) gave a residue of crude strong base (Total Residue IIA, 12.22 g.). Hydrochloric acid (500 ml., 8 x 250 ml.) gave a small residue of crude weak base (Total Residue IIB, 1.51 g.).

The remaining 30 l. of initial percolate were concentrated to 2.5 l. and treated as above, giving the following yields of crude base: IA, 1.46 g.; IB, 0.72 g.; IIA, 0.70 g.; IIB, 0.02 g. The volumes of solvent employed are given in Fig. 10.

Purification of strong bases.

Residue IA was almost completely soluble in 25 ml. of hot benzene.

1. Neutral Extraction - Solution I.



2. Extraction at pH 9 - Solution II.

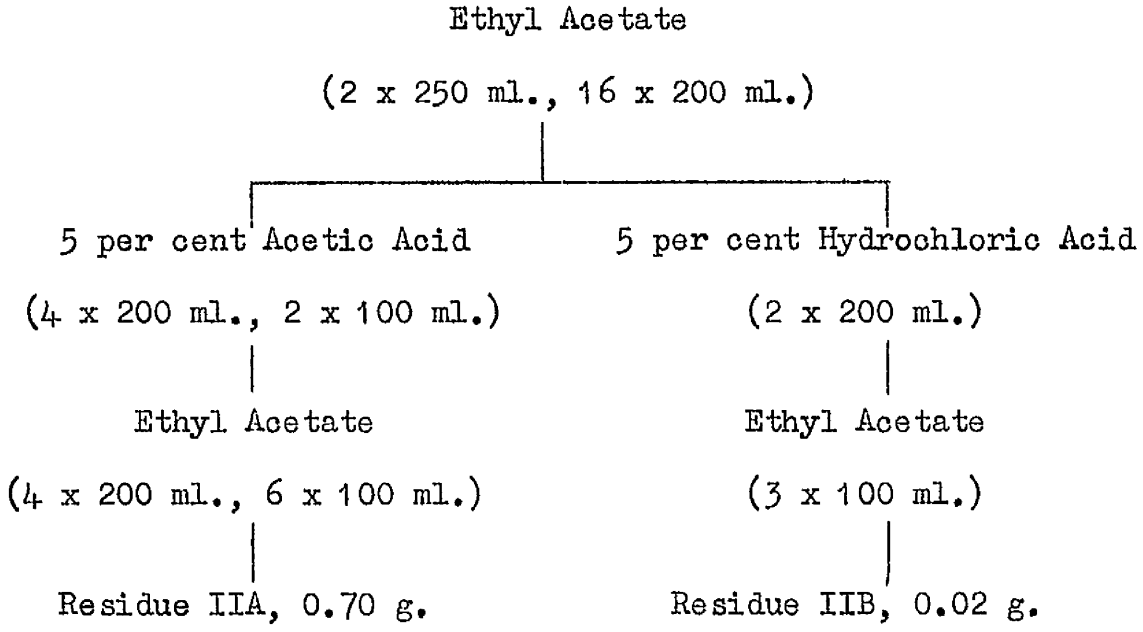


Fig. 10 : Purification of final runnings of percolate.

A small amount of insoluble, non-basic material was removed by passing the cooled solution through a No. 3 sintered glass filter. The benzene solution was then evaporated to dryness under reduced pressure and the residue redissolved in 15 ml. of benzene to obtain as concentrated a solution as possible. This was then chromatographed on a column of alumina.

The column was prepared by adding 100 g. of dry alumina (Merck) to a suitable tube containing benzene, to produce a column of dimensions 14 x 3.25 cm. The alkaloidal solution was added to the column and elution commenced with benzene. When the band of colour had travelled about half way down the alumina, the collection of fractions was started. Fractions of 100 ml. were collected, each fraction being evaporated to dryness under reduced pressure in a tared flask and the residue weighed. This residue was then washed into a tube with hot methanol and reserved in a refrigerator in an attempt to induce crystallisation.

It was soon clear that benzene was only removing traces of material from the column, so the eluting solvent was changed to ether. Immediately a considerable weight of material was eluted (Table 5). Elution was continued with ether for some time until it was removing very little from the column, when elution with ether containing 1 per cent methanol was begun. Again there was a sudden increase in material being removed from the column. Development of the column

Table 5 : Results of the chromatography of Residue IA.

Fraction, 100 ml.	Eluant	Weight of residue, mg.	Bulked fractions	Crystals	Melting-point	Blue with nitric acid
1	benzene	7	f(i)	-		
2		60				
3		88				
4		73				
5		51				
6		35				
7		48				
8	ether	75	f(ii)	+	228-230	+
9		136	f(iii)	+	230	+
10		129	f(iv)	+	232-233	+
11		90	f(v)	+	246	+
12		83				
13		89				
14		86				
15	ether with 1 per cent methanol	61	f(vi)	+	247-283	+
16		80				
17		342	f(vii)	+	286-293	+
18		256	f(viii)	+	264-286	+
19		129				
20		69				
21		38				
22		39				
23	ether with 3 per cent methanol	32	f(ix)	-		
24		112				
25		54				
26		32				
27	ether with 5 per cent methanol	27				
28		43				
29		71				
30		25				
31	ether with 10 per cent methanol	23				
32		63				
33		37				
34	methanol	167				

was continued in this manner, increasing the proportion of methanol (3, 5, 10 per cent) in the ether, and finally pure methanol was used. The column was extruded and checked to ensure that all the base had been removed. With the addition of methanol to the eluant, increasing amounts of colouring matter were eluted but most of the colour remained until the final elution with pure methanol.

As indicated in Table 5, crystals were only produced in the fractions eluted by ether and ether containing 1 per cent of methanol. The mother liquors in the tubes containing crystals were transferred by pipette to other tubes, concentrated slightly and returned to the refrigerator. The tubes containing the crystals were heated momentarily in a boiling-water bath, under reduced pressure, to remove the residual solvent and then, for each batch of crystals, melting-points were determined on a hot bar, and the colour produced with nitric acid noted. Thus the crystalline yield was shown to consist of at least two substances; one, melting around 230°, and giving no colour reaction with nitric acid, and another, melting above 280°, and giving a deep blue colouration with the acid. It was thus clear that the ether had completely eluted the first substance, and that the second substance was eluted partly in the last ether fractions, but mostly in ether containing 1 per cent of methanol.

The first substance, being only sparingly soluble in methanol, was recrystallised several times from this solvent, and dried <u>in vacuo</u>,

yielding white acicular crystals, later shown to be voacamine.

The second substance proved insoluble in methanol, and was dissolved in methylene chloride, in which it was readily soluble. The subsequent addition of methanol followed by warming, produced white crystals which were further purified by repeating the above process. The crystals were dried in vacuo and later shown to be vobtusine.

The mother liquor from which vobtusine had separated was concentrated (by blowing a current of nitrogen through the tube to minimise oxidative degradation) and on standing, large rosettes of white feathery crystals formed. These were separated, recrystallised several times from methanol and finally from acetone, dried in vacuo, and later shown to be voacorine.

Residue IIA was then treated in a similar manner to IA. The residue was largely dissolved in 75 ml. of benzene, the solution cooled, filtered through sintered glass, evaporated to dryness, and taken up in 50 ml. of benzene. This solution was chromatographed on alumina (360 g.; 25 x 4.25 cm.). Elution commenced with benzene, then ether, then ether containing 1 per cent methanol, as before, but subsequently only with ether containing 5 per cent methanol before finishing with pure methanol. The results followed the same pattern as with IA, and are detailed in Table 6.

The crystals were harvested, dried and examined. Again it was found that ether had eluted voacamine, and ether containing 1 per cent

Table 6 : Results of the chromatography of Residue IIA.

Fraction, 300 ml.	Eluant	Weight of residue, mg.	Bulked fractions	Crystals	Melting-point	Blue with nitric acid
1	benzene	11	f(i)	-		
2		20				
3		14				
4	ether	232				
5		282				
6		181	f(ii)	+	227	-
7		411	f(iii)	+	237	-
8		278	f(iv)	+	234	-
9		457	f(v)	+	235	-
10		150	f(vi)	+	248	+
11		93				
12		66				
13		42				
14	ether with 1 per cent methanol	60	f(vii)	-		
15		46				
16		264				
17		905	f(viii)	+	261-293	+
18		1,121				
19		439				
20		347	f(ix)	+	291-295	+
21		193				
22		196				
23		130				
24	ether with 5 per cent methanol	138	f(x)	+	289-298	+
25		859				
26		103				
27		141	f(xi)	-		
28	methanol	1,121				

methanol had removed vobtusine and voacorine, the latter crystallising from the methanolic mother liquor after removal of the insoluble vobtusine.

Purification of weak bases.

The two crude weak base fractions (IB and IIB) although very small, especially the former, were treated by the same purification process as the strong bases.

Residue IB was digested with hot benzene and it was found that a much smaller proportion went into solution than with the strong base fractions. After filtering through sintered glass, the insoluble material was shown to be non-basic. The filtrate was evaporated to dryness, taken up in 7 ml. of benzene and chromatographed on 15 g. of alumina, 30 ml. fractions being collected. Elution was carried out in exactly the same manner as with IA. The results, as detailed in Table 7, showed that crystalline material was obtained only in the initial benzene fraction and on separation from the mother liquor, which was only very faintly positive to Meyer's reagent, the crystals were found to be non-alkaloidal. It was a white wax-like substance, melting at 42°.

The reaction of the bulked fractions to Meyer's reagent showed that base was present in all fractions up to, and including, that eluted by ether containing 3 per cent methanol. Weights of residue

Table 7 : Results of the chromatography of Residue IB.

Fraction, 30 ml.	Eluant	Weight of residue, mg.	Bulked fractions	Crystals	Melting-point	Blue with nitric acid	Meyer's reagent
1	benzene	39	f(o)	+	42	-	+
2		0					
3		6	f(i)	-			+
4		7					
5		2					
6		30	f(ii)	-			+++
7	ether	9					
8		7					
9		1	f(iii)	-			+
10		7					
11		0					
12	ether with 1 per cent methanol	1					
13		2	f(iv)	-			+++
14		8					
15		1					
16	ether / 3p.c. methanol	8	f(v)	-			-
17		3					
18	ether / 5p.c. methanol	11	f(vi)	-			-
19		8					
20	ether / 10p.c. methanol	28	f(vii)	-			-
21		12					
22	methanol	67	f(viii)	-			-

Table 8 : Results of the chromatography of Residue IIB.

Fraction, 50 ml.	Eluant	Weight of residue, mg.	Bulked fractions	Crystals	Melting-point	Blue with nitric acid	Meyer's reagent
1	benzene	34	f(o)	+	43	-	-
2		18	f(i)	-			+
3		52					
4		9					
5		22					
6		7					
7		25					
8	ether	49	f(ii)	+	299	+	++
9		63					
10		37					
11		33	f(iii)	+	294	+	++
12		58					
13		13					
14		20					
15		12					
16		7					
17	ether with 1 per cent methanol	11	f(iv)	+	293	+	++
18		16					
19		49					
20		26					
21		22					
22		20					
23		10					
24		8					
25	ether with 3 per cent methanol	11	f(v)	-			-
26		26					
27		21					
28		16					
29		13					
30	ether with 5 per cent methanol	20					
31		21					
32		11					
33		13					
34		15					
35	ether with 10 per cent methanol	31					
36		26					
37		21					
38	methanol	149					

obtained from the various fractions, and visual estimations of the strength of reaction with Meyer's reagent, showed clearly that there were two main concentrations of alkaloid, the first in the first fraction eluted by ether, and the second in the fractions eluted by ether containing 1 per cent methanol. Thus it appeared that this weak base fraction was following the same pattern as the two strong base fractions.

The somewhat larger Residue IIB was treated similarly, and digestion with hot benzene again showed a large proportion of insoluble, non-basic material. After concentration of the filtered benzene solution, the resultant dried basic residue was dissolved in 10 ml. of benzene and chromatographed on 35 g. of alumina, 50 ml. fractions being collected. Again the initial benzene fractions yielded a white, non-basic material (M.P. 43°). A small amount of crystalline material was obtained in the final benzene eluates, more in the ether eluates, and some also in the ether containing 1 per cent methanol. From the high melting-points and the blue colouration with nitric acid, all these materials appeared to be composed largely of vobtusine. So again a similarity with the strong base pattern emerged, as shown in Table 8.

Paper chromatography of the weak bases.

The alkaloidal fractions from the chromatography of the weak

base residues were examined in greater detail. Fractions giving a positive Meyer's reaction were bulked and designated as shown in Tables 7 and 8, and the following series of peper chromatograms run, using the circular method previously described (page 78, pH 4.0). Direct comparisons were made with pure samples of various known Voacanga alkaloids as reference compounds.

Bulked fraction IIB/f(i) was compared with pure samples of voacamine, vobtusine and voacangine and it gave a single line coinciding with voacangine.

IB/f(i) and IB/f(ii) were compared with voacangine and vobtusine. IB/f(i) gave a single line coinciding with voacangine, and IB/f(ii) gave a heavy concentration of staining at the solvent front, a line coinciding with voacangine, and another between the reference voacangine and vobtusine.

IB/f(ii) was again run, this time accompanied by pure voacangine, voacamine, vobtusine and some of the voacorine isolated from the strong base residue (Residue IIA). Again IB/f(ii) gave the intense stain at the solvent front, a line coinciding with voacangine, the third line which corresponded to voacamine, and also a further faint line coinciding with vobtusine. The voacorine behaved in an unusual manner producing a wedge of staining; apex at the spot, and base carried a short distance forward (Fig. 11, pH 4.0).

The isolated voacorine was compared with an authentic sample,

and the two substances behaved in an identical manner, producing the wedge of staining described above.

The three bulked fractions IB/f(ii), IB/f(iii) and IB/f(iv) were compared directly with the corresponding bulked fractions from IIB. An insufficient quantity of IB/f(ii) was used and no vobtusine or voacangine was found, only voacamine and the frontal stain. These were also present in IIB/f(ii), and also vobtusine and a further very faint line to the inside of the vobtusine stain. In both IB/f(iii) and IIB/f(iii) was a single line corresponding to vobtusine. IB/f(iv) gave no distinct staining, but IIB/f(iv) showed two lines, corresponding to voacamine and vobtusine.

Heavier concentrations of IB/f(iv) and IIB/f(iv) were compared with voacamine and vobtusine. The two bulked fractions gave identical results, showing the presence of both voacamine and vobtusine, and also giving the concentration of staining at the front, and, further, a faint line to the inside of the vobtusine position, corresponding to that previously shown by IIB/f(ii).

Two spots each of voacorine (isolated), IB/f(ii), and voacangine were run. The paper was halved and one half only was sprayed to locate the positions of the bases. Areas corresponding to these on the other half of the chromatogram were cut out, eluted with chloroform, and tested with Meyer's reagent. This confirmed that the frontal staining with IB/f(ii) was due to an alkaloid, and that

Table 9 : Summary of the results of the paper chromatographic analysis of Residues IB and IIB, alkaloids being listed in descending order of Rf values.

Fraction	Residue IB	Residue IIB
f(i)	voacangine	voacangine
f(ii)	U1 voacangine voacamine vobtusine	U1 voacamine vobtusine U2
f(iii)	vobtusine	vobtusine
f(iv)	U1 voacamine vobtusine U2	U1 voacamine vobtusine U2

voacangine was also present in this bulked fraction. From the Rf values of two other bases present it was concluded that traces of voacamine and vobtusine also occurred.

From these results, summarised in Table 9, it was clear that some residual voacamine and vobtusine had persisted in the weak base fraction, though no trace of voacorine was found. In addition, the presence of at least two other bases was indicated, one following the solvent front, and which will be referred to subsequently as U1, and the other behaving as did a genuine sample of voacangine. From their positions on the chromatograms it was reasonable to refer to these two bases as the weak bases. The presence of another strong base, designated U2, with an Rf value between that of voacorine and vobtusine, was suggested by the faint staining with both IB/f(iv) and IIB/f(iv).

The pattern shown by both weak and strong bases seemed to indicate that no advantage was achieved by the initial extraction of the concentrated percolate with ethyl acetate prior to adjustment to pH 9. It might, therefore, be justifiable to omit this step and basify the percolate immediately after concentration, before ethyl acetate extraction. For the purpose of investigating this modification of the extraction process, and at the same time to examine a second sample of the bark for conformity to the pattern of alkaloidal content set by the first sample, a second extraction was carried out.

Sample VS/2.

Extraction of crude base.

In this case only 1.2 kg. of powdered bark were extracted with 70 per cent ethanol, and the total percolate collected was 23 l., which was then concentrated under reduced pressure to 1.5 l. This concentrate was then transferred to a 5 l. separating funnel containing 1 l. of ethyl acetate, and solid sodium carbonate was added, with shaking, to adjust to pH 9. The basified aqueous fraction was further extracted with ethyl acetate (500 ml., 13 x 200 ml.) until free from alkaloid, the bulked ethyl acetate being reduced to about 2 l., washed with distilled water (400 ml.), and then extracted with 5 per cent acetic acid (400 ml., 20 x 200 ml.). These acid fractions, together constituting Solution A, were all washed with the same 500 ml. of ethyl acetate, and the washings returned to the main ethyl acetate fraction which was then extracted with 5 per cent hydrochloric acid (400 ml., 9 x 200 ml.). Each fraction of this latter acidic extraction was also washed with the same 500 ml. of ethyl acetate, and after bulking constituted Solution B.

Solution A was then basified with 20 per cent ammonia solution in the presence of ethyl acetate (1 l.), and further extracted with ethyl acetate (2 x 500 ml., 8 x 200 ml.), each fraction being washed with the same 400 ml. of distilled water. These combined extracts were concentrated under reduced pressure to about 2 l., dried over

anhydrous sodium sulphate, filtered and evaporated to dryness as described previously. Solution B was treated similarly, the alkaloids being removed by ethyl acetate (1 l., 2 x 500 ml., 1 x 200 ml.) after basifying with ammonia solution, and the fractions washed, concentrated, dried and evaporated to dryness.

The weight of crude strong base (Residue A) from the 1.4 kg. of bark was 18 g., and of weak base (Residue B) only about 120 mg. Thus although the quantity of bark used in this extraction was only half that used previously, almost exactly the same amount of crude strong base resulted. The weak base fraction was negligible, much less than before, but as the crude weak base fraction of the first extraction proved to be largely non-basic, and the basic portion to be largely voacamine and vobtusine (i.e. strong bases), this is of no significance.

These results indicated that the modified extraction was more efficient and that this sample (VS/2) was considerably richer in alkaloids than the first (VS/1).

Purification of strong bases.

The solution of bases for chromatography was prepared as before. About 16 g. of the crude base were dissolved in hot benzene, and the filtered solution evaporated to dryness. The residue was redissolved in 80 ml. of benzene and chromatographed on 600 g. of

Table 10 : Results of the chromatography of Residue A.

Fraction, 300 ml.	Eluant	Weight of residue, mg.	Bulked fractions
1	benzene	0	f(i)
2		89	
3		3	
4		15	
5		2	
6		28	
7		0	
8	ether	85	f(ii)
9		272	f(iii)
10		918	
11		337	
12		100	f(iv)
13		164	
14		170	
15		174	
16		189	
17		206	f(v)
18		257	
19		321	
20		494	
21		755	f(vi)
22		670	
23		497	
24		396	
25		224	
26		150	
27		140	f(vii)
28		125	
29		74	
30		97	
31		70	
32		28	
33		58	
34	ether with 0.5 per cent methanol	52	
35		41	
36		45	
37		56	

Table 10 - continued.

Fraction, 300 ml.	Eluant	Weight of residue, mg.	Bulked fractions
38		36	f(vii)
39		38	- contd.
40		349	
41	ether with	1,355	f(viii)
42	1 per cent	544	
43	methanol	289	
44		162	
45		98	f(ix)
46		48	
47		44	
48		125	
49	ether with	519	
50	3 per cent	388	
51	methanol	295	f(x)
52		358	
53		267	
54		15	
55	ether / 5p.c.	56	
56	methanol	14	
57		120	
58	ether with	306	f(xi)
59	10 per cent	1,106	
60	methanol	611	
61		203	
62		151	
63		768	
64		138	
65	methanol	205	f(xii)
66		250	
67		104	
68		93	
69		16	

alumina in a column of dimensions 50 x 4.3 cm.

Eluate fractions of 300 ml. were collected. Elution of the column was carried out in a manner similar to that previously described, with one important difference. This was that the ether-elution was continued for a much longer period and this affected the results as will be shown below. Little colour appeared in the eluted fractions until 1 per cent methanol in ether was used, and varying amounts of crystalline material separated in all fractions from eight to sixty-one (Table 10).

Paper chromatography of the fractions collected from the alumina column.

Paper chromatograms were run at pH 4.7, examining each individual fraction from the column. From the results obtained, like fractions were bulked, and the reduced number of fractions again run on paper, this time alongside samples of the known reference alkaloids. Thus it was seen that benzene had removed small quantities of voacangine and voacamine, traces of voacorine, and a small amount of basic material, running almost with the solvent front, which was clearly the substance previously referred to as U1 (Table 9). The initial ether fraction (f(ii)) repeated this result with the U1 and voacangine arcs somewhat stronger, and vobtusine was also present. Additionally there was the slightest trace of another base with a smaller Rf value than voacorine.

The early ether fractions eluted voacamine, vobtusine and in-

creasing amounts of voacorine, the last voacamine coming off in f(v). The unknown strong base mentioned in f(ii) recurred in strength in f(iv), and persisted in f(v). Most of the vobtusine was eluted by ether simply, in fractions f(ii) to f(vi), but some persisted, accompanied by increasing amounts of voacorine, to f(viii), which was ether containing 1 per cent methanol. The next fraction, f(ix), was unusual in that it revealed the recurrence of traces of voacangine and voacamine. The explanation of this would seem to be that slight traces of these bases had been coming off the column continuously, and on the addition of this increased concentration of methanol, the final traces were washed off in a sufficient concentration to be detectable.

The final fractions, which were strongly coloured, gave results which were difficult to interpret. Alkaloidal stains were always prominent at the starting-point of the chromatogram and frequently streaks were obtained along most of the chromatogram length. It would seem that some strong bases were present in small quantity but whether these were the same as those described previously or not could not be determined.

The crude weak base fraction, considered too small to be chromatographed on alumina, was examined by paper chromatography and found to contain only traces of voacorine.

In summing up these results, it is clear that voacamine, vob-

tusine and voacorine are the three main alkaloids present. The weak base voacangine is also present, in small amounts, accompanied by the even weaker base, U1. At least one other strong base is present in trace amounts as was also found in the previous extract. However in this case the Rf value was well short of that of voacorine, instead of being between those of voacorine and vobtusine as in the earlier experiment. This anomaly may be explained by the fact that the previous paper chromatography was carried out at pH 4.0 and the more recent work at pH 4.7. If this were the same base, then it had been held back by decreasing the acidity of the paper, which would be very unusual behaviour for a base. Evidence to support this, however, was obtained by running f(iv) and f(v) at pH 4.0 as in the earlier experiment, whereupon voacorine was held back, and another base was shown between it and vobtusine, exactly as had happened previously.

Another possible explanation of this behaviour is that there are two strong bases present beside the known ones. At pH 4.0, the stronger of the two unknowns (U2) is clear as an arc behind vobtusine but well ahead of voacorine. At this pH the voacorine is shown as a wedge of staining, so this may also contain the other base (designated U3). At pH 4.7, voacorine moves much further, until it is close behind vobtusine, and it is suggested that U2 is also in this region but undetected as a separate arc. Further, U3 is then revealed as a compact arc well behind voacorine, and no wedge of staining is

present. This second explanation, although in no way verified, seems the more creditable (Table 11 and Fig. 11).

It is also likely that other strong bases are present in extremely small amounts.

Table 11 : Rf and Rvb values of the alkaloids from paper disc chromatography. The Rvb value is the ratio of the movement of the alkaloid to that of pure vobtusine, determined simultaneously.

(Paper - Whatman No. 1 ; Solvent - Ether.)

Alkaloid	pH 4.0		pH 4.7	
	Rf	Rvb	Rf	Rvb
U1	1.00	1.54	0.99	1.52
voacangine	0.88	1.35	0.87	1.34
voacamine	0.74	1.14	0.74	1.14
vobtusine	0.65	1.00	0.65	1.00
U2	0.50	0.77	undetected	
voacorine	0.24	0.37	0.55	0.85
U3	undetected		0.32	0.52

To face p. 107

Fig. 11.

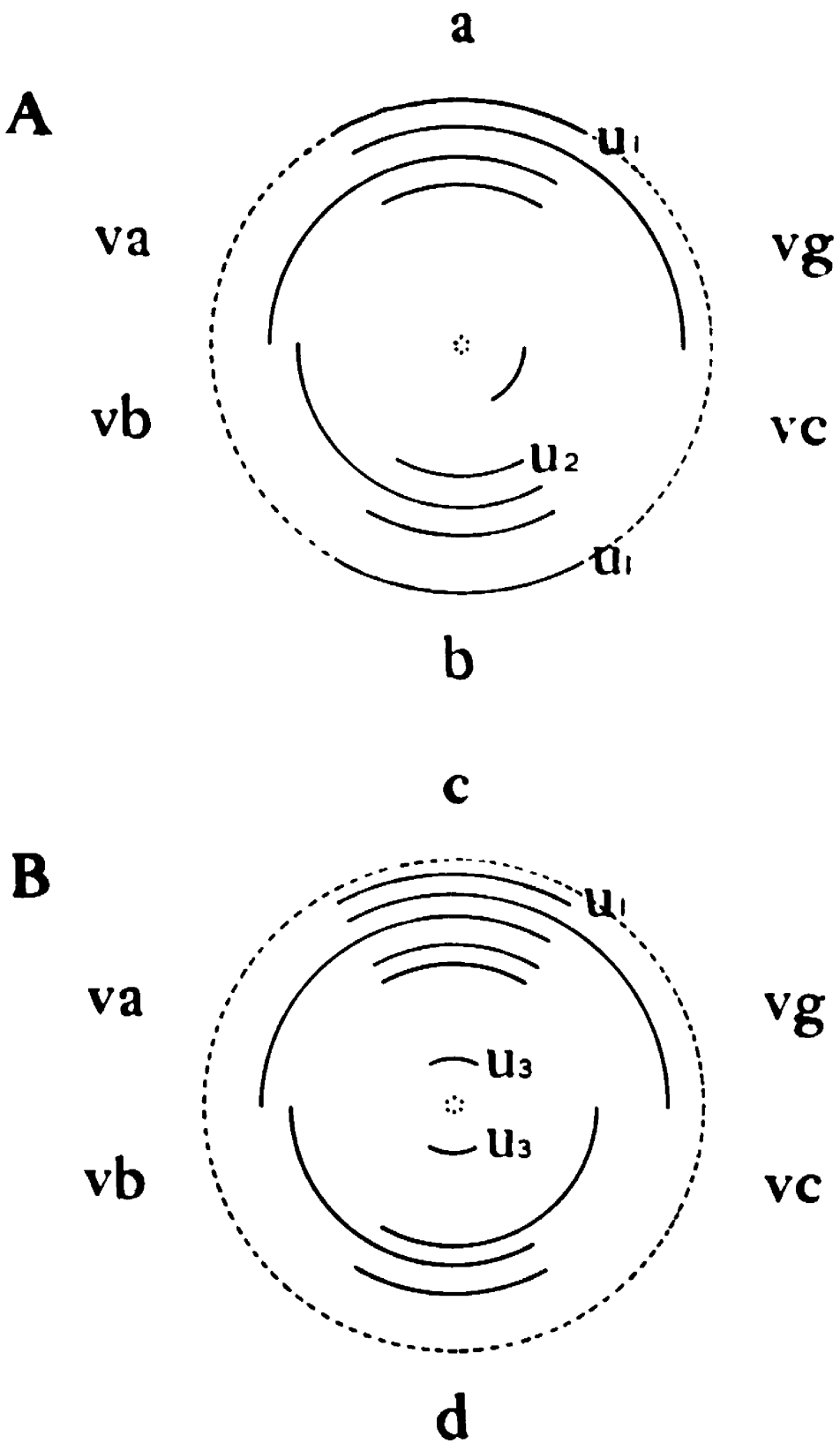
Diagram to show the results of paper disc chromatography of the Voacanga alkaloids.

A. results at pH 4.0.

B. results at pH 4.7.

(Paper - Whatman No. 1; Solvent - Ether.)

a, VS/I-IB/f(ii); b, VS/1-IIB/f(iv); c, VS/2-A/f(ii); d, VS/2-A/f(iv); u1, U1; u2, U2; u3, U3; va, voacamine; vb, vobtusine; vc, voacorine; vg, voacangine.


a
A
u1
va
vg
vb
vc
u2
u1
b
c
B
u1
va
vg
u3
u3
vb
vc
d

Thin-layer chromatography

Attempts were made using the increasingly important technique of thin-layer chromatography, to confirm the presence of voacangine and isolate, in the pure state, the unidentified alkaloids.

The first plates were made by the method of Demole[124], using silicic acid bound with starch. A large number of tests were carried out to find a suitable solvent system, but although many solvents with widely varying properties were tried, it was found that the base nearly always stayed at the original spot. However, ether proved effective in moving the weak bases and the results were improved by mixing ether with half its volume of chloroform.

Detection of the bases posed a problem, due to the presence of starch as a binding agent. When stained with iodine vapour the bases present in fairly large amounts were readily seen but the heavily stained background obscured those less prominent. On spraying with Dragendorff's reagent the whole plate stained dark brown. In an attempt to overcome this difficulty, the plates were washed with 1 per cent solution of sodium thiosulphate after spraying with Dragendorff's reagent; the background immediately became colourless leaving the bases stained orange. However, it was found that some of the staining of the bases was removed also and it was feared that trace materials might thus be missed. The results with iodine-stained

plates were very unsatisfactory.

An alternative solution was to replace starch as the binding agent and this was successfully done by using powdered tragacanth. The tragacanth (0.7 g.) and silicic acid (28.5 g.) were intimately mixed in a mortar and about 60 ml. of water added, followed by vigorous trituration. This gave a smooth paste which when diluted with about 40 ml. of water produced a mucilage of a suitable viscosity for spreading on the plates. If the viscosity is correct, then manipulation of the plate by hand is sufficient to distribute it evenly over the surface, but if necessary, spreading can be done using a broad palette knife. The plates were then dried in an oven but it was found necessary to dry them more slowly than advocated by Demole, in order to minimise cracking of the surface. After drying at 85° for several hours the plates were stored in a desiccator until required. On spraying with Dragendorff's reagent, the bases separated on such plates stained orange, the background becoming pale yellow, as with paper chromatograms.

Using the tragacanth-silicic acid plates and ether/chloroform (2:1) as solvent, the four known alkaloids were studied. In the case of the three strong bases, the original spots all stained deeply with short streaking upwards from the points of application. By contrast, voacangine moved well with the solvent and produced a fairly compact spot, Rf 0.74.

With this knowledge the fractions from the column chromatography of Residue A(VS/2) were then examined. As was expected it was the initial fractions that proved the most interesting. The first fraction stained at the starting-point with some streaking to about Rf 0.3. The next fraction, f(ii), showed two strong tailing spots at Rf 0.92 and 0.72, plus staining at the starting-point, and f(iii) showed three well-defined areas at Rf 0.72, 0.61 and 0.39. The remainder of the fractions showed staining only at the starting-points with a little streaking, somewhat more pronounced in f(ix), which reflected the results found by paper chromatography.

Thus the presence of U1 and voacangine had again been demonstrated in f(ii), and it also seemed that f(iii) contained some voacangine and other two weak bases in trace amounts, previously unsuspected.

As U1 and voacangine were present in f(ii) in some quantity, it was decided to try to isolate them using this technique. Thus a broad plate was loaded with a spot of the fraction towards one side, and a heavy streak towards the other side. After development the plate was examined under ultra-violet light and the relative positions of the fluorescent components from spot and streak were marked. The developed spot was then sprayed (protecting the rest of the plate from the spray) and the positions of the two alkaloids determined. The corresponding sections from the developed streak were then scraped

off, eluted with acetone, filtered and evaporated to dryness. Unfortunately in neither case was there sufficient residue to carry out a melting-point determination, but both yielded sufficient material to allow examination of the ultra-violet spectra which confirmed the presence of voacangine but gave no hint of the identity of U1.

The alkaloids

Final purification.

Accurate physical constants are only obtained from substances with a high degree of purity. The achievement of such purity in the isolation of the three alkaloids, voacamine, vobtusine and voacorine, proved extremely difficult due to their instability. Solutions of all three rapidly produced a violet colouration and to avoid this in the final purification by repeated crystallisation or precipitation (in the case of vobtusine), it was found necessary to maintain conditions as inert as possible. Three principles were adopted,

i, the bases were kept in solution for as short a time as possible;

ii, the temperature was kept as low as was practicable;

and, iii, contact with atmospheric oxygen was minimised.

Thus recrystallisation was carried out as quickly as possible by solution in the minimum amount of warm solvent, and then concentrating the solution by passing nitrogen through under pressure, while maintaining the temperature at about 30 to 35°. At room temperature the solvent came off too slowly, leading to colour formation.

The harvested crystals then required to be thoroughly dried and although only dry solvents were used during recrystallisation, only prolonged treatment at about 60°, under vacuum, over phosphorous

pentoxide (using a drying pistol, with chloroform as the heating solvent), produced samples pure enough to give analytical figures in agreement with those previously published. In this manner the last traces of solvent were removed fairly quickly from voacamine and vobtusine (four hours and twenty-four hours, respectively) but very slowly from voacorine (several days).

Melting-point.

First attempts at determination of the melting-points of the alkaloids resulted only in charring, generally starting just below 200° and worsening progressively with increase in temperature. The successful method adopted was to seal the substances in evacuated glass capillary tubes and begin heating at about 15° below the suspected melting-point. In this manner quite sharp decomposition-points were achieved.

Specific rotation.

This was determined on 1 per cent (approximately) solutions in chloroform at 20°.

Voacamine.

Recrystallised from methanol, corrected m.p. 223° (decomp.), readily soluble in chloroform, sparingly soluble in methanol. $(\alpha)_D^{20}$, -50° (c = 1, chloroform). Ultra-violet spectrum (absolute

ethanol) with absorption peaks at 225 mμ (log ε, 4.72), 285 mμ (log ε, 4.27) and 295 mμ (log ε, 4.28). Infra-red spectrum coincided with that obtained using authentic voacamine. Found: C, 71.00; H, 7.5; N, 7.8 per cent, $C_{45}H_{56}O_6N_4$ requires C, 72.3; H, 7.6; N, 7.8 per cent. These figures are in agreement with those already published[9,16,88,105].

Vobtusine.

Precipitated from methylene chloride, uncorrected m.p. 305° (decomp.), readily soluble in chloroform but insoluble in methanol and ethanol, deep blue colour with concentrated nitric acid. $(\alpha)_D^{20}$, -295° (c = 1, chloroform). Ultra-violet spectrum (absolute ethanol) with absorption peaks at 220, 265, 300 and 325 mμ. Infra-red spectrum coincided with that obtained using authentic vobtusine. Found: C, 70.3; H, 7.1; N, 7.65 per cent, $C_{42}H_{48}O_6N_4$ requires C, 71.6; H, 6.9; N, 7.95 per cent. These figures agree in general with those published[9,18,94], although the specific rotation is low and also the analytical figures. The latter agrees with the observation of Schuler et al.[18] who found that slight traces of moisture affected the figures. The value obtained for specific rotation (-295°), although higher than the published figure (-321°), was the same as that obtained by the author on a genuine sample of vobtusine. Due to vobtusine being almost completely insoluble in ethanol, no values for log ε could be calculated but the maxima were in approximately the

same proportion to each other as the published extinction coefficients[16].

Voacorine.

Recrystallised from acetone, uncorrected m.p. 271° (decomp.), readily soluble in chloroform, slightly soluble in acetone, very slightly soluble in methanol. $(\alpha)_D^{20}$, -50° (c = 1, chloroform). Ultra-violet spectrum (absolute ethanol) with absorption peaks at 225 mμ (log ε, 4.66), 286 mμ (log ε, 4.20) and 295 mμ (log ε, 4.21). Infra-red spectrum coincided with that obtained using authentic voacorine. Found: C, 70.25; H, 7.39; N, 8.07 per cent, $C_{41}H_{50}O_6N_4$ requires C, 70.87; H, 7.25; N, 8.06 per cent. These figures are in agreement with those already published[12,20]. Although, as shown, the analytical figures are very close to those given initially by Goutarel and Janot[12], they subsequently[20], from revised figures, suggested the formula was more likely $C_{45}H_{54}O_7N_4$ or $C_{46}H_{56}O_7N_4$.

Voacangine.

This alkaloid was not obtained in the crystalline state and thus the evidence for its presence was much less complete than for the previously described alkaloids, although sufficiently strong to report its presence.

The base was eluted from the alumina column by benzene and the initial ether fractions, as reported by other workers. It moved in an identical manner to genuine voacangine when run on paper at

both pH 4.0 and 4.7, and on silicic acid plates. The ultra-violet spectrum of the ethanolic solution of the base showed absorption peaks at 225 and 287 mμ, in agreement with published results[10]. The concentration of the alkaloid in the solution was unknown and hence no values for log ϵ could be obtained but the maxima were in the same proportion relative to each other as the published extinction coefficients.

U1.

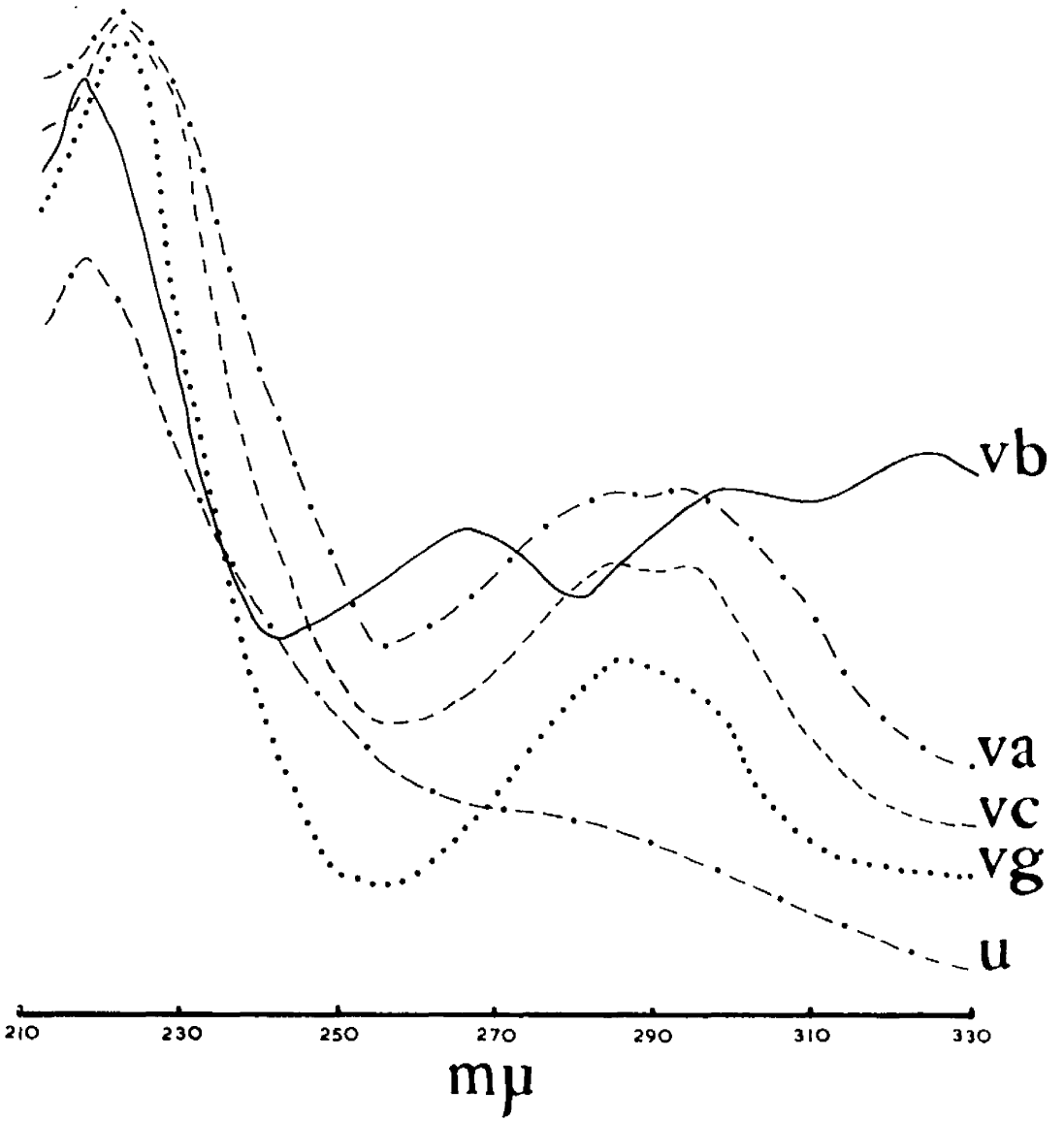
All that can be said of this base is that from its behaviour on paper and silicic acid, it is extremely weak and in ethanol its ultra-violet spectrum showed a single absorption peak at 220 mμ. This latter fact suggests it differs from the Voacanga alkaloids described to date, and it may be significant that 220 mμ is the wave-length of the first of the four vobtusine maxima.

To face p. 117

Fig. 12.

Ultra-violet spectra of the Voacanga alkaloids.

va, voacamine; vb, vobtusine; vc, voacorine;
vg, voacangine; u, U1.

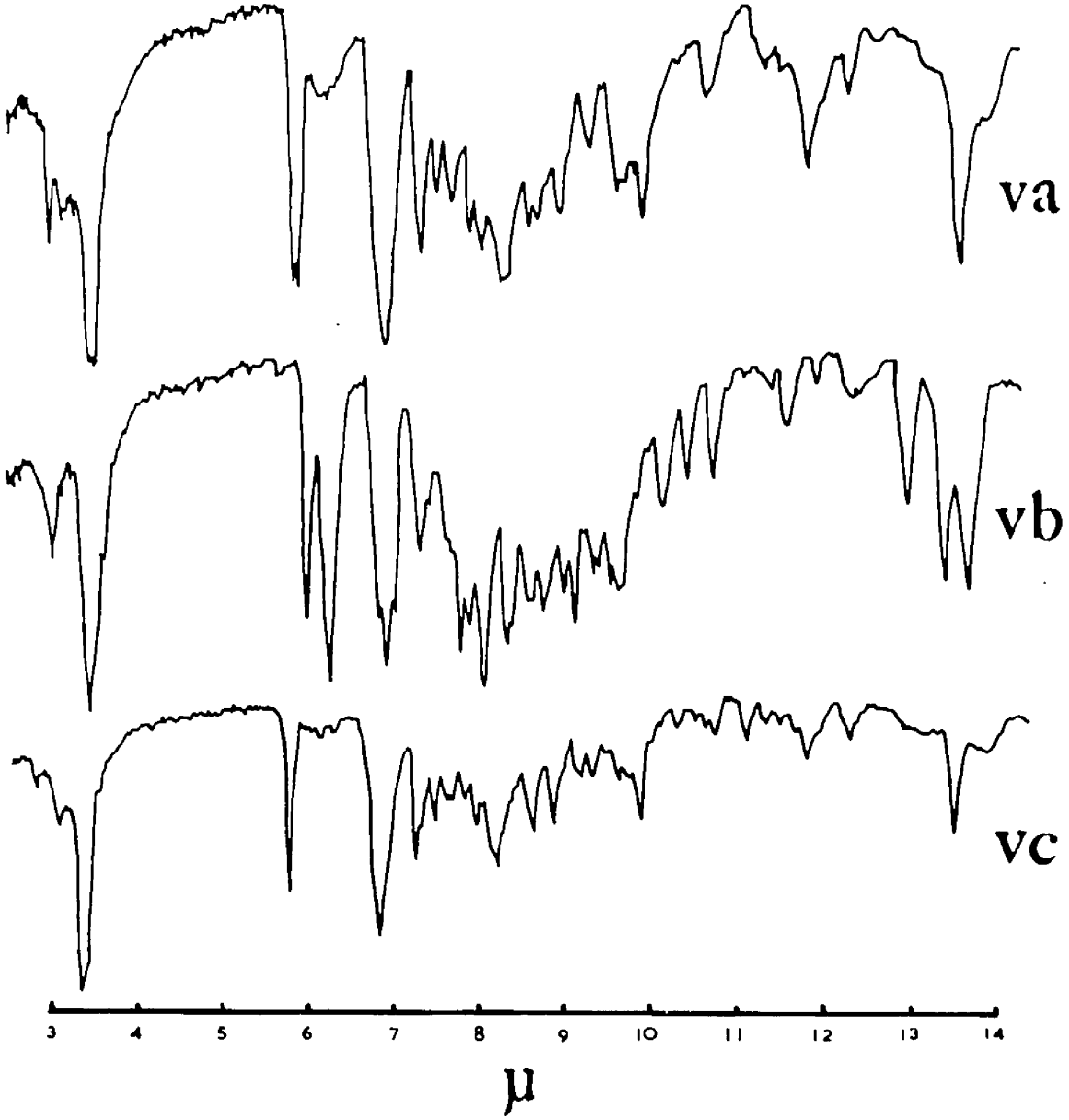

vb
va
vc
vg
u
210
230
250
270
290
310
330
mμ

To face p. 118

Fig. 13.

Infra-red spectra of the Voacanga alkaloids.

va, voacorine; vb, vobtusine; vc, voacorine.


va
vb
vc
3
4
5
6
7
8
9
10
11
12
13
14
μ

Assay of the alkaloids

Alkaloidal assay.

Both samples of V. schweinfurthii were assayed for total alkaloidal content by the following method, which was adapted from the alkaloidal assay of Nux Vomica described in the British Pharmacopoeia, 1963[125]. This method was chosen as the alkaloids of Nux Vomica, like the Voacanga alkaloids, are indole derivatives.

100 ml. of a mixture of ether (two parts) and chloroform (one part) were added to about 10 g. (accurately weighed) of finely powdered bark and allowed to stand for ten minutes. 5 ml. of dilute solution of ammonia were added and the flask shaken continuously for six hours. After standing overnight, the contents of the flask were transferred to a continuous extraction apparatus and extraction continued for another two hours with more of the same solvent mixture.

The extract was then transferred to a separating funnel and extracted with 20 ml. portions of N sulphuric acid until free from alkaloid. The bulked acid fractions were basified with strong solution of ammonia and the alkaloid extracted with 20 ml. portions of chloroform. The bulked chloroform extracts were concentrated in a boiling-water bath, under reduced pressure, to about 2 ml., 5 ml. of 95 per cent ethanol added and the extract evaporated to dryness. The residue was dried at 100° for 30 minutes.

Finally the extract was dissolved in 10 ml. of N/10 sulphuric acid and titrated with N/10 sodium hydroxide, using solution of methyl red as indicator. Each ml. of N/10 sulphuric acid was equivalent to 0.0352 g. of vobtusine (calculated for a M.W. of 704).

The alkaloidal contents of samples VS/1 and VS/2 were thus found to be 0.4 per cent and 1.6 per cent respectively, calculated as vobtusine.

Quantitative analysis of the extraction results.

An analysis was made of the yields of crude base, total alkaloids and of individual alkaloids obtained by the extraction of both samples of V.schweinfurthii, described above. The yields of crude base were 0.9 per cent and 1.5 per cent for VS/1 and VS/2 respectively.

Assessment of the total alkaloidal contents of the two samples was made from the results of column chromatography of the crude bases; VS/1 contained about 0.34 per cent, and VS/2 about 1.0 per cent of alkaloids.

Accurate values for the content of individual alkaloids could not be obtained as they were not distinctly separated by chromatography, but an approximate estimation could be made and it was concluded that VS/1 contained about 0.1 per cent voacamine, 0.2 per cent vobtusine and 0.1 per cent voacorine; and VS/2 contained about 0.2 per cent voacamine, 0.5 per cent vobtusine and 0.3 per cent voacorine.

Conclusions.

The results from the alkaloidal assays and the actual extractions of the alkaloids were in reasonable agreement, both indicating that sample VS/2 contained considerably more alkaloid than VS/1.

Detailed quantitative analyses of the alkaloidal content of V.africana have not been published, but the yield of crude base from the stem bark has been reported as 3.5 per cent[9]. This is considerably higher than the values obtained for V.schweinfurthii but fuller quantitative studies would be necessary before it could be concluded whether there was any consistent difference between the alkaloidal contents of the two species. Evidence contrary to this was obtained by assaying one of the samples of V.africana, VA/4, by the method described above. The alkaloidal content was found to be about 1.5 per cent, calculated as vobtusine, which was very similar to the content of VS/2. This figure could only be considered approximate, however, as it was obtained from a single determination of one sample of particularly thin bark.

P A R T V

ANATOMICAL STUDY OF THE STEM BARK OF VOACANGA SCHWEINFURTHII

Macroscopical structure

Individual pieces of bark varied greatly in appearance. Most pieces were 2 to 4 cm. broad, a very few being less than 1 cm. or up to 6 cm. The length was extremely variable, the majority being 5 to 7 cm. long, but several were only 2 to 3 cm. and a few up to 10 cm. long. Most pieces were 1 to 3 mm. thick, the larger ones 6 to 8 mm. and, occasionally, up to 11 mm. The pieces were all more or less curved, the degree of curvature being inversely proportional to the thickness of the bark; the majority of the pieces were therefore channelled but the thinner, branch bark occurred in quills.

The outer surface was greyish-brown, being pale on the thinner pieces and dark on the thicker ones; occasionally it was fairly smooth with transverse ridges at frequent intervals and exhibiting numerous small, warty spots, but usually it was extremely rough with relatively fine or, occasionally, coarse, longitudinal striations and more prominent, irregular ridges and cracks. The presence of warts, mostly 1 to 2 mm. in diameter but up to 5 mm. in larger pieces, was a constant feature. Occasionally, the rather soft, spongy cork exfoliated, exposing the hard brown outer cortex. (Fig. 14).

Various epiphytes were present on some pieces; these included a closely adherent, white lichen, sometimes with abundant round, black apothecia, and, less commonly, dark green lichen, or yellowish green

or brown moss (Fig. 14).

The inner surface was fairly smooth on the very thin pieces with faint, longitudinal striations; thicker pieces frequently showed much coarser striations. Thin pieces were light brown and thick ones dark greyish brown. Glistening points, due to the presence of crystals of calcium oxalate, were frequently apparent, though these were more clearly seen on the freshly fractured surface. (Fig. 15A).

Large pieces of bark were hard and tough, with a fracture which was short and granular in both the cortex and phloem, although also slightly fibrous in the cortex. Thin pieces were much more brittle and the fracture, though short internally, was extremely fibrous in the cortex.

On the smoothly cut surface, the cork appeared as a dark brown line to the outside of the somewhat paler cortical and phloem tissues. The sclerenchyma appeared as light brown points, on the thinner pieces, and striations, on the thicker pieces.

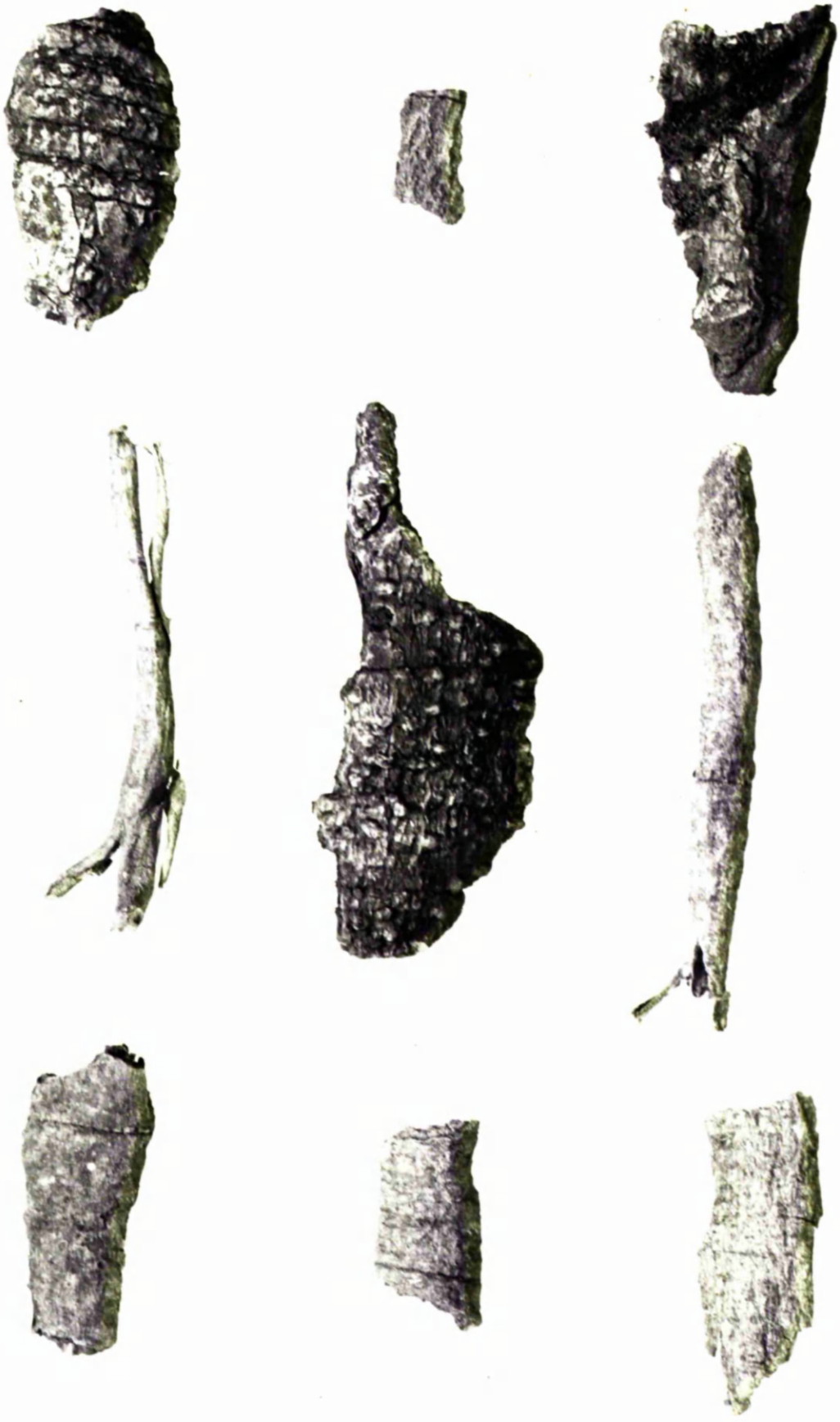
The bark had little odour and a slightly bitter taste.

To face p. 125

Fig. 14.

The outer surface of the stem bark of V. schweinfurthii.

(x $\frac{2}{3}$)



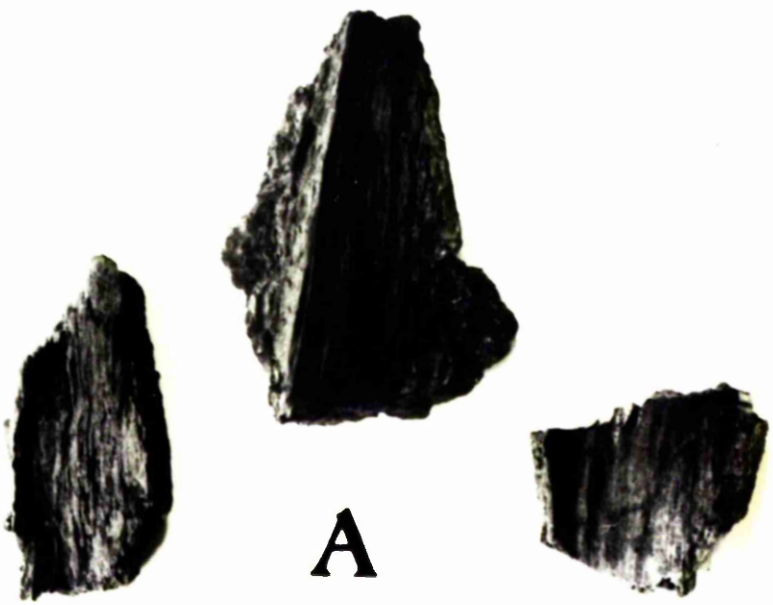
To face p. 126

Fig. 15.

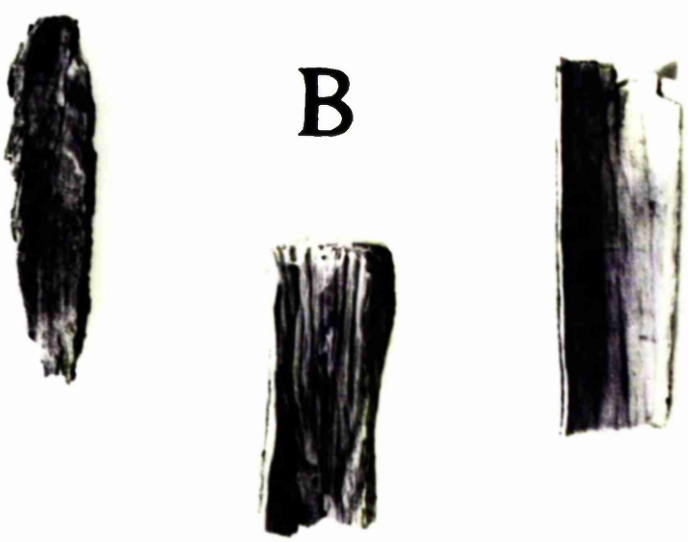
A. The inner surface of the stem bark of *V. schweinfurthii*.

B. The inner surface of the stem bark of *V. africana*.

(Both x $\frac{2}{3}$)



A



B

Microscopical structure

For the microscopical examination of the bark, several pieces of varying size and appearance, that is of colour and surface markings, were chosen from each sample and a series of mounts of transverse, radial longitudinal and tangential longitudinal sections was made. Because of the strongly lignified nature of the material it was difficult to prepare wax blocks of this type of structure due to poor penetration of embedding media[44], therefore the following technique was developed and adopted for the production of the majority of the sections.

Small pieces of the bark were prepared for sectioning by soaking them overnight in Mucilage of Tragacanth containing 0.1 per cent w/w of cetrimide to prevent mould growth, which otherwise occurred very quickly. The perforated metal object holder of a Cambridge rocking microtome was plugged by adding several drops of the mucilage and freezing this in a mixture of solid carbon dioxide and acetone. A small piece of trimmed bark was placed on top of the plug, liberally coated with mucilage from a pipette, and then frozen quickly by dipping into the freezing-mixture. When hard, the whole was fixed to the rocking microtome and the sections cut. Sections of varying thickness were used, from about 5 to 20 μ. Since no method was employed of keeping the microtome head cool during cutting, the block melted

quickly but, with practice and by keeping the knife cool, the procedure could be conveniently carried out.

The sections were collected in water and were ready for mounting, clearing in chloral hydrate solution, or staining, without any further treatment. The method was, therefore, convenient and quicker than wax-embedding techniques. The majority of the sections were cleared in 70 per cent chloral hydrate solution on a slide and mounted in glycerin jelly. Lignin was detected by staining with alcoholic solution of phloroglucinol and hydrochloric acid, and suberin by staining with Tincture of Alkanet or Sudan III.

Crystals of calcium oxalate present were slowly soluble in 66 per cent sulphuric acid without effervescence.

Note: Conventions used for recording cell dimensions are as follows: R, radial; T, tangential; H, axial; B, wall thickness; and D, diameter. Where a full range of values is given, they have resulted from a total of 600 measurements taken from twelve different pieces of bark and expressed as 'a to M1 to M2 to b', where 'a' was the smallest value, 'b' the highest value, and 'M1 to M2' represents the arithmetic mean $\pm$ the standard deviation. When the measurement of such a number of values was impracticable, as large a number as possible was determined, such values not being expressed in the above fashion.

The amount of cork present varied from 5 layers of cells to over 100 in a very few cases; normally about 12 layers were seen. Except in very thin pieces of bark, patches of parenchymatous cells with slightly thickened, brownish walls were dispersed among the cork cells, generally in association with small groups of stone cells. When a thick cork was present, the cork cells sometimes occurred in distinct layers alternating with layers of this parenchyma; there were usually about 12 rows of cork cells in each layer. (Figs. 16A, B and C, and 17A, B and C).

Occasionally a well-formed rhytidome was present, where a large piece of cortex had been cut off, and this usually consisted of dead parenchymatous cells, sclereids, and, frequently, cortical fibres. (Figs. 16A, 17A and 18).

The cork cells occurred in fairly regular radiate rows and were characteristically tabular, and elongated axially and tangentially (Figs. 18 and 19). The tangential walls were much thicker (many being up to 5μ thick) than the radial walls, which were usually wavy, and were sometimes beaded when seen in surface view (Fig. 28). Although this beading was relatively rare, when present it was usually well-marked. The cells were lignified, staining pink to deep red with solution of phloroglucinol and hydrochloric acid and measured R 8 to 16 to 28 to 56 μ, T 14 to 27 to 45 to 70 μ and H 10 to 21 to 39 to 62 μ.

The phellogen was seldom well-defined but, when apparent, consisted of regular thin-walled cells, mostly measuring about R 10 μ, T 28 μ and H 28 μ. These cells frequently contained more or less cubical prismatic crystals of calcium oxalate of about 21 μ in edge. (Figs. 18 and 19).

The phelloderm was well-defined, consisting largely of a band of sclereids of about 5 to 7 (Figs. 18 and 19), or, occasionally, numerous (Fig. 16C) horizontal rows of cells which were also radiating in more or less uniform rows from the cells of the phellogen, forming an almost complete, lignified sheath below the cork. The cells were isodiametric or somewhat elongated tangentially, measuring R 14 to 26 to 54 to 128 μ, T 16 to 34 to 62 to 110 μ, H 18 to 30 to 50 to 80 μ and B 4 to 5 to 15 to 28 μ. They exhibited fairly heavily and uniformly lignified and striated walls having numerous, simple, tubular pits. Associated with the groups of sclereids were groups of thin-walled parenchymatous cells of similar size, containing occasional prismatic crystals of calcium oxalate (Fig. 19).

The cortex was obvious in many sections and occupied up to one third of the width of the bark in thin pieces (Fig. 16B), but in thick pieces it was frequently ill-defined and accounted for a much smaller proportion of the bark (Fig. 16C). It consisted largely of parenchyma, the cells of which had walls of varying thickness. The cells were elongated tangentially, sometimes very markedly, and in

places were ceratenchymatous (Figs. 20A and B, and 21A and B). When the cortex was obvious, sclereids were either absent, or present in small amounts towards the inner layers. In thick pieces of bark the sclereid layer of the phelloderm was well-defined and exterded very close to the sclereid bands of the phloem. In such cases, a few tangentially and axially elongated groups of sclereids occurred in the cortex similar to those of the phloem, though rather smaller, such that the two could not always be differentiated. Extremely long fibres occurred singly or in groups of two or three. They were sparse in thick bark but abundant in thin pieces, and, in these, tended to be in radial rows (Figs. 16A and B, and 20B); they were thick-walled but generally unlignified, though many showed slight lignification in the primary wall only and in some very rare cases the entire wall was lignified. The complete wall showed concentric rings in transverse sections (Fig. 20B) and longitudinal striations, which were not always distinct, in longitudinal sections (Fig. 21B). The walls exhibited single spiral markings, or complex multiple spirals running in opposite directions on the same wall giving a criss-cross appearance. It was presumed that this effect resulted from the striations in the various lamellae of the secondary wall running in different directions, as described by Mühlethaler[126]. The fibres had a fairly uniform diameter, though occasionally bulbous swellings appeared near the ends and, very occasionally, at intervals

along the lengths of the fibres. The walls were more or less uniformly thick, so that the shape of the lumen followed the outline of the fibre, and generally closed some length from the end of the fibre. At the end, part of the wall was frequently continued as a thread-like tail. In longitudinal sections and with isolated fibres, the primary wall was occasionally broken off at the tip, the secondary wall projecting to give a characteristic appearance (Fig. 28). The walls were pitted with simple pits which were particularly obvious in the swellings, and nodes were present similar to those found in flax fibres. As these nodes were only observed in isolated fibres, that is not in sections, they probably resulted from distortion of the fibres during their isolation. (Fig. 22).

Occasionally, generally when occurring in a group, the fibres were surrounded by a sheath of parenchymatous cells containing prism crystals of calcium oxalate (Fig. 29).

Similar fibres have been reported in Rauwolfia species[51,52].

Due to the difficulty in isolating these very long fibres intact, a full range of measurements could not be compiled. Although some were fairly short, about 2 mm., the length frequently exceeded 10 mm., values of about 13.5 mm. being quite common, with occasional readings up to 22 mm., with D 10 to 21 to 39 to 62 μ and B 4 to 8 to 16 to 30 μ.

More or less regular prismatic crystals of calcium oxalate,

measuring 6 to 13 to 31 to 52 μ, occurred in the cortical parenchyma in greatly varying amounts (Figs. 20A and 21B).

In the phloem were numerous, large, tangentially and axially elongated groups of sclereids, which in most pieces of bark were so large and numerous that they ran together forming a heavy mass. After softening the tissues by heating with 5 per cent aqueous potassium hydroxide solution, these sheets of sclereids could be readily removed from the phloem intact, showing oval perforations corresponding to the positions of the medullary rays. The thicker the bark, the greater was the proportion of sclereids present. The innermost phloem was usually free from lignification. Most individual sclereids were larger than those of the phelloderm, measuring R 14 to 36 to 76 to 156 μ, T 18 to 34 to 78 to 156 μ, H 18 to 37 to 115 to 300 μ and B 6 to 12 to 24 to 48 μ. The walls were heavily lignified, the lumen being correspondingly very much reduced, and the striations which were uniform in the small cells, were much distorted in the larger sclereids. Small cells were uniform and isodiametric, but they became increasingly irregular with increase in size, the largest being tortuous and branched, intermeshing and imparting great strength to the sheets. This tortuous appearance was best seen after disintegration and examination of the isolated elements. The walls exhibited numerous simple and branched pits. (Figs. 23, 24A, 25A and 27).

Narrow, elongated, lignified sclereids occurred on the edges of

some of the sclereid groups towards the inner phloem, or in separate small groups (Fig. 25A). In a very few sclereids the lumen was filled with an orange, amorphous substance (Fig. 29).

Cells of the abundant phloem parenchyma varied from thin to thick-walled, the walls being occasionally beaded. The cells were somewhat elongated tangentially and markedly elongated axially, especially those of the inner phloem. (Figs. 23, 24A and B, 25A, B and C, and 26A and B).

Numerous latex vessels were also present in the phloem, the widest occurring in the outer phloem. They consisted of simple, unbranched tubes running axially, of indeterminate length, with slightly thickened, somewhat refractive walls. Only those with abundant granular content could be positively identified and the diameters of those measured varied from about 20 μ to 60 μ, the majority being between 22 and 28 μ. (Figs. 23 and 26A).

Prismatic crystals of calcium oxalate occurred infrequently in the outer phloem (Figs. 24A and B), but were almost always present in the innermost phloem as long, prismatic needles lying in markedly axially elongated cells of the phloem parenchyma, there frequently being more than one crystal per cell (Figs. 25A and 26B - Type B). These crystals were responsible for the glistening points visible on the inner surface of the bark. Intact needles were either pointed at both ends or pointed at one end and notched at

the other. They measured 32 to 59 to 165 to 356 μ in length and 2 to 9 to 27 to 58 μ in breadth. (Fig. 29).

The medullary rays were more or less straight, widening only slightly towards the cortex, as seen in transverse section. They were markedly heterogeneous, most clearly observed in tangential longitudinal sections, consisting of two sizes of cells, one (Type A) being much smaller than the other (Type B). In transverse section, the small cells had slightly thickened, frequently wavy walls with few air spaces between the cells and formed rays 1 to 6 cells wide, though normally 3-celled throughout most of their length (Figs. 23 and 24A and B). The large cells had slightly thicker walls which were rarely wavy and formed uniseriate rays (Fig. 24B). In radial longitudinal section, the small cells exhibited the characteristic 'brick-wall' formation being in rays, occasionally only a few cells deep, but normally about 10 to 12 cells deep (Figs. 25A and 26A). The large cells had a similar arrangement and were situated above and below the rays of small cells, though they were frequently difficult to distinguish. In tangential longitudinal section, the small cells appeared as normal sub-oval areas, generally about 4 to 6 cells wide and 6 to 12 cells deep, and the large cells occurred as uniseriate, axial rays of axially elongated cells, linking groups of small cells (Fig. 26B). Occasionally both types of cell had some yellow, granular content. Cells of Type A measured R 20 to 36 to 60

to 94 μ, T 6 to 13 to 23 to 40 μ and H 10 to 14 to 22 to 30 μ, and those of Type B measured R 8 to 18 to 34 to 56 μ, T 14 to 26 to 50 to 86 μ and H 18 to 32 to 56 to 90 μ.

Sieve-tubes, in thinner pieces of bark, were thin-walled, fairly uniformly wide structures, with oblique end-walls having large sieve-areas (Figs. 25A and B, and 26B). In thicker bark, the tube walls were thicker, and numerous, large, oval sieve-areas occurred on the side-walls as well as on the end-walls; the former were frequently distributed along the entire length of the tube (Fig. 25C). Since only tubes showing sieve-plates clearly could be positively identified, the full range of measurements could not be compiled; however, the diameters of those measured varied from about 11 μ to 37 μ, the majority being in the range 18 to 22 μ.

Starch grains occurred in very variable quantities in the cortical parenchyma, phloem parenchyma and medullary rays, the bulk usually being in the cortex but the distribution was as variable as the quantity; in some cases it was virtually confined to the parenchyma of the cortex and the phloem, but elsewhere it was confined to the cells of the medullary rays. The grains were sub-spherical, or polyhedral with rounded edges, but many were compound with two to four components. The hilum appeared as a central point or, in the case of larger grains, as a short line, although it was frequently indistinct. Striations were absent. Single grains measured 2 to 4

to 8 to 18 μ in diameter; compound grains had a maximum diameter of about 8 to 14 μ, most being about 12 μ. (Fig. 28).

To face p. 138

Fig. 16.

Diagrammatic transverse sections of the bark of V. schweinfurthii illustrating the distribution of tissues.

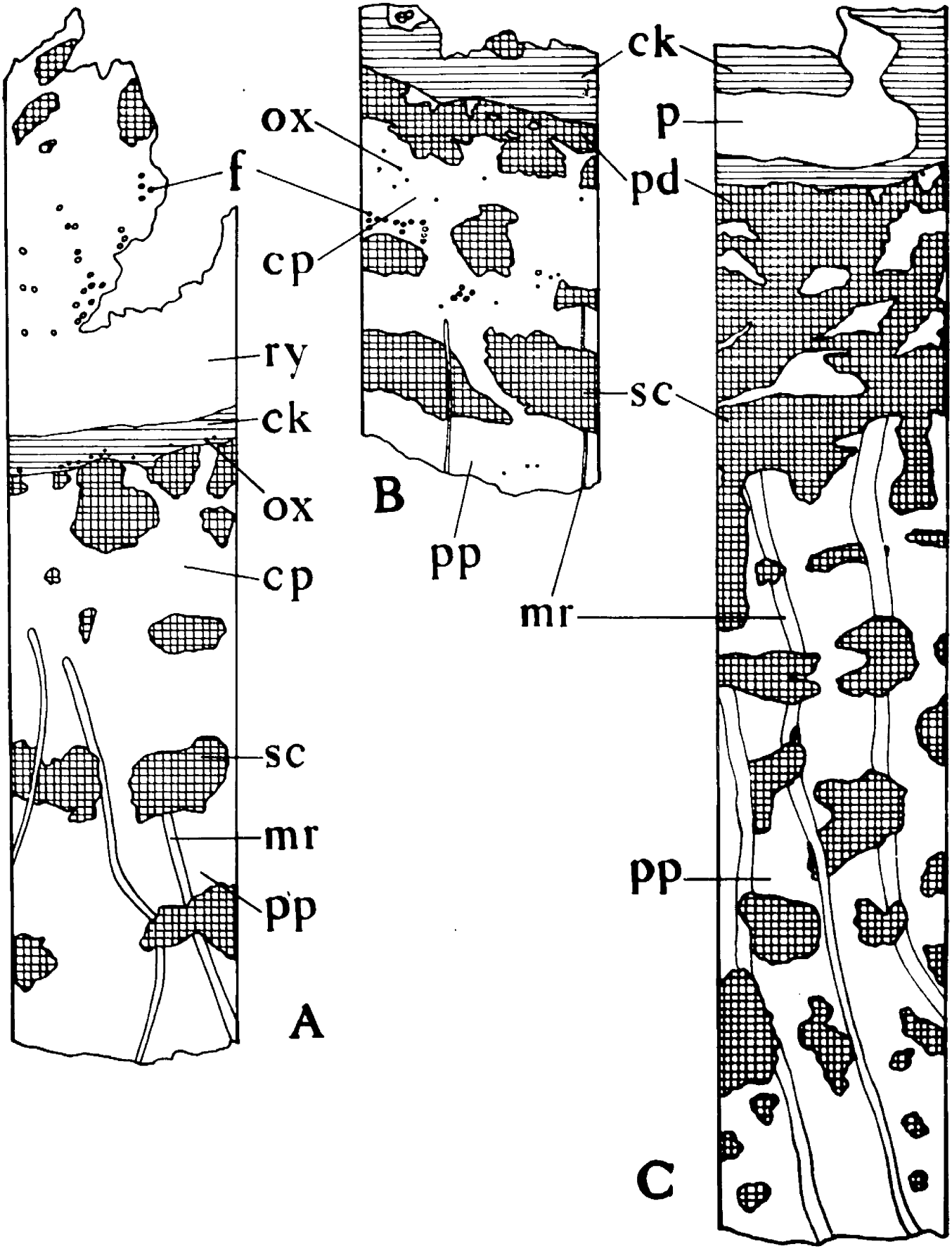
A. bark with well-developed rhytidome.

B. thin bark.

C. thick bark.

(x 32)

ck, cork; cp; cortical parenchyma; f, fibre; mr, medullary ray; ox, crystal of calcium oxalate; p, parenchyma; pd, phelloderm; pp, phloem parenchyma; ry, rhytidome; sc, group of sclereids.


f
ry
ck
ox
cp
sc
mr
pp
A
ox
cp
B
pp
ck
p
pd
sc
mr
pp
C

To face p. 139

Fig. 17.

Diagrammatic radial longitudinal sections of the bark of *V. schweinfurthii* illustrating the distribution of tissues.

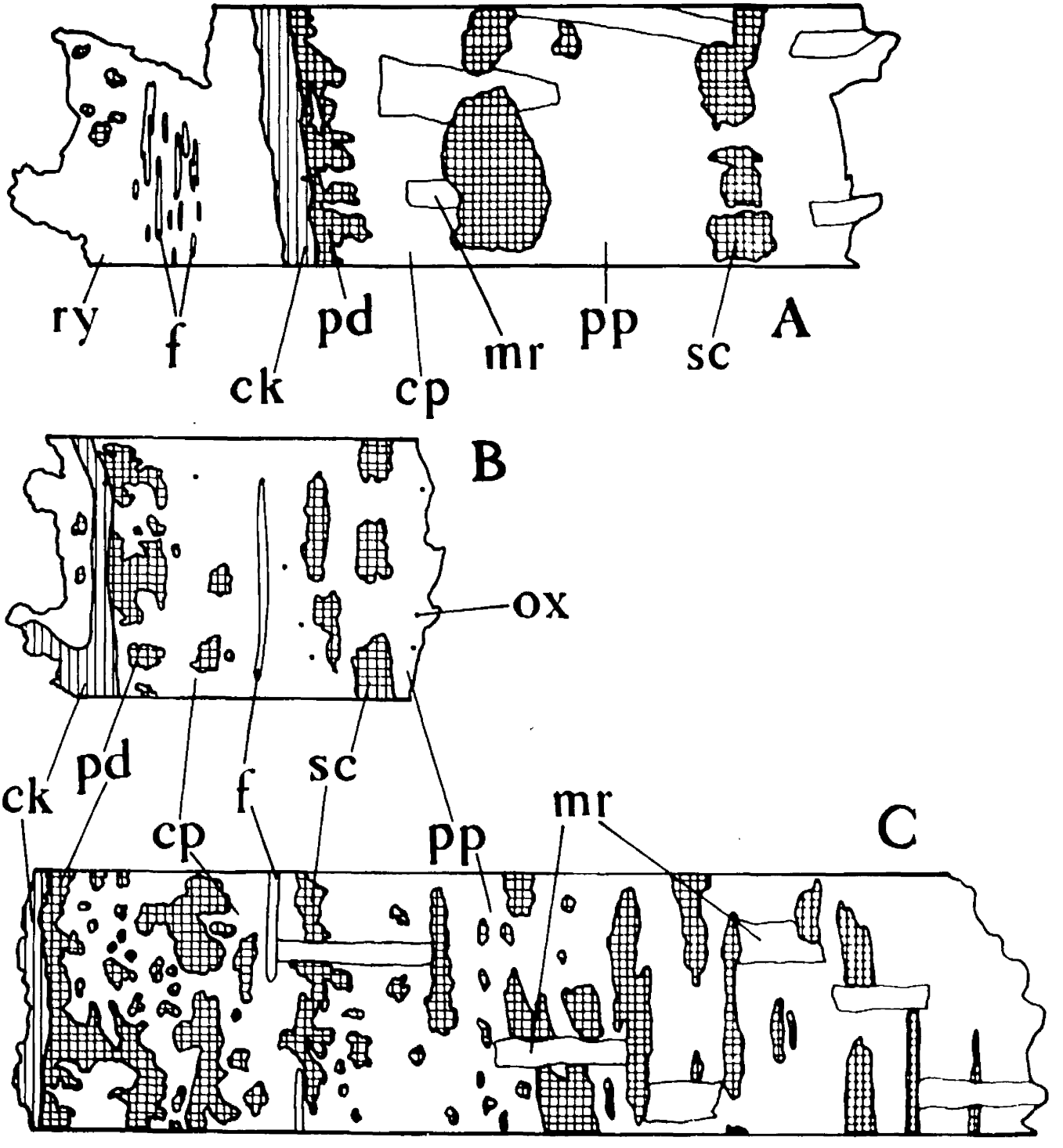
A. bark with well-developed rhytidome.

B. thin bark.

C. thick bark.

(x 22)

ck, cork; *cp*, cortical parenchyma; *f*, fibre; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *pd*, phelloderm; *pp*, phloem parenchyma; *ry*, rhytidome; *sc*, group of sclereids.


ry
f
ck
pd
cp
mr
pp
sc
A
B
ox
ck
pd
f
sc
cp
pp
mr
C

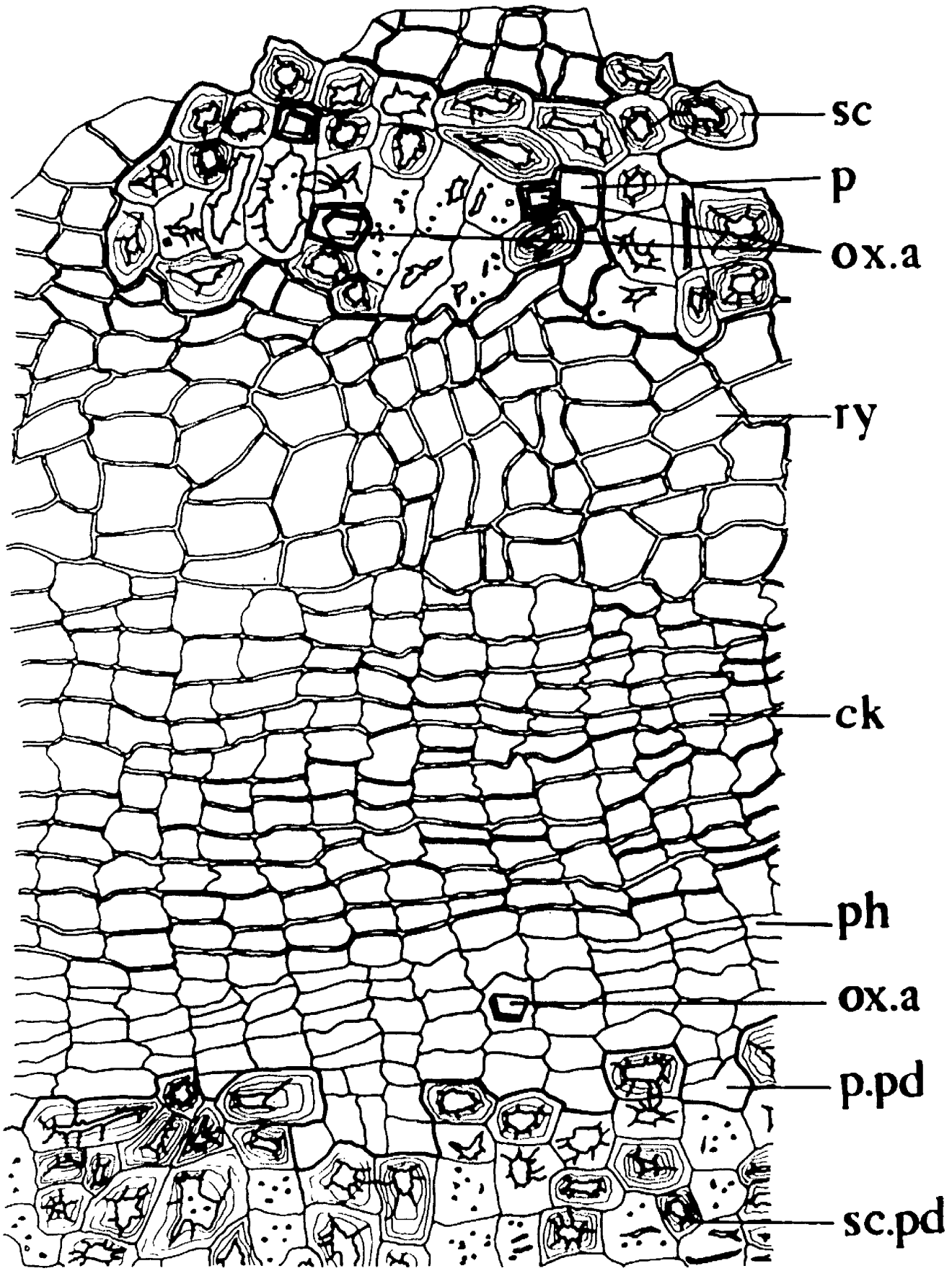
To face p. 140

Fig. 18.

Transverse section of the rhytidome, cork, phellogen and phelloderm of the bark of *V. schweinfurthii*.

(x 250)

ck, cork; ox.a, crystal of calcium oxalate (Type A); p, parenchyma; ph, phellogen; p.pd, parenchyma of the phelloderm; ry, rhytidome; sc, sclereid; sc.pd, sclereid of the phelloderm.


sc
p
ox.a
ry
ck
ph
ox.a
p.pd
sc.pd

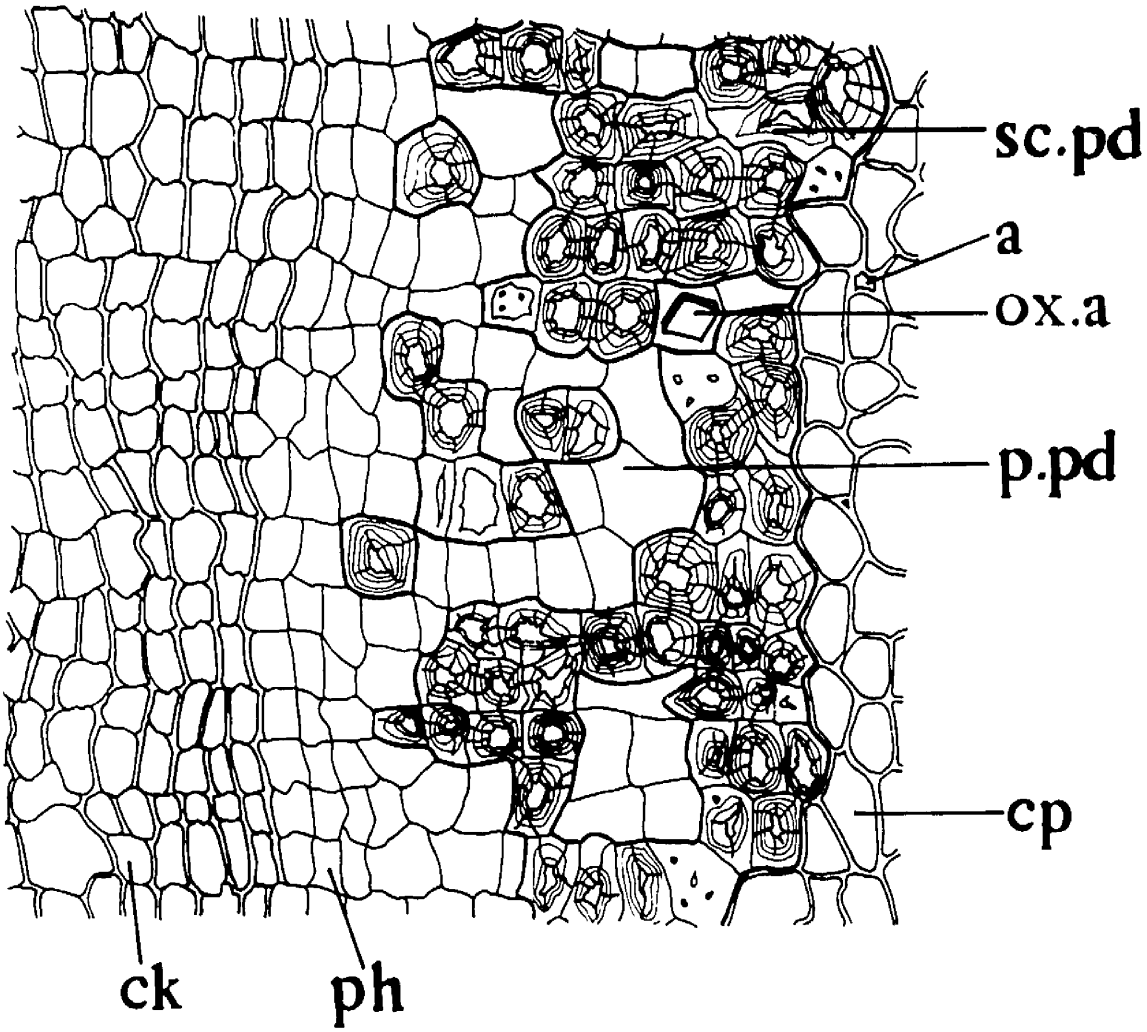
To face p. 141

Fig. 19.

Radial longitudinal section of the cork, phellogen, phelloderm and outermost cortex of the bark of V. schweinfurthii.

(x 250)

a, air space; ck, cork; cp, cortical parenchyma; ox.a, crystal of calcium oxalate (Type A); ph, phellogen; p.pd, parenchyma of the phelloderm; sc.pd, sclereid of the phelloderm.


sc.pd
a
ox.a
p.pd
cp
ck
ph

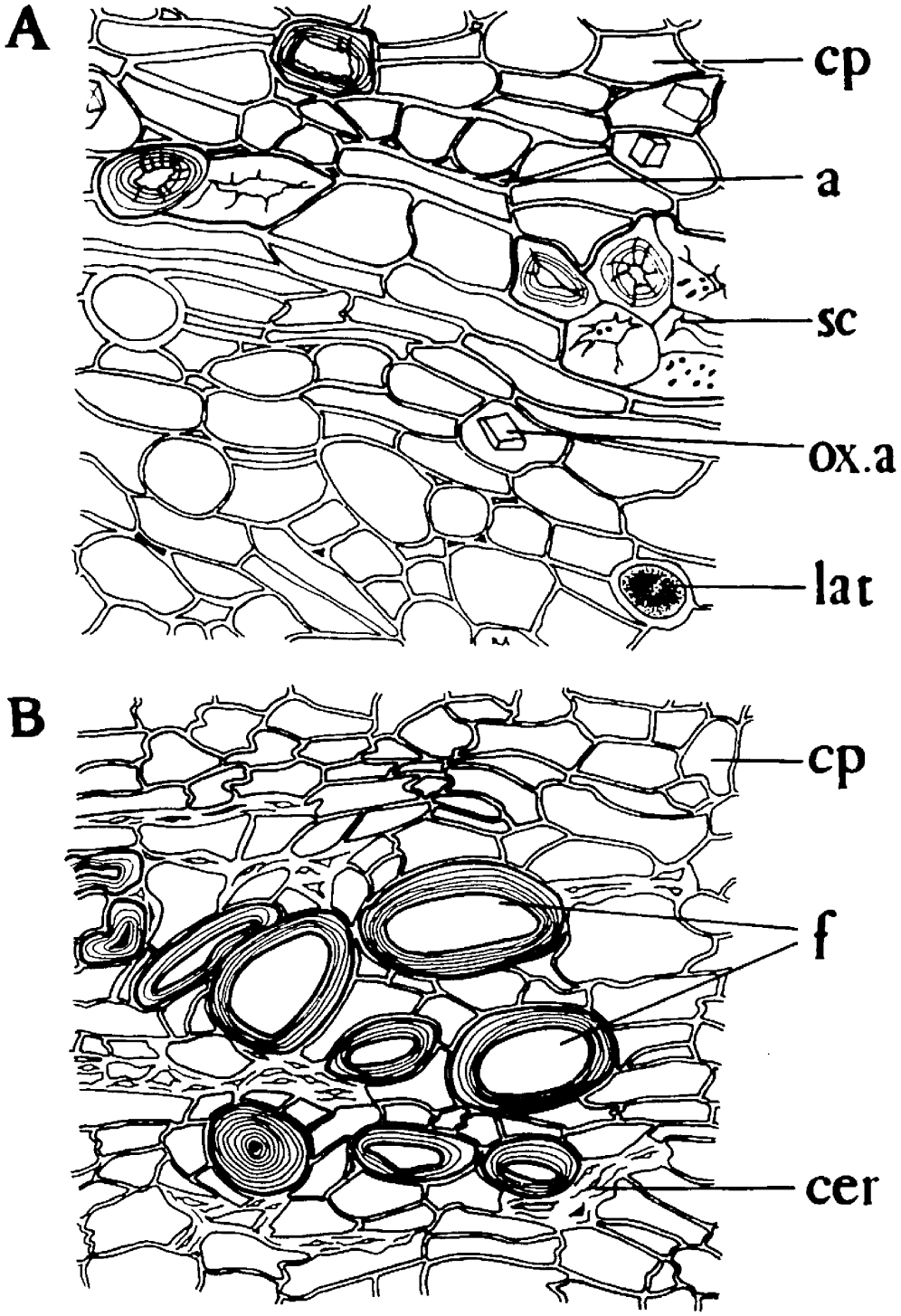
To face p. 142

Fig. 20, A and B.

Transverse sections of the cortex of the bark of *V. schweinfurthii*.

(x 250)

a, air space; *cer*, ceratenchyma; *cp*, cortical parenchyma; *f*, fibre; *lat*, latex vessel; *ox.a*, crystal of calcium oxalate (Type A); *sc*, sclereid.


A
cp
a
sc
ox.a
lat
B
cp
f
cer

To face p. 143

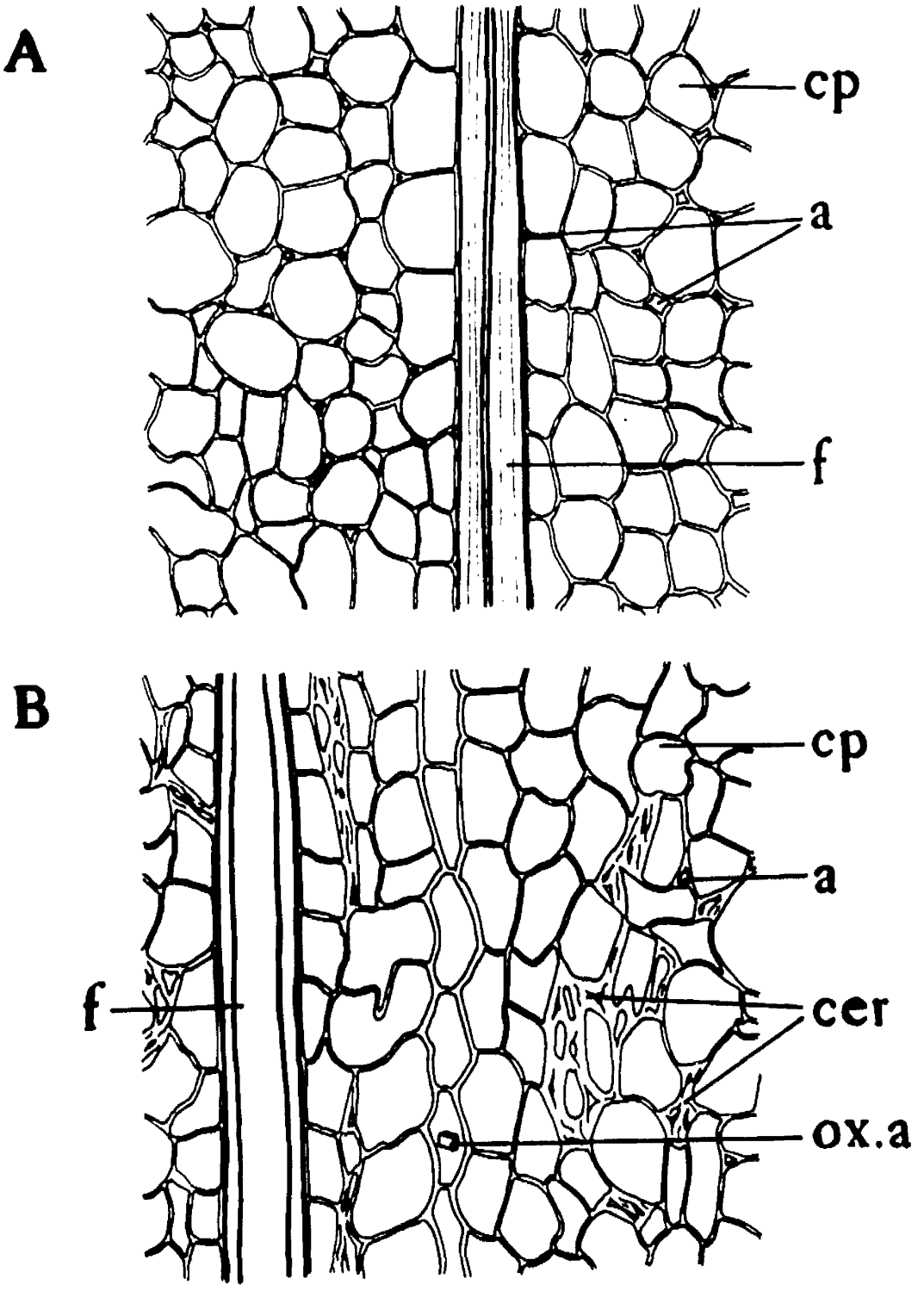
Fig. 21, A and B.

Radial longitudinal sections of the cortex

of the bark of *V. schweinfurthii*.

(x 250)

a, air space; *cer*, ceratenchyma; *cp*, cortical parenchyma; *f*, fibre; *ox.a*, crystal of calcium oxalate (Type A).


A
cp
a
f
B
cp
a
f
cer
ox.a

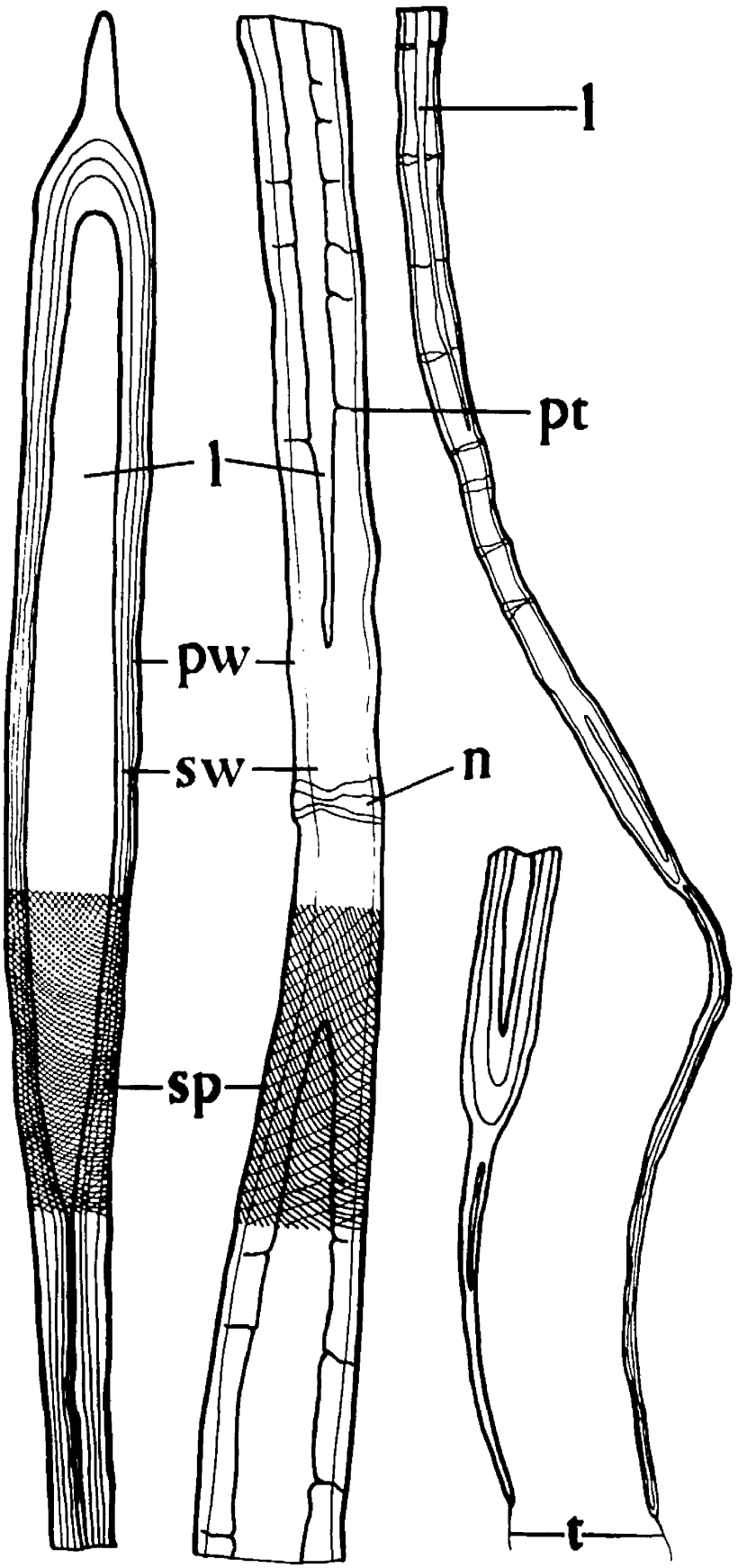
To face p. 144

Fig. 22.

Fragments of fibres isolated from the cortex of the bark of *V. sohweinfurthii*.

(x 250)

l, lumen; *n*, node; *pt*, pit; *pw*, primary wall; *sp*, spiral markings; *sw*, secondary wall; *t*, thread-like tail.


l
pt
l
pw
sw
n
sp
t

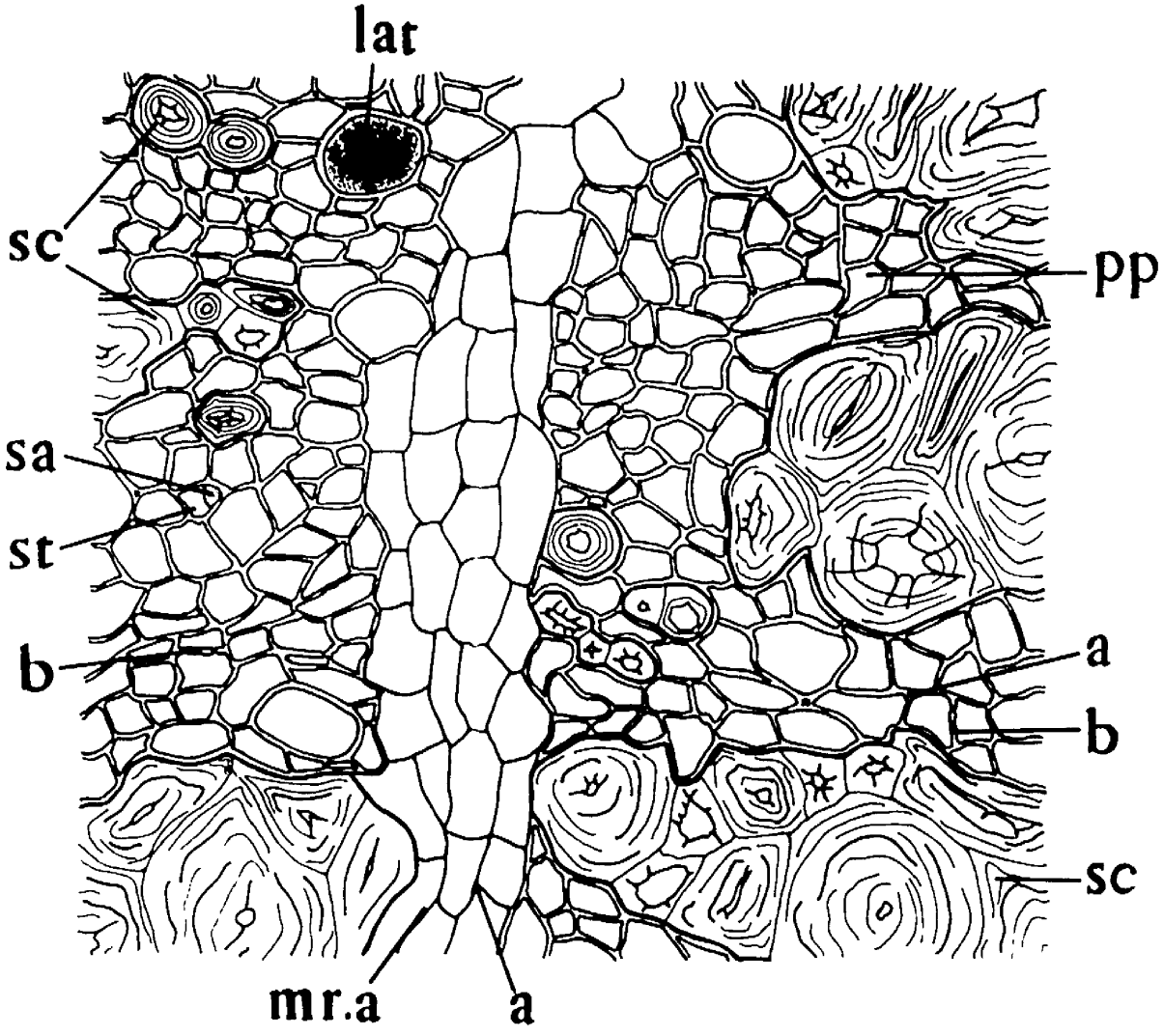
To face p. 145

Fig. 23.

Transverse section of the outer phloem of the bark of *V. schweinfurthii*.

(x 250)

a, air space; *b*, beaded wall; *lat*, latex vessel; *mr.a*, medullary ray (cells of Type A); *pp*, phloem parenchyma; *sa*, sieve-area; *sc*, sclereid; *st*, sieve-tube.


lat
sc
pp
sa
st
b
a
b
sc
mr.a
a

To face p. 146

Fig. 24.

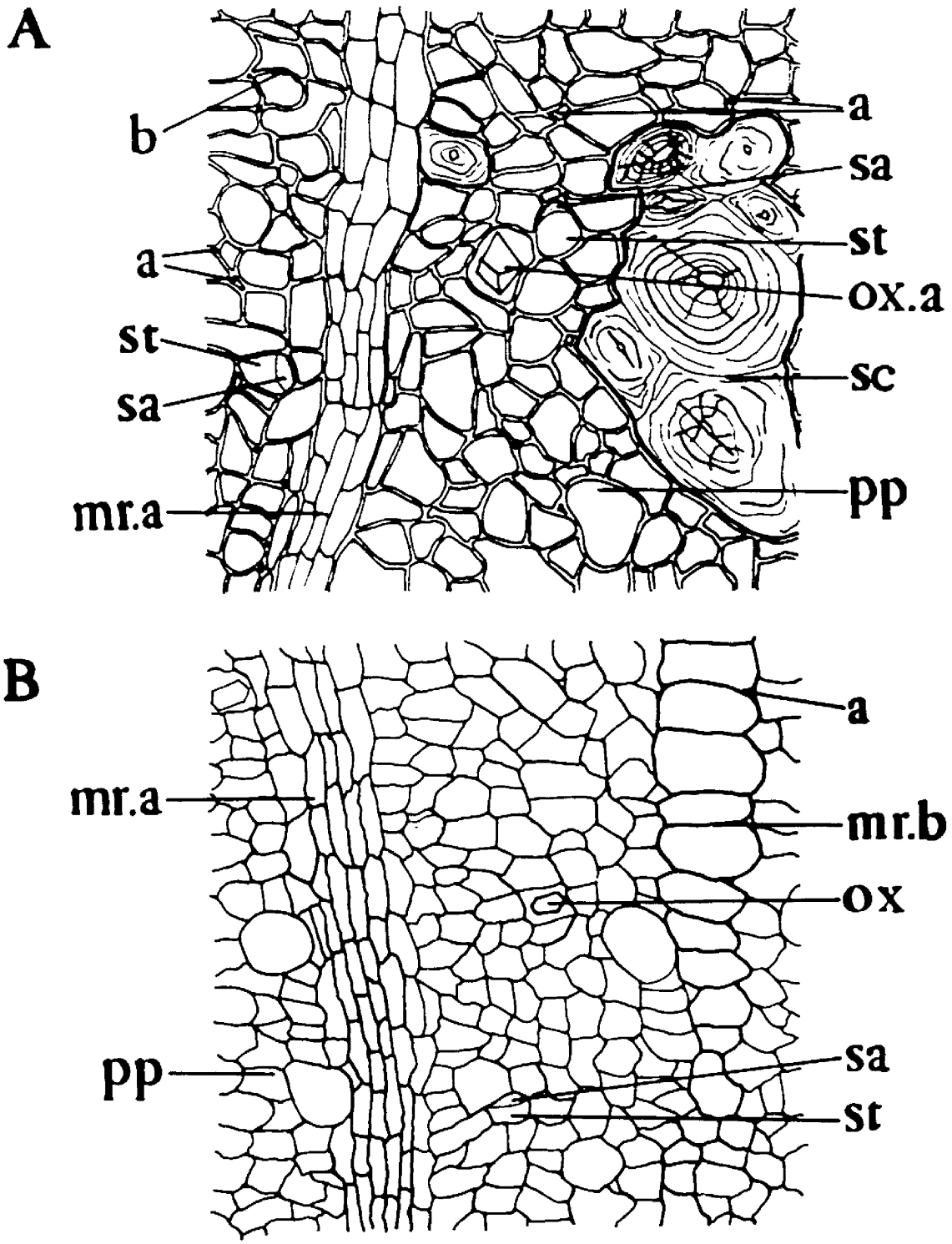
Transverse sections of the phloem of the bark of V. sohweinfurthii.

A. middle phloem.

B. inner phloem.

(x 250)

a, air space; b, beaded wall; mr.a, medullary ray (cells of Type A); mr.b, medullary ray (cells of Type B); ox, crystal of calcium oxalate; ox.a, crystal of calcium oxalate (Type A); pp, phloem parenchyma; sa, sieve-area; sc, sclereid; st, sieve-tube.


A
b
a
sa
st
ox.a
sc
pp
mr.a
B
a
mr.a
mr.b
ox
pp
sa
st

To face p. 147

Fig. 25.

Radial longitudinal sections of the phloem of the bark of *V. schweinfurthii*.

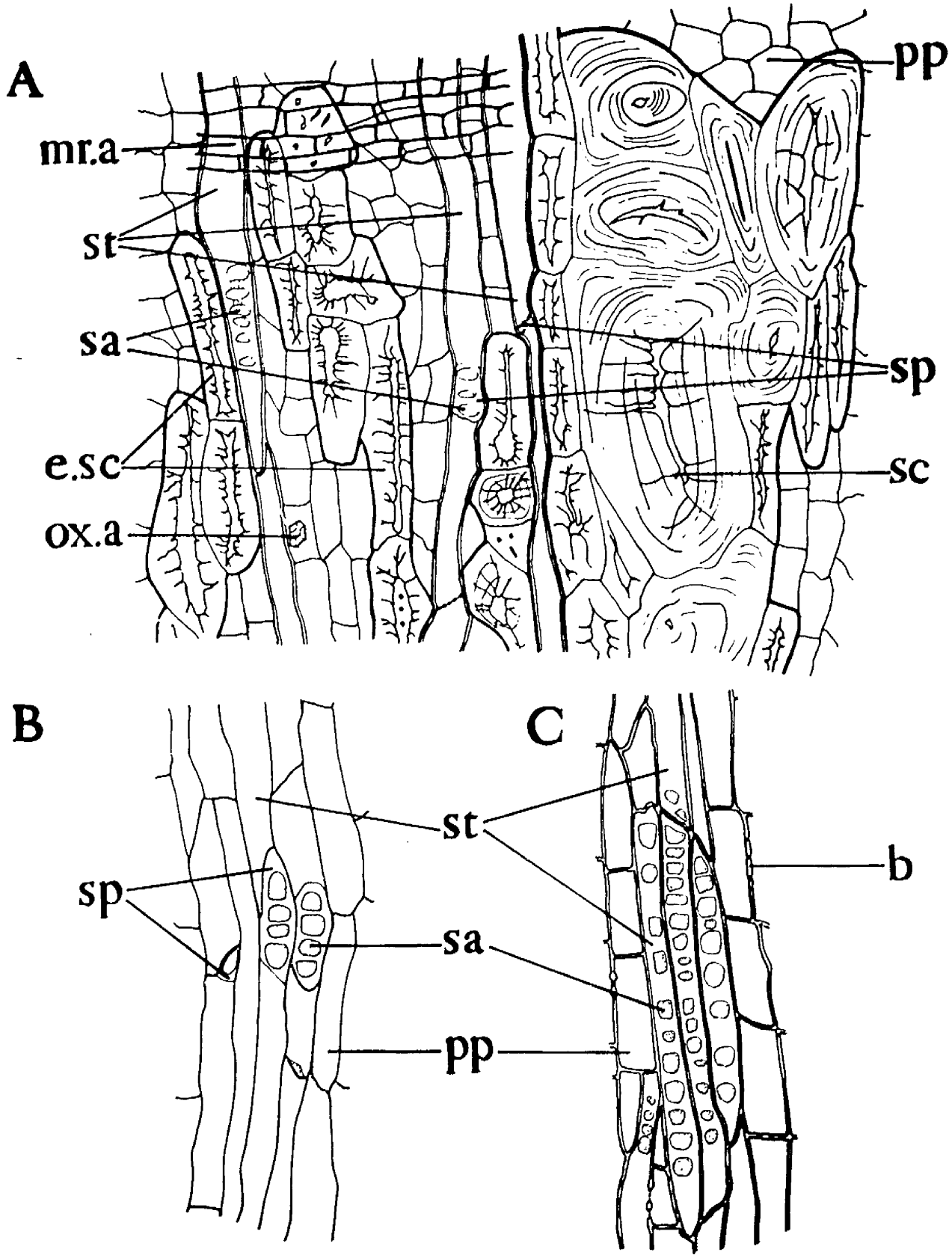
A. outer phloem.

B. inner phloem from thin bark.

C. inner phloem from thick bark.

(x 250)

b, beaded wall; *e.sc*, elongated sclereid; *mr.a*, medullary ray (cells of Type A); *ox.a*, crystal of calcium oxalate (Type A); *ox.b*, crystal of calcium oxalate (Type B); *pp*, phloem parenchyma; *sa*, sieve-area; *sc*, sclereid; *sp*, sieve-plate; *st*, sieve-tube.


A
mr.a
st
sa
e.sc
ox.a
pp
sp
sc
B
sp
st
sa
pp
C
st
b

To face p. 148

Fig. 26.

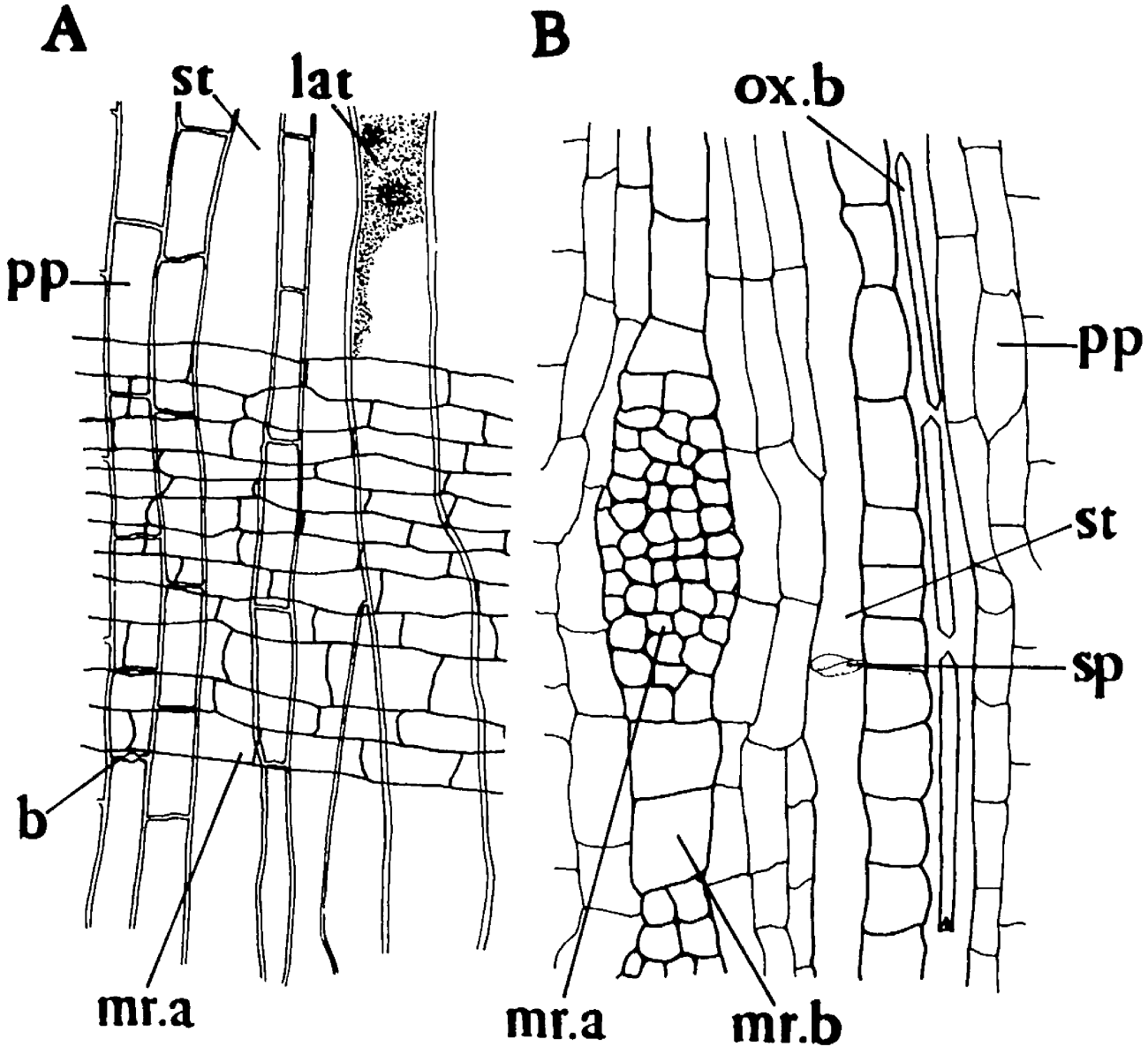
Longitudinal sections of the inner phloem of the bark of *V. schweinfurthii*.

A. radial longitudinal section.

B. tangential longitudinal section.

(x 250)

b, beaded wall; *lat*, latex vessel; *mr.a*, medullary ray (cells of Type A); *mr.b*, medullary ray (cells of Type B); *ox.b*, crystal of calcium oxalate (Type B); *pp*, phloem parenchyma; *sp*, sieve-plate; *st*, sieve-tube.


A
st
lat
pp
b
mr.a
B
ox.b
pp
st
sp
mr.a
mr.b

To face p. 149

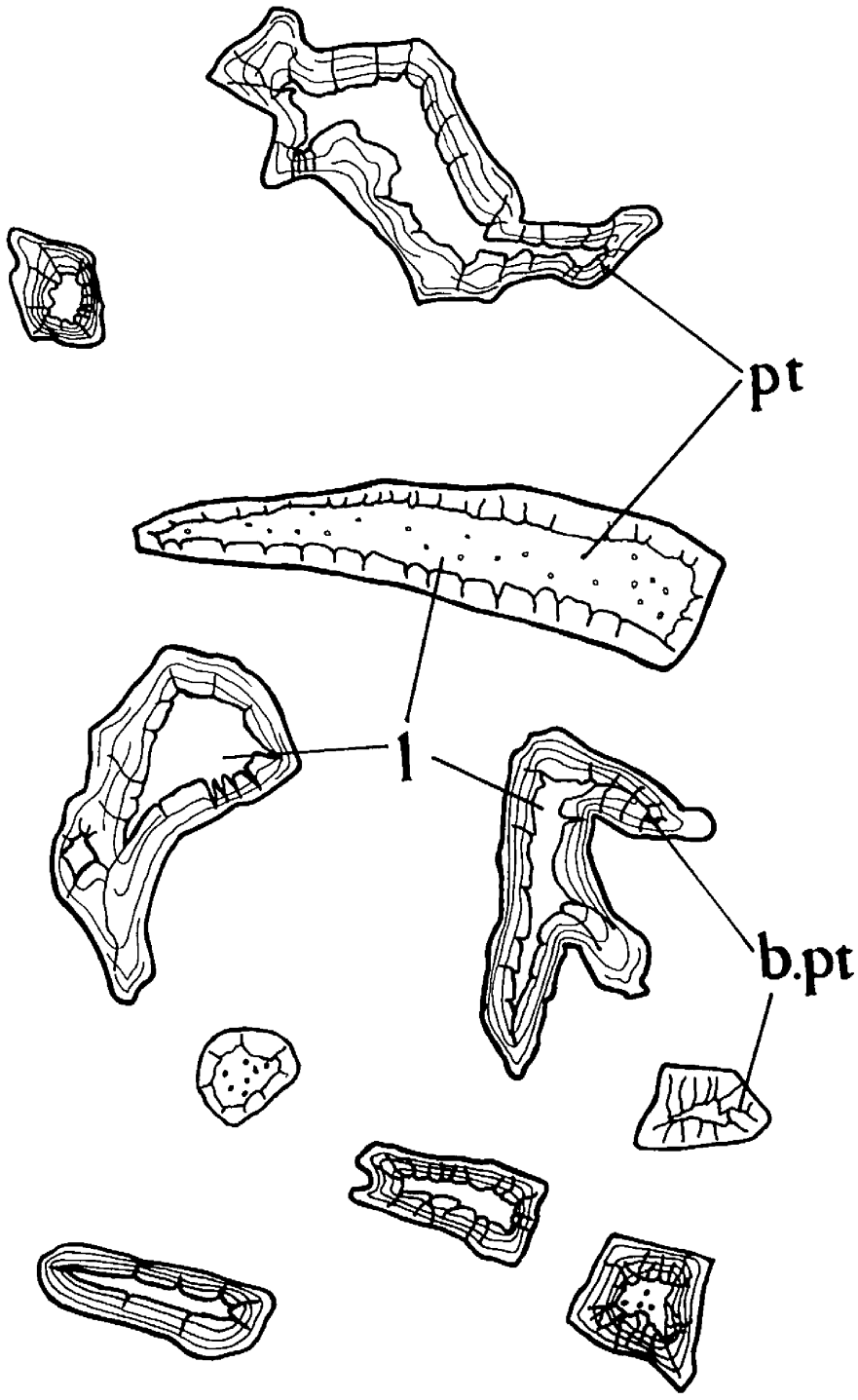
Fig. 27.

Isolated sclereids from the bark of

V. sohweinfurthii.

(x 250)

b.pt, branched pit; *l*, lumen; *pt*, pit.


pt
l
b.pt

Powder

The colour of a No. 60 powder of the stem bark of V. schweinfurthii varied from light brown to pale yellowish brown. The odour was slight and musty, and the taste faintly bitter. In texture it was very gritty and fibrous, its fibrous nature being accentuated by the occurrence of occasional tangled masses.

When mounted in dilute glycerol, the powder was seen to consist of greyish masses of parenchyma, orange to brown sheets of cork, pale yellow groups of sclereids and elongated pieces of fibres. Also present were numerous prismatic crystals, both needle-like and cubical, and numerous granules which stained blue with dilute solution of iodine, showing them to be starch. These starch grains occurred both free and enclosed in parenchymatous cells. They occurred primarily as single grains, subspherical, oval or polyhedral with rounded edges, and as compound grains of two to four components. When apparent the hilum was a central point, or a short line in some of the larger, more oval grains.

When stained with solution of phloroglucinol and hydrochloric acid, abundant lignified tissue was seen to be present. Most strongly stained were the groups of sclereids and also some isolated stone cells; the cork stained pink to deep red; and the fibres, although frequently showing no staining, generally were pink in the primary wall and

occasionally stained red in the entire wall. A very few fragments of vessels, from traces of adherent xylem, were stained red.

To allow a more detailed study of the cell structures, the powder was cleared with chloral hydrate solution. Abundant masses of cork were present. These varied in colour from being almost colourless to brownish orange or brown. The cells were moderately thick-walled and polygonal in outline, when seen in surface view, but in section the masses consisted of regular rows of tabular cells. Very occasional, but marked, beading was observed on some of the cell walls when seen in surface view.

Numerous masses of parenchymatous cells were present, a few having brownish yellow, wavy walls, presumably from rhytidome, but most being colourless. The walls were mainly smooth though of variable thickness, but some of the thicker-walled cells had marked and abundant beading. Occasionally the cells had strap-like, unlignified thickening. The cells were usually subspherical but with relatively few air spaces, or elongated, sometimes markedly, as when associated with sieve-tissue. A few cells contained regular, prismatic crystals of calcium oxalate.

Abundant sclereids were present as single cells, small clumps of a few cells, and large masses of numerous cells. Individual cells varied greatly in appearance from being rather small with quite uniformly thickened, colourless walls and simple tubular pits, to very

large cells with heavily thickened, yellowish and, occasionally, brownish walls, irregularly shaped lumens, and numerous branched pits. The smaller cells were usually more or less isodiametric with uniformly striated walls but the larger cells were frequently very irregular in shape with correspondingly distorted striations. A few cells were elongated, giving them a fibrous appearance. Occasionally the lumen of some of the smaller cells was filled with a brownish orange, amorphous content.

Many pieces of phloem tissue were seen, consisting mainly of elongated parenchyma and straight, uniformly wide sieve-tubes. The sieve-tubes had fairly thin walls or slightly thickened walls and several showed oval sieve-areas on their oblique end-walls and, much less frequently, on their side-walls. Many axially elongated cells of the phloem parenchyma contained elongated prismatic crystals of calcium oxalate, these being frequently pointed at either end or, in the larger ones, pointed at one end and cleft at the other. Many were broken.

The pieces of phloem tissue were frequently crossed at right angles by medullary rays, both types of medullary ray cells being obvious (vide supra). Type A cells were small, with thin, often wavy walls, and crossed the phloem in regularly arranged 'brick-wall' formation, 1 to 12 rows deep. Type B cells were larger, but also thin-walled, and frequently appeared above and below the Type A cells, in

a similar arrangement. More often, however, they appeared on one side of the Type A cells only. The rays often appeared cut tangentially, the Type A cells forming an oval plate of small, round cells with frequent small air spaces, with a single row of cells of Type B extending above and below, which often connected with a second group of Type A cells. Both types of cells occasionally had yellowish content.

Very occasional pieces of latex vessels were seen associated with phloem parenchyma. These had slightly thickened, smooth walls and, in parts, had greyish granular content.

Numerous fragments of cortical fibres were present. These were very long, uniformly thick-walled cells with occasional bulbous swellings, particularly near the ends. They occurred most often singly but were sometimes in small groups. The lumens were uniformly wide except in the swellings. The walls were longitudinally striated and, in many cases, were marked by compound criss-cross spirals. The ends were sometimes blunt but more often tapered finely. At many broken ends the secondary wall projected beyond the broken-off primary wall. Very occasional groups of fibres were surrounded by a sheath of parenchymatous cells containing small prism crystals of calcium oxalate.

Numerous crystals of calcium oxalate of both types, acicular and cubical, were scattered free in the powder, the former frequently being broken.

A very few wide vessels were observed, with numerous, regularly

arranged, slit pits. It is presumed that these came from fragments of wood formerly adhering to the inner surface of some pieces of bark, although the presence of adherent wood was not observed in the macroscopical examination of the bark.

The characters of the powder are illustrated in Figures 28 and 29.

To face p. 155

Fig. 28.

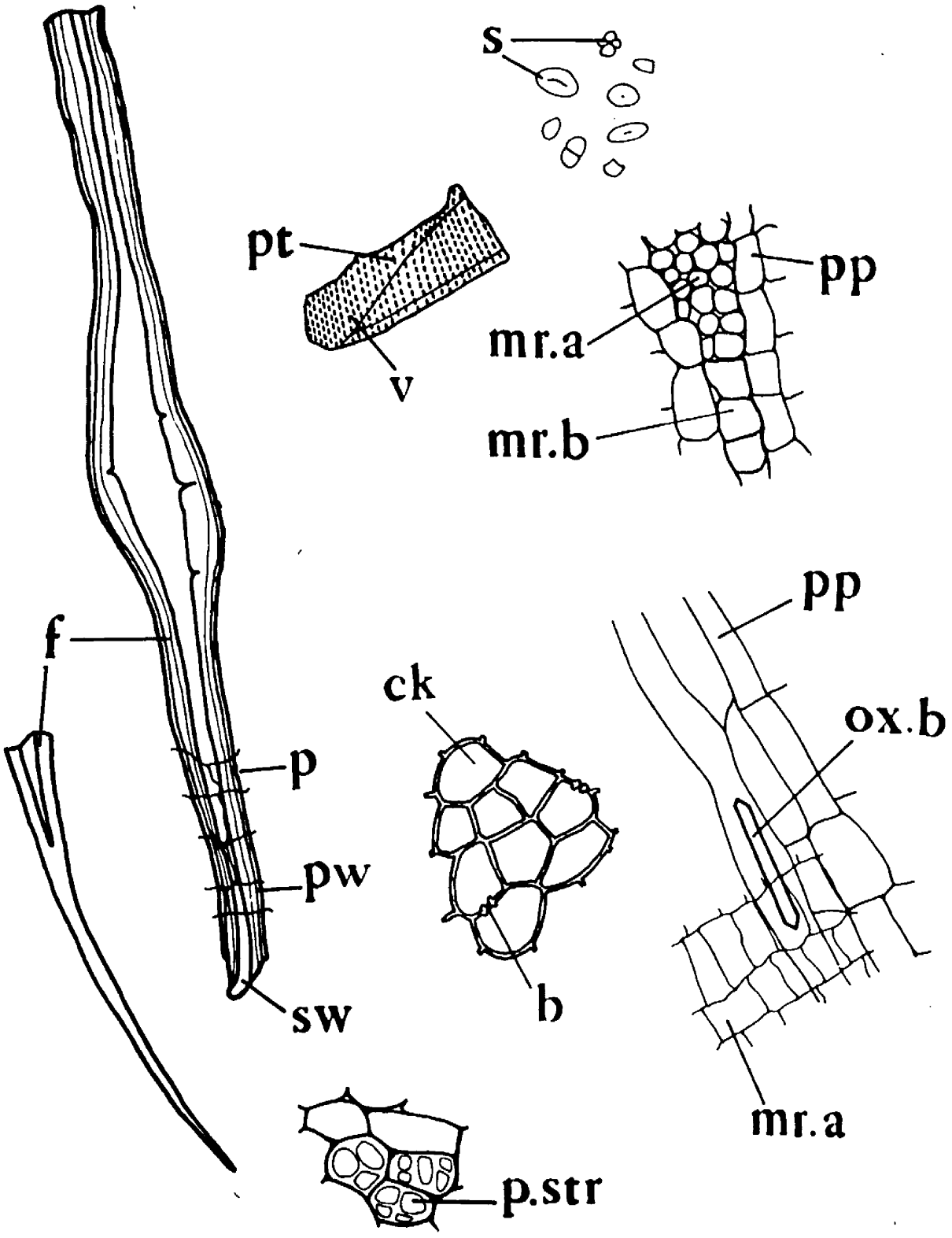
Structures present in the powdered bark of

V. schweinfurthii (1).

b, beaded wall; ck, cork; f, fibre fragment; mr.a, medullary ray (cells of Type A); mr.b, medullary ray (cells of Type B); ox.b, crystal of calcium oxalate (Type B); p, parenchyma; pp, phloem parenchyma; pt, pit; pw, primary wall; p.str, parenchyma with strap-like thickening; sw, secondary wall; v, vessel fragment.

(ALL x 250)

s, starch (x 375).


s
pt
v
pp
mr.a
mr.b
f
p
pw
sw
ck
b
pp
ox.b
mr.a
p.str

To face p. 156

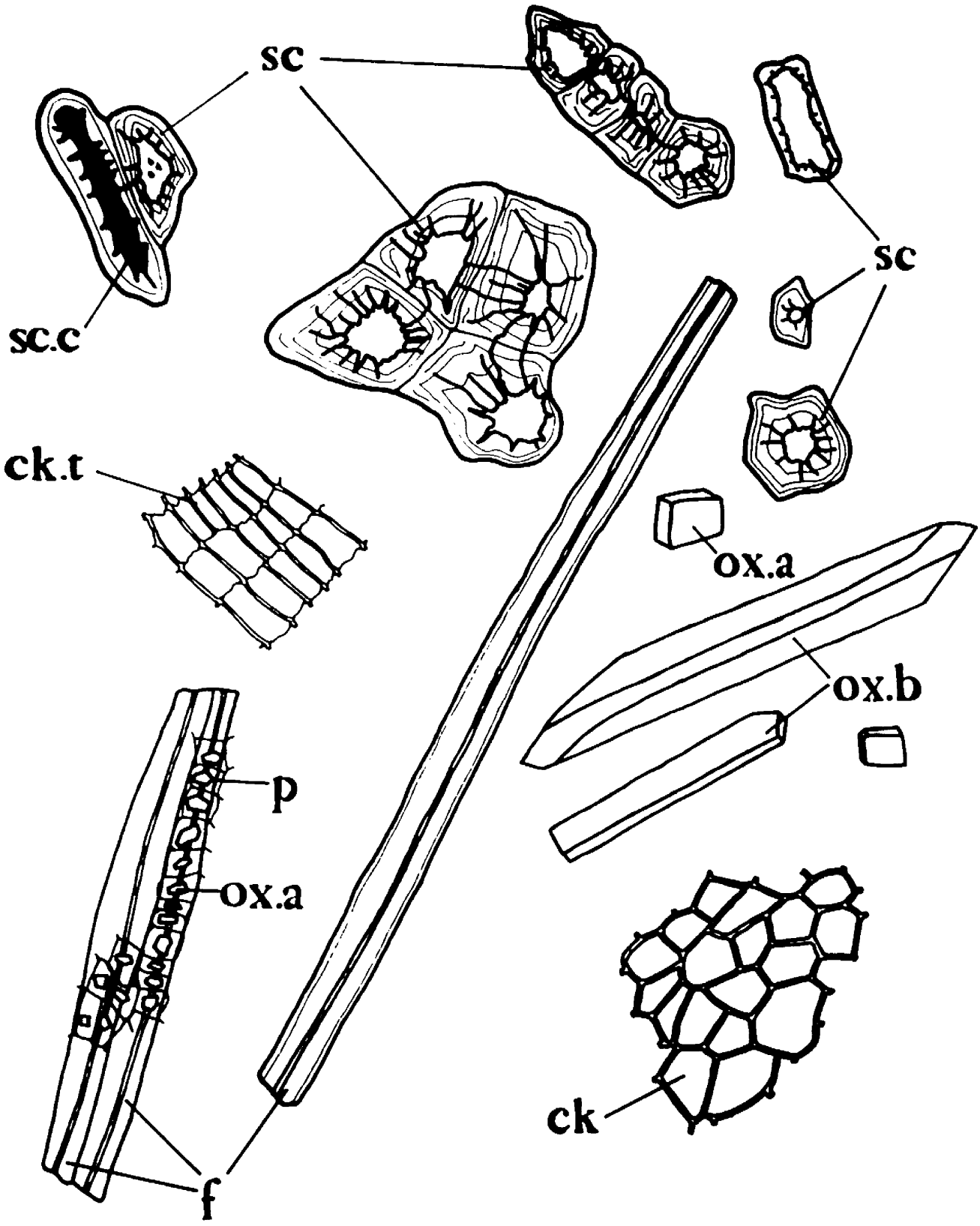
Fig. 29.

Structures present in the powdered bark of

V. schweinfurthii (2).

(x 250)

ck, cork; *ck.t*, cork in transverse section; *f*, fibre fragment; *ox.a*, crystal of calcium oxalate (Type A); *ox.b*, crystal of calcium oxalate (Type B); *p*, parenchyma; *sc*, sclereid; *sc.c*, sclereid with amorphous content.


sc
sc.c
sc
ck.t
ox.a
ox.b
p
ox.a
ck
f

P A R T V I

ANATOMICAL STUDY OF THE STEM BARK

OF *VOACANGA AFRICANA*

Macroscopical structure

Less variation was shown among the various individual pieces of bark of this species than among those of V.schweinfurthii and they were clearly all from fairly slender stems, none corresponding to the large thick pieces, obviously from stems of considerable girth, prevalent in the samples of this other species. Most pieces were 1 to 2 cm. broad, a few being less than 1 cm., down to 0.3 cm. in some very thin pieces of bark, and occasional pieces up to 2.5 cm. The length was not very variable, most pieces being 6 to 10 cm. long, though some pieces were only 2 to 3 cm. and a few pieces of branch bark were up to 15 cm. long. Most pieces were 1 to 2 mm. thick, with occasional pieces up to 4 mm. The pieces were all more or less curved, the majority of the pieces being channelled and the thin branch bark quilled.

The outer surface was greyish brown and relatively smooth, except for occasional patches of rough, brown rhytidome which was present on some of the larger pieces. Numerous fine, or, occasionally, coarse longitudinal striations were present on most pieces and some pieces had occasional marked transverse ridges. Small warty spots were present, though sometimes indistinct, most being about 1 mm. in diameter with a very few up to 1.5 mm. and some, on the thinner pieces, being less than 1 mm. The cork showed no tendency to exfoliate. (Fig. 30).

Various epiphytes were present on most pieces, including, most commonly, a closely adherent, white lichen, frequently with abundant, round, black apothecia and, less commonly, a pale green lichen.

The inner surface was fairly smooth with faint, irregular longitudinal striations which were occasionally accentuated to form corrugations. Almost all pieces were pale fawn but a few were dark brown. Glistening points, due to the presence of crystals of calcium oxalate, were frequently apparent. (Fig. 15B).

Large pieces of bark had a fracture which was short and granular in both cortex and phloem, although also slightly fibrous in the cortex, thin pieces were generally more brittle and the fracture, though short internally, was extremely fibrous in the cortex. In some very thin pieces of branch bark the high concentration of fibres made it extremely difficult to break them.

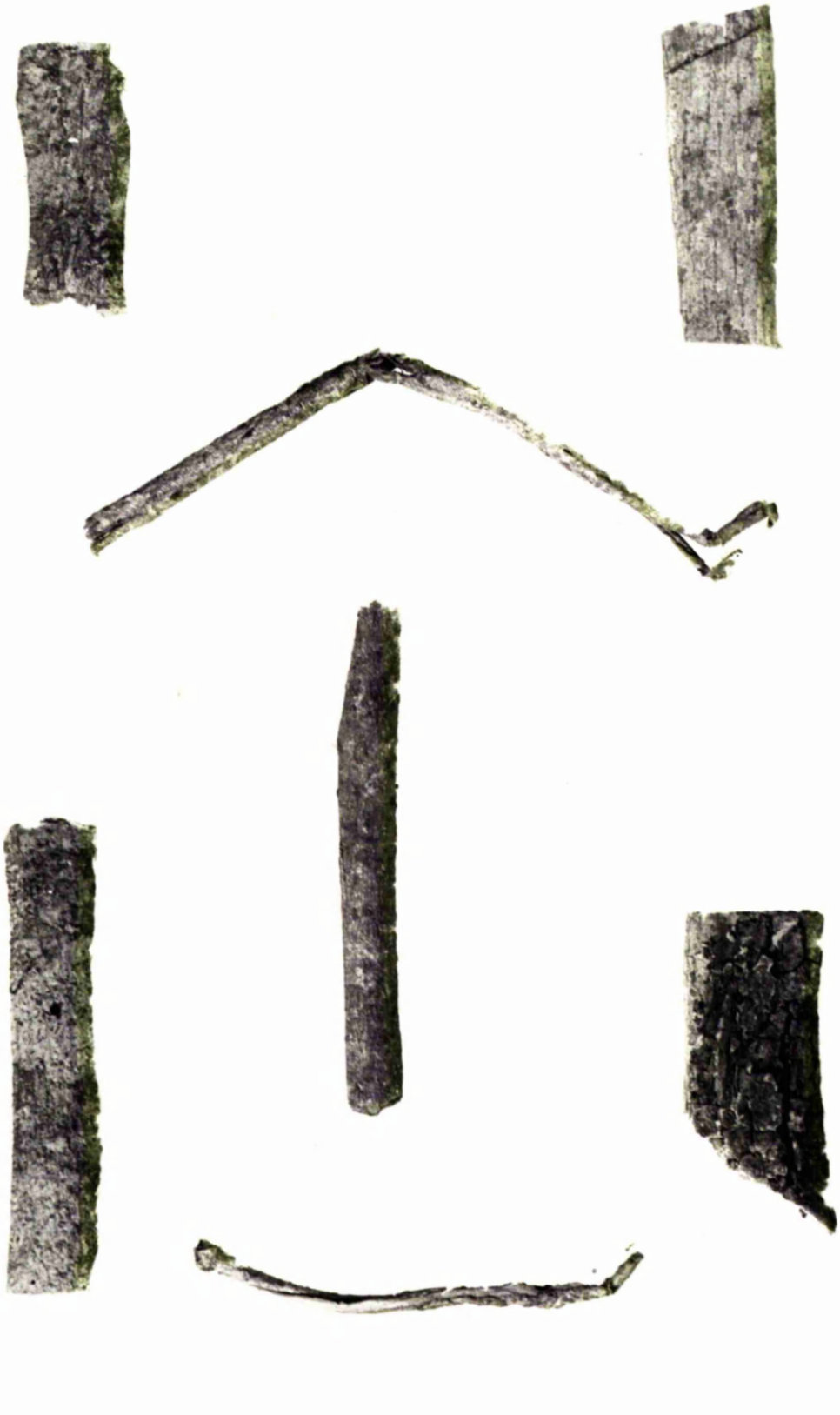
The bark had little odour and a faintly bitter taste.

To face p. 160

Fig. 30.

The outer surface of the stem bark of V.africana.

(x 2/3)



Microscopical structure

From four separate samples of V.africana representative pieces of bark were chosen, the number of pieces chosen from each sample depending on its size, until twelve pieces were obtained. A similar range of sections was prepared as described for V.schweinfurthii, the methods of sectioning and subsequent treatment also being as previously described.

The amount of cork present varied from 5 layers of cells up to about 24 in a few cases; normally about 12 layers were seen. In most pieces of bark small groups of parenchymatous cells with slightly thickened, brownish walls were present on the outer side of the cork, occasionally with associated, small groups of sclereids. In pieces of bark with prominent patches of rhytidome on the outer surface, sectioning through those areas showed the groups of brown cells to be larger than elsewhere, being up to about 12 layers thick.

The cork cells occurred in regular radiate rows and were characteristically tabular, elongated axially and tangentially. The tangential walls were much thicker (many being up to 4 μ thick) than the radial walls, the latter usually being wavy. On rare occasions, viewing of the cells in surface sections showed slight beading on the tangential walls. The cells were lignified, staining pink to deep red with solution of phloroglucinol and hydrochloric acid, and measured R 4 to 16 to 24 to 36 μ, T 10 to 27 to 45 to 66 μ and H 8 to 23 to 37 to 60 μ.

The phellogen was seldom well-defined but, when apparent, consisted of regular thin-walled cells, mostly measuring about R 7 to 10 to 21 μ, T 14 to 24 to 38 μ and H 10 to 28 to 45 μ. These cells frequently contained prism crystals of calcium oxalate, measuring about 21 μ in diameter.

The phelloderm was well-defined, consisting largely of thin-walled parenchymatous cells containing occasional prismatic crystals of calcium oxalate. It contained a discontinuous band of sclereids, adjacent to the cork, the cells rarely occurring singly, more often as groups of about 4 cells, but most often in large tangentially elongated groups of 20 cells or more. The amount of sclerenchyma was directly proportional to the thickness of the bark, such that in the thicker pieces the band of sclereids was almost continuous. The cells were more or less isodiametric or somewhat elongated tangentially, measuring R 16 to 29 to 51 to 80 μ, T 16 to 40 to 80 to 134 μ, H 16 to 30 to 54 to 98 μ and B 6 to 10 to 18 to 32 μ. They exhibited fairly heavily and uniformly lignified walls having numerous, simple, tubular pits.

The cortex was well-defined, occupying up to almost half the width of the bark in some pieces, more often occupying about one third. It consisted largely of parenohyma, the cells of which had walls of varying thickness. The cells were frequently elongated tangentially, sometimes very markedly, and in places were ceratenchymatous. Occasionally sclereids were present, either singly or in small groups,

mostly in the inner layers of the cortex. Extremely long fibres occurred either singly or in groups of two or three. They were most numerous in the thinner pieces of bark, being particularly abundant in the very thin branch bark, tending to be in radial rows when present in large numbers. Their appearance and structure were exactly as described for the cortical fibres of V.schweinfurthii. Although some were fairly short, about 2 mm., the length frequently exceeded 10 mm., values about 14 mm. being quite common, with occasional readings up to 20 mm., with D 8 to 26 to 54 to 80 μ and B 4 to 9 to 23 to 40 μ.

More or less cubical prismatic crystals of calcium oxalate, 6 to 14 to 26 to 42 μ in breadth, occurred in the cortical parenchyma, in greatly varying amounts.

In the phloem were numerous large groups of sclereids which were frequently fused to form sheets of lignified tissue, as described for V.schweinfurthii. The appearance of the individual cells was also as described for the phloem sclereids of that species. Normally sclereids were absent from the innermost phloem. The sclereids measured R 18 to 34 to 74 to 156 μ, T 20 to 32 to 68 to 134 μ, H 20 to 35 to 81 to 182 μ and B 6 to 12 to 24 to 40 μ.

Cells of the abundant phloem parenchyma varied from thin to moderately thick-walled, the walls frequently being beaded; they were tangentially elongated and markedly axially elongated, especially in the inner phloem.

Numerous latex vessels were present in the phloem, being very similar in appearance to those of V. schweinfurthii. In diameter they varied from about 21 μ to 48 μ, the majority being between 24 and 28 μ. Calcium oxalate occurred infrequently in the outer phloem as cubical crystals, but was almost always present in the innermost phloem as stout acicular crystals lying in axially elongated cells of the parenchyma. Their appearance was very similar to the Type B crystals in V. schweinfurthii and they measured 20 to 92 to 172 to 278 μ in length and 4 to 9 to 19 to 30 μ in breadth.

The medullary rays were generally straight as seen in transverse section, though a few curved at the cortical end, widening only slightly towards the cortex. Seen in longitudinal sections, these were formed of two distinct sizes of cells and formed a similar pattern to the medullary ray cells of V. schweinfurthii. The individual cells of both types were similar in appearance and content to those of V. schweinfurthii. The smaller cells, Type A, measured R 22 to 34 to 50 to 74 μ, T 8 to 18 to 30 to 46 μ and H 12 to 18 to 26 to 48 μ and the larger cells, Type B, R 14 to 24 to 36 to 56 μ, T 16 to 27 to 45 to 82 μ and H 24 to 35 to 65 to 124 μ.

Sieve-tubes were mainly thin-walled, uniformly wide structures with oblique end-walls having large sieve-areas. In some of the thicker pieces of bark several of the larger tubes had fairly thick walls with oval sieve-areas on the side-walls as on the end-walls.

The diameters of those measured varied from about 10 μ to 28 μ, the majority being in the range 15 to 20 μ.

Starch grains occurred in very variable quantities in the cortical parenchyma, phloem parenchyma and, less commonly, medullary ray cells. The grains were subspherical or polyhedral with rounded edges, but a considerable number of compound grains, with two to four components, were also present. The hilum, when distinct, was most frequently a central point but appeared as a short line in some of the larger grains. Striations were absent. Single grains measured 2 to 4 to 8 to 14 μ in diameter; compound grains had a maximum diameter of about 9 to 15 μ, most being about 12 μ.

Due to the observation that the anatomical structure of the bark of V.africana was very closely similar to that of the bark of V.schweinfurthii, and to avoid unnecessary repetition, no drawings of the various sections of V.africana bark are recorded. However, a brief description of the histological structures of this bark, as seen in sections, is given and, to illustrate the similarities, drawings accompany the description of the microscopical characters of the powdered bark.

Conventions used for recording cells dimensions are as before.

Powder

The colour of a No. 60 powder of the stem bark of V.africana was pale yellowish brown. The odour was slight and musty and the taste only slightly bitter. In texture it was somewhat gritty and extremely fibrous, frequent tangled masses of fibres being present.

When mounted in dilute glycerol the powder consisted of greyish masses of parenchyma, brownish orange sheets of cork, pale yellow groups of sclereids and elongated pieces of fibres. Also present were numerous prismatic crystals, both acicular and cubical, and numerous granules which stained blue with dilute solution of iodine, showing them to be starch. The appearance and distribution of the starch granules were similar to those of the starch granules present in the powder of V.schweinfurthii.

When stained with solution of phloroglucinol and hydrochloric acid, a large amount of lignified tissue was seen to be present. Most strongly stained were the groups of sclereids, and the many isolated stone cells; the cork stained pink to deep red; the fibres, although most frequently showing no staining or staining pink in the primary wall only, very occasionally stained red in the entire wall.

To allow a more detailed study of the cell structures, the powder was cleared with chloral hydrate solution. Numerous masses of cork were present, varying from colourless to brownish orange.

In structure the cells were similar to the cork cells of powdered V. schweinfurthii but beaded walls were even rarer than in this other species.

Abundant masses of parenchymatous cells were present, a very few having brownish yellow, wavy walls but most being colourless. The walls were mainly smooth, varying only slightly in thickness, some of the thicker walls being beaded. The cells were similar in shape and arrangement to the parenchyma in powdered V. schweinfurthii and a few of the cells contained cubical crystals of calcium oxalate.

Numerous sclereids were present, many as single cells, some in groups of a few cells and frequently in large masses of numerous cells. Individual cells showed the same variation in size and appearance as the sclereids of powdered V. schweinfurthii but there was a somewhat larger proportion of the smaller, single cells and groups of a few rather small cells, both types probably mainly from the phelloderm. Occasionally the lumen of some of the smaller cells was filled with a brownish orange, amorphous content.

Many pieces of phloem were present, consisting mainly of elongated parenchyma, which frequently contained elongated, prismatic crystals of calcium oxalate, and associated sieve-tubes. When seen in radial longitudinal section, the phloem tissue was often crossed at right angles by compound medullary rays, and, when seen in tangential longitudinal section, the rays appeared as oval plates of

small, round cells with a single row of larger cells extending above and below the plate. Very occasional pieces of latex vessel were seen, associated with phloem parenchyma. The distribution, appearance and content of all these phloem structures were similar to the phloem structures described for powdered V.schweinfurthii.

Abundant fragments of cortical fibres were present and numerous crystals of calcium oxalate, of both Type A and Type B, were scattered in the powder. The fibres and crystals were also similar to those present in powdered V.schweinfurthii, though the fibre fragments were more numerous in V.africana.

The characters of the powder are illustrated in Figures 31 and 32.

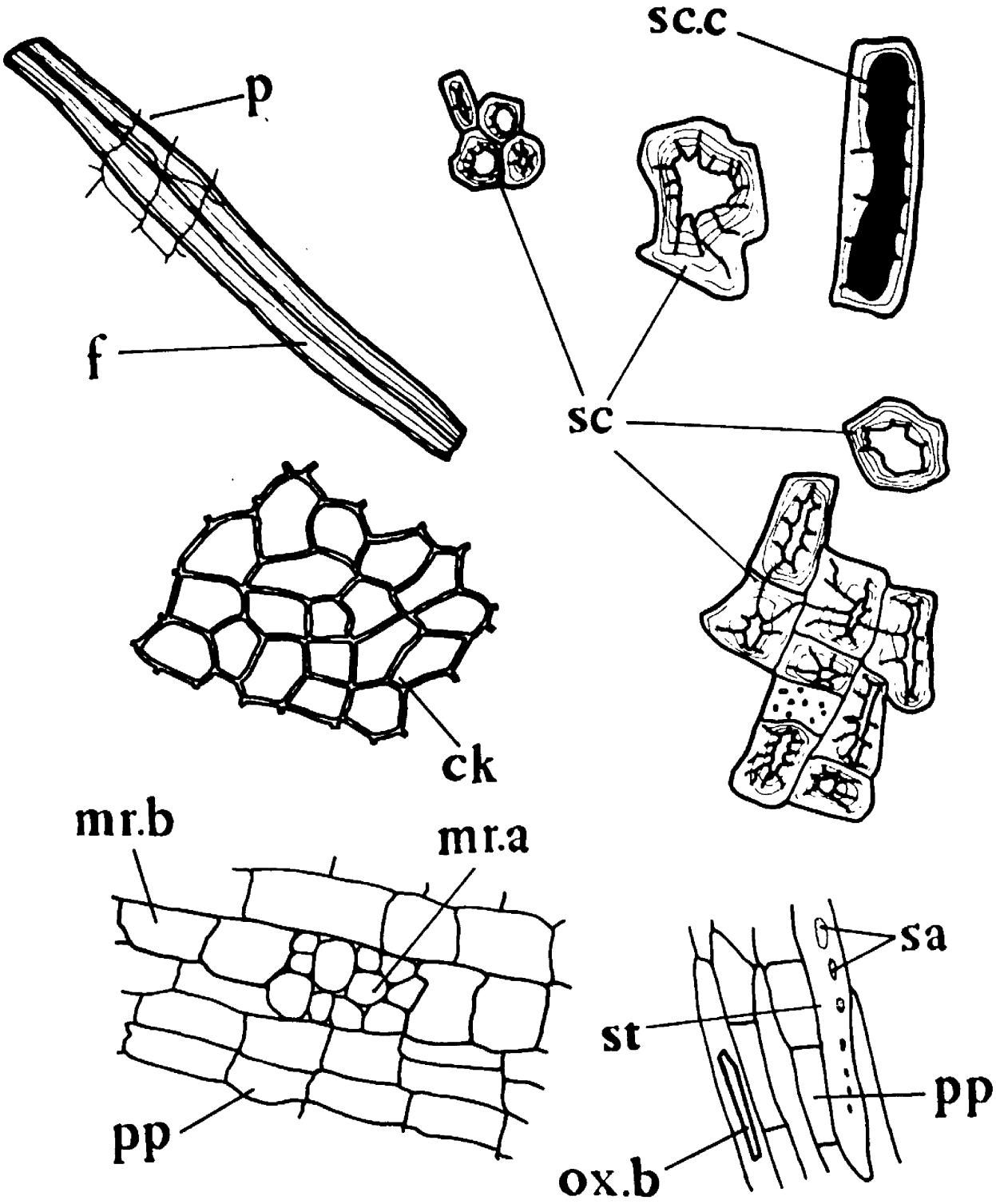
To face p. 169

Fig. 31.

Structures present in the powdered bark of V.africana (1)

(x 250)

ck, cork; f, fibre fragment; mr.a, medullary ray (cells of Type A); mr.b, medullary ray (cells of Type B); ox.b, crystal of calcium oxalate (Type B); p, parenchyma; pp, phloem parenchyma; sa, sieve-area; sc, sclereid; sc.c, sclereid with amorphous content; st, sieve-tube.


sc.c
p
f
sc
ck
mr.b
mr.a
pp
sa
st
pp
ox.b

To face p. 170

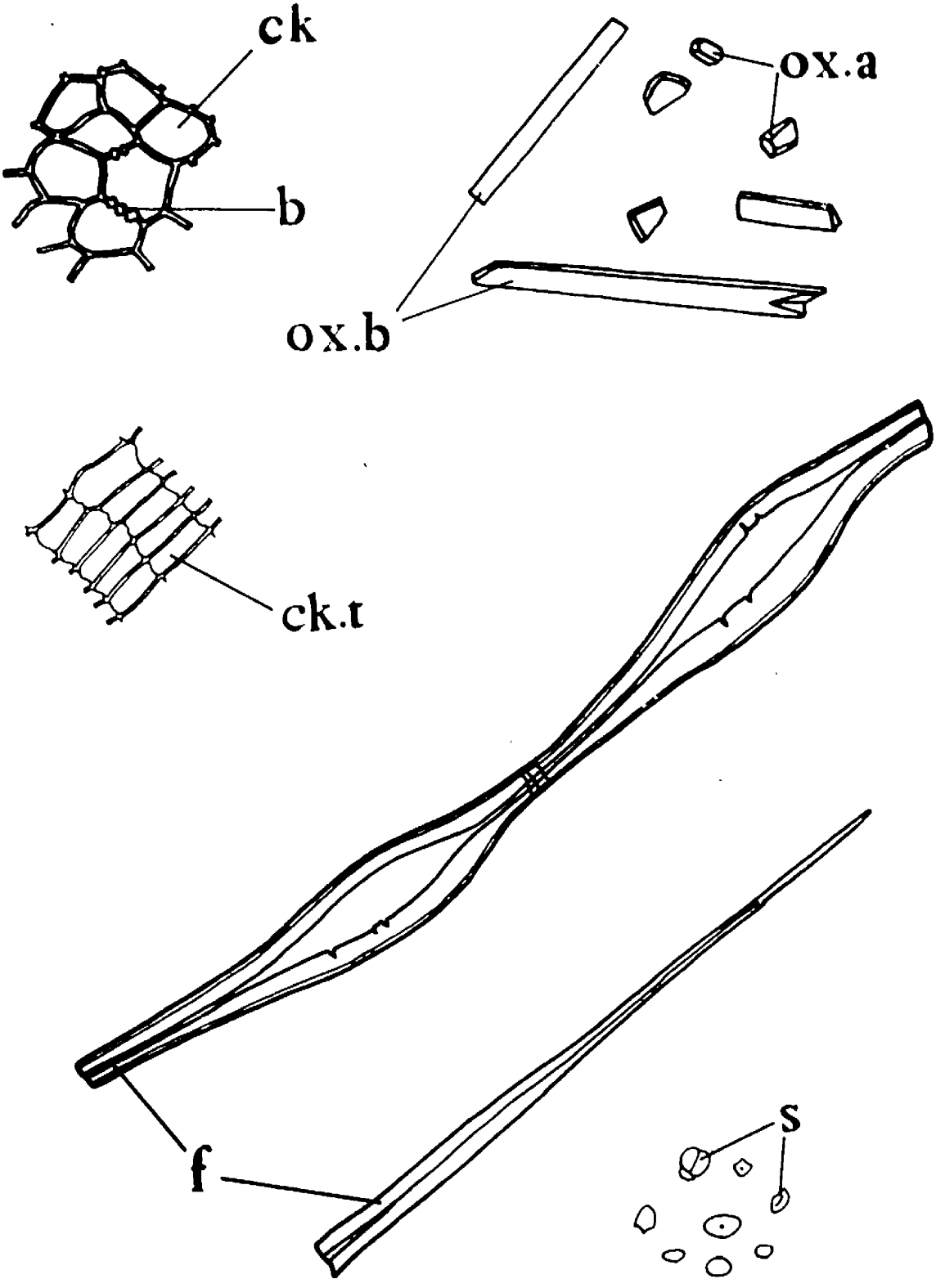
Fig. 32.

Structures present in the powdered bark of V.africana (2).

b, beaded wall; ck, cork; ck.t, cork in transverse section; f, fibre fragment; ox.a, crystal of calcium oxalate (Type A); ox.b, crystal of calcium oxalate (Type B).

(ALL x 250)

s, starch (x 375).


ck
b
ox.a
ox.b
ck.t
f
s

P A R T V I I

N U M E R I C A L V A L U E S

As the barks of the two species V.schweinfurthii and V.africana could not be distinguished from one another by means of the appearance or size of their anatomical structures, attempts were made to establish suitable numerical values whereby these two species could be differentiated.

Only fairly discrete, readily distinguishable, histological structures are of potential use in the establishment of numerical values and the structures of this type present in the stem barks of V.schweinfurthii and V.africana were the cork, sclereids, fibres, starch and crystals, and these were considered in turn.

Cork

The possibility of devising a method to measure the amount of cork per unit weight of bark was considered, but such was the variation in the thickness of the cork in the bark of V.schweinfurthii, it was felt that this would be of little value. Also, as the dimensions of the cork cells were so similar in both species, it appeared extremely unlikely that the determination of the number of cork cells per unit area of cork, used by Melville[127] to differentiate Atropa belladonna and A.acuminata, would be of value.

Sclereids

During the anatomical studies of the stem barks, the impression had been formed that, in general, there tended to be more sclereids in V.schweinfurthii than in V.africana, although in both species the amount varied, apparently directly with the thickness of the bark. Attempts were made to estimate the total weight of sclereids per unit weight of bark. Two methods were considered, firstly, determination of the crude fibre of the barks, and, secondly, direct dissection and weighing of the sclereids. The first method tends, in general, to be inaccurate and in this particular case was thought likely to be markedly so due to the relatively small amount of material available for the estimations, and, more particularly, due to the variation in the amount of lignified cork present. The second method, while being considerably more tedious to perform, appeared likely to afford more accurate and consistent results with the small amount of material available.

Direct application of this method to the examination of the powdered barks would be impracticable, so carried out concurrently with the estimation of the total weight of sclereids in whole barks, was the determination of the area per unit weight of sclereid layer and the area of the sclereid layer per unit weight of bark. The intention was that should there be a direct relationship between the weight of sclereid present and the total area of the sclereid sheets,

then a standard figure for the area of sclereid per unit weight of bark could be used, employing the lycopodium spore technique, for the differentiation of the powders of the barks of the two species. Such a reference figure was used successfully in quantitative estimations of powdered linseed[128] and cardamom seed[129].

Estimations based on the number of sclereids present were also considered. The number of sclereids per unit area has been applied in studies of drugs containing sclereid layers which were one cell thick[129-131], but as the sheets of sclereids in the barks of the two species of Voacanga were of variable thickness, and also their dimensions in both species were so similar, it was concluded that this method would not be applicable. The method was extended to include sclereids occurring in oval masses[132-134], the number of sclereids per unit weight being determined, and, due to the uniformity of the masses, the application of the method was considerably facilitated by estimating the number of cells present in a mass from its dimensions. However, due to the irregularity of the sclereid sheets in the barks being studied, no similar geometrical calculation could be made, and attempts to disintegrate the sheets to allow counting of individual sclereids would be extremely difficult to effect efficiently due to the tortuous, interlocking nature of the individual cells.

Determination of the percentage weight of sclereids in the bark.

Method.

From each piece of bark examined, four separate sample pieces of about 0.1 g. were cut, measured for thickness, and accurately weighed. Each sample was transferred to a boiling-tube containing 15 ml. of 5 per cent solution of potassium hydroxide, suspended in a boiling-water bath and allowed to digest for exactly three hours. The conditions of the experiment were carefully controlled. The tube of solution was stabilised at the temperature of the water-bath before adding the sample, and the volume of the alkaline solution kept constant throughout the experiment.

Immediately the digestion period was completed, the sample was transferred to a basin of water and rinsed with several changes of cold water to avoid any further degradation of the tissues. The bark was carefully dissected, the sclereid content separated and the fragments laid out on a weighed slide. The separation of the sclereids from all other tissues was greatly facilitated by their occurrence in large fused sheets, the most difficult task being the separation of the phelloderm from the cork.

Finally, the isolated tissue was allowed to dry overnight and the slides reweighed. The percentage weight of sclereids in each piece of bark was then calculated.

Results.

These are listed in Tables 12 and 13.

Conclusions.

As the quadruplicate results for each individual piece of bark were in fairly close agreement, it was concluded that the method gave a satisfactory estimation of the sclereid content of the bark.

The bark of V. schweinfurthii showed a wide variation in sclereid content, from about 17 to 65 per cent by weight, and the previous observation of a tendency for the amount of sclereid to be directly proportional to the thickness of the bark, was confirmed.

Although only three pieces of the bark of V. africana were examined, the results obtained, all about 40 per cent, were in the centre of the range of values of V. schweinfurthii and very close to the values obtained with pieces of similar thickness of this species. This indicated that the analysis had no value as a method of differentiating the two species.

Determination of the area per unit weight of sclereid layer and the area of sclereid layer per unit weight of bark.

Method.

The areas of the sclereid fragments from the previous determination were determined by tracing their outlines, at a magnification of

Table 12 : Sclereid content of the bark of V. schweinfurthii.

Bark thickness mm.	Amount of sclereid in bark p.c.	
	Individual readings	Mean
20	35.4 34.9 38.1 33.8	35.6
18	34.7 35.2 39.6 40.6	37.5
10	17.9 16.4 17.5 16.4	17.1
40	49.5 47.4 50.7 48.8	49.1
10	42.1 38.6 42.3 39.9	40.7
36	66.3 66.1 66.1 61.7	65.1
25	40.6 39.8 43.7 34.1	39.6

Table 13 : Sclereid content of the bark of V.africana.

Bark thickness mm.	Amount of sclereid in bark p.c.	
	Individual readings	Mean
18	42.4 43.4 43.0 44.9	43.4
18	37.4 42.4 44.6 40.2	41.2
20	38.7 36.6 38.4 40.4	38.5

about x 35, using a camera lucida attached to a binocular microscope. The traced areas were then measured using a planimeter and the total area per unit weight of sclereid layer and per gramme of whole bark calculated.

Results.

These are listed in Table 14.

Conclusions.

Values for the area of sclereid layer per unit weight varied from about 53 to 110 sq.cm. per g., with twelve estimations, and thus was of no value as a standard reference figure.

Fibres

Numerical values based on fibres which had been applied in qualitative microscopy were the area of fibres per unit weight[135,136] and the length of fibre per unit weight[134,136]. From the dimensions of the fibres present in the two barks under examination, determination of their areas was impracticable. The length of fibre per unit weight was unlikely to be of diagnostic value due to the great variation in the number of fibres present in different pieces of bark. However, as previously stated, the amount of fibre in the bark of both species varied inversely with the thickness of the bark, while the converse was true of the sclereids, therefore it was considered possible that the product (K) of

Table 14 : Area of sclereid determinations in the bark of V. schweinfurthii.

Sclereid in bark, per cent (W)		Bark thickness (mm.)	Sclereid layer, area per g. (sq.cm.)		Area of sclereid per g. of bark (sq.cm.)	
values	mean		values	mean	values	mean
35.4 34.9 38.1 33.8	35.6	20	60.7 53.2 62.9 63.3	60.0	21.5 18.6 23.9 21.4	21.4
34.7 35.2 39.6 40.6	37.5	18	83.8 87.4 92.8 95.3	89.8	29.1 30.8 36.7 38.7	33.8
17.9 16.4 17.5 16.4	17.1	10	82.1 108.7 97.7 110.5	99.8	14.7 17.8 17.2 18.2	16.9

the weight of sclereid, in grammes, and the length of fibre, in metres, present in 1 g. of bark, was a potentially useful value.

Determination of K.

Method.

From each of three of the pieces of bark previously analysed for sclereid content, another four sample pieces were cut, weighed, digested with 5 per cent solution of potassium hydroxide, and washed as before. The samples were carefully dissected, the fibres separated from the other tissues and placed on a slide, and their lengths traced on paper by the same method employed for tracing the sclereid areas. The total length of fibre was measured using a map-measurer and the length of fibre per gramme of bark calculated. Using the previously determined values for the percentage weight of sclereid in the corresponding pieces of bark, the value K was calculated.

Results.

These are listed in Table 15.

Conclusions.

There was considerable variation between the values of length of fibre per gramme of bark obtained for different samples of the same piece of bark and average values also varied widely, from about 27 to 61 m. per g., in only three estimations. Thus, as expected, this

Table 15 : Values for K for the bark of V. schweinfurthii.

Sclereid in bark, per cent (W)		Bark thickness (mm.)	Length of fibre per g. (m.) (L)		K = W.L / 100
values	mean		values	mean	
35.4 34.9 38.1 33.8	35.6	20	34.3 25.5 21.7 29.9	27.9	9.9
34.7 35.2 39.6 40.6	37.5	18	72.8 63.0 28.0 31.8	48.9	18.3
17.9 16.4 17.5 16.4	17.1	10	64.8 58.6 41.6 82.6	61.3	10.5

figure itself had no significance as a reference value.

The results confirmed that the length of fibre per gramme of bark was inversely proportional to the thickness of the bark.

The values obtained for K varied from about 10 to 18; and from the width of this variance and, particularly, the degree of variation of the length of fibre per gramme within each piece of bark, it was concluded the method had no diagnostic value.

Starch

As the starch present in the barks of both species was of similar size and form, they could not be differentiated on appearance. Quantitative estimations of starch were considered unlikely to be of value due to the great variation in starch content amongst individual pieces of bark within each species.

Crystals

The appearance of the crystals of calcium oxalate present in both species was so similar that they could not be differentiated. However, although the dimensions of the Type B crystals in both species were very similar, there was a slight tendency for those of V. schweinfurthii to be shorter and broader than those of V. africana. This difference was accentuated by determining the length to breadth ratio

of these crystals but estimation of this value for 200 crystals of each species showed no significant difference. The results, calculated to the nearest whole number, are given in Fig. 33.

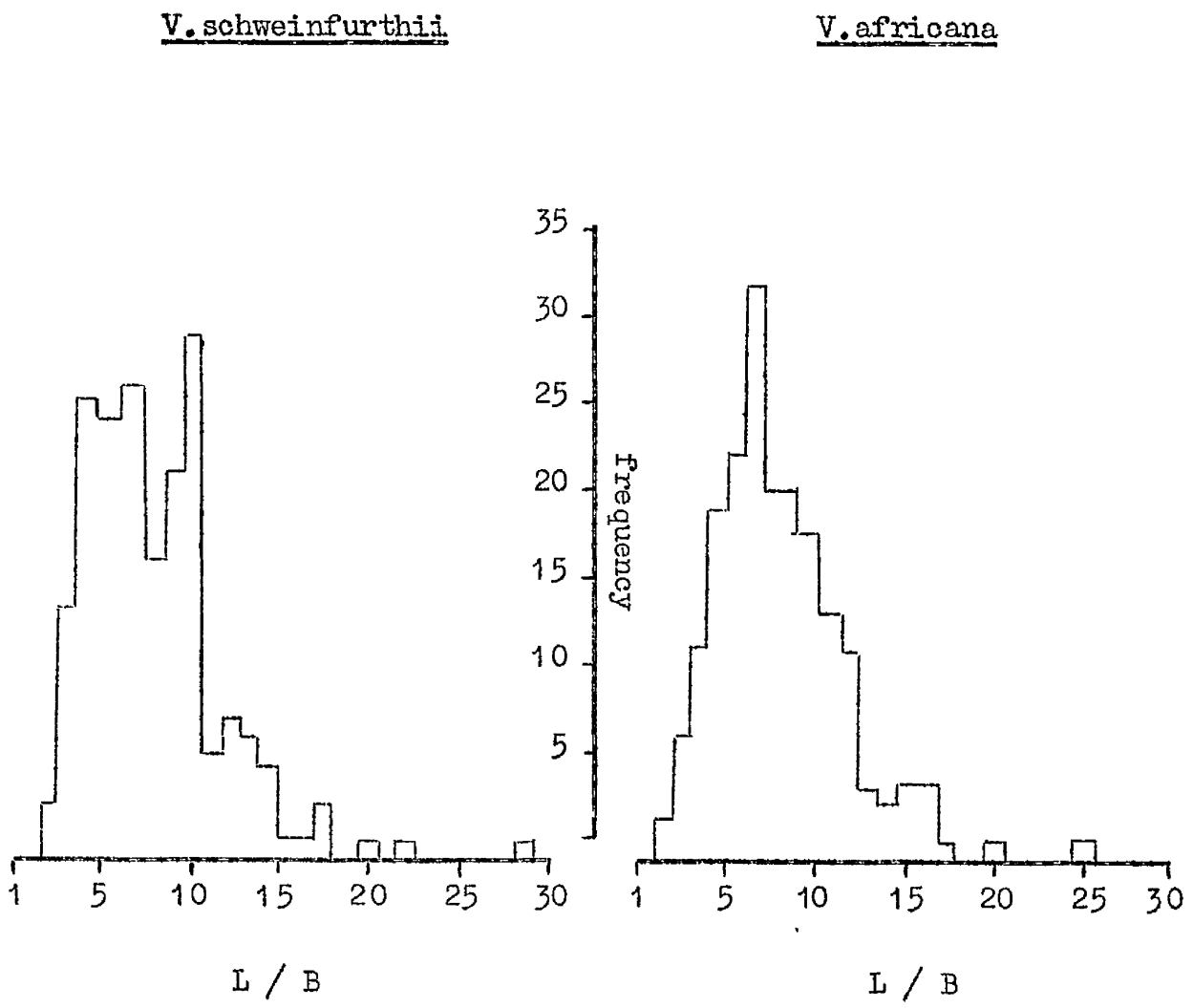


Fig. 33 : Nomograms of length to breadth ratios of crystals, Type B.

P A R T V I I I

D I S C U S S I O N

Chemistry of Voacanga schweinfurthii

Analysis of the composition of the alkaloidal fraction of the stem bark of Voacanga schweinfurthii has shown it to consist of a mixture of about ten alkaloids, which may be classified as follows.

The major alkaloids.

The three major alkaloids of the bark, which have been isolated in the pure crystalline state, are voacamine, vobtusine and voacorine. These substances were identified by their physical constants and by a comparison of their chromatographic properties with authentic samples of the alkaloids.

The minor alkaloids.

Two other alkaloids were present in small amounts, although these were not isolated in the pure state. One was shown to be voacangine, the identification being primarily from comparison of its chromatographic properties with those of an authentic sample of the alkaloid. It behaved similarly to authentic voacangine when examined by paper chromatography under conditions of varying pH, and when examined by thin-layer chromatography. Also, in the separation of the individual alkaloids from the total alkaloidal fraction of the bark by column chromatography on alumina, it was eluted in the first fractions by benzene, as reported in the analysis of the alkaloids of V.africana. Confirmatory evidence

of the identity of this alkaloid was obtained from its ultra-violet spectrum.

The presence of a second minor alkaloid, designated U1, was established by paper and thin-layer chromatography. From the intensity of the staining of the spots of this alkaloid, it appeared to be present in somewhat larger amounts than voacangine. The Rf value of this alkaloid, when examined by paper chromatography at both pH 4.0 and 4.7, was about one. Its ultra-violet spectrum, which proved quite different from those of the other Voacanga alkaloids, was obtained.

The trace alkaloids.

Paper and thin-layer chromatographic studies indicated the presence, in trace amounts, of several other alkaloids. Paper chromatography showed that at least one other strong base was present, designated U2, although it appeared more probable that two or more additional strong bases were present (including that designated U3).

The thin-layer chromatographic system used provided a more efficient analysis of the weak base fraction of the total alkaloids than paper chromatography, and revealed the presence of very small amounts of two alkaloids whose presence was previously unsuspected. It is possible that had a system been devised suitable for examining the strong bases, the number of these observed may also have been increased.

Extraction and isolation of the alkaloids.

The methods used for the extraction of the total alkaloidal fraction of the bark and the separation of the individual alkaloids, followed the classical pattern. The success of any particular extraction technique is generally dependent on the selectivity of the solvents employed, under the particular conditions, for example of pH, of their use. The superiority of the extraction method involving alcoholic percolation over that based on benzene percolation, was probably related to this principle. The two main differences between the methods were in the solvents used in percolation and the solvents used in the preliminary purification of the basic fraction prior to its analysis by chromatography. Thus the success of the method employed could have been due either to a superior selectivity of 70 per cent ethanol over benzene, or to the superior selectivity of ethyl acetate over chloroform, or of a combination of these two factors.

However, as indicated above, from the preliminary investigations it appeared, from the colours of the percolates and marcs, that benzene was more selective than 70 per cent ethanol, and thus it would seem more likely that the selective action of the ethyl acetate was the vital factor in the successful method. The importance of this solvent illustrates the empirical nature of much phytochemical analysis.

The efficiency of the extraction procedure was considerably improved by omission of extraction of the concentrated percolate by

ethyl acetate under neutral conditions. The separation of the total basic fraction into Solutions I and II fulfilled no useful function as similar alkaloids were present in both. It would also appear that the attempted separation into strong and weak base fractions, by extraction of the ethyl acetate solution of the bases with dilute acetic acid and dilute hydrochloric acid respectively, was equally valueless, a single extraction with dilute hydrochloric acid would have sufficed. From the successful purification of the fractions extracted by hydrochloric acid, it is unlikely that this would give rise to any difficulties and the efficiency of any extraction process is enhanced by minimising the number of operations involved.

Isolation of the alkaloids present in very small amounts is most likely to be effected using counter-current separation, as used for the isolation of the trace alkaloids of V.africana.

Comparison with Voacanga africana.

Eleven alkaloids have been isolated from the stem bark of V. africana and it has now been shown that a similar number of alkaloids are present in the stem bark of V.schweinfurthii. The major alkaloids of V.africana are voacamine, vobtusine, voacangine and voacorine, all of which are present in V.schweinfurthii in appreciable quantities, and they may be isolated from either species by exactly the same process.

These facts indicate that the alkaloidal contents of the barks of

the two species are very similar. It seems likely that a fuller study of the unidentified alkaloids of V. schweinfurthii might show them to be the same as the minor alkaloids of V. africana, in which case the alkaloidal contents of the barks of the two species would be identical. The sole piece of evidence in conflict with this conclusion is that the ultra-violet spectrum of U1 differs from all those previously described for the alkaloids of V. africana, but its nature would have to be more fully investigated before the significance of this fact could be assessed. It could prove, for example, to be a breakdown product of vobtusine, the ultra-violet spectra of both alkaloids having maxima at 220 mμ.

Comparison of the structures of the stem barks of *V. schweinfurthii* and *V. africana*

The structures of the stem barks of the two species *V. schweinfurthii* and *V. africana* have been described in detail and shown to fit the general pattern of Apocynaceous barks given by Metcalfe and Chalk. Both barks had all the main features typical of the family, namely 'white fibres', latex tissue and markedly heterogeneous medullary rays.

The comparison of the barks of these two species indicated that structurally they were very similar indeed and the small differences which had been observed are now discussed.

Macroscopy.

The main difference in the macroscopical appearance of the barks examined was the presence in *V. schweinfurthii* of a considerable number of pieces over 4 mm. thick, a few being up to 11 mm. thick, whereas in *V. africana* the thickest pieces were only up to 4 mm. thick. In general the bark of *V. schweinfurthii* was in broader pieces, but in length the bark of *V. africana* tended to exceed that of *V. schweinfurthii*. Some pieces of *V. africana* were up to 15 cm. long and these were invariably very thin.

Differences in length and breadth would arise from the method of collection of the bark, and from the particularly fibrous nature of thin pieces one would expect that the species with many pieces of thin bark

would also tend to have a preponderance of long pieces, as found in V.africana. The axially orientated fibres would not affect the breadth of the pieces. Differences in the thickness of the bark could arise from collection from stems of differing girths or plants of differing age. Both plants are bushes or low trees up to 5 m. in height.

Some pieces of the bark of V.schweinfurthii had both outer and inner surfaces which were more coarsely marked than any pieces of V. africana, but these were almost exclusively pieces of greater thickness than found in this latter species.

It is thus seen that there were no significant differences in macroscopical appearance between the barks of the two species.

Microscopy.

Cork.

The only difference in the cork of the two species was in amount, up to 100 layers being observed in the very thick pieces of V.schweinfurthii, but only up to 24 layers being present in V.africana. In both species about 12 layers were commonly present.

Phelloderm.

The phelloderm of V.schweinfurthii contained, in general, more numerous sclereids, frequently forming an almost complete sheath, than that of the phelloderm of V.africana. As the number of sclereids was directly proportional to the thickness of the bark in both species, this

was in agreement with the differences in thickness already discussed.

Cortex.

The size of the cortex in both species also varied with the thickness of the pieces of bark, being large and obvious in thin pieces but smaller and frequently ill-defined in thicker pieces, the latter also containing a greater number of sclereids. Thus in V.africana the cortex was more often well-defined and less sclerotic than in V.schweinfurthii. There were more pieces of bark with abundant cortical fibres in V.africana than in V.schweinfurthii, but again this may be related to the thickness of the pieces, as the number of fibres present was inversely proportional to the thickness of the bark in both species.

Phloem.

As in the phelloderm and cortex, the sclereid content of the phloem of both species was directly proportional to the thickness of the pieces of bark, and again phloem sclereids were generally more abundant in V.schweinfurthii.

Summary.

The barks of the two species examined are thus very similar in structure and any slight differences which have been noted may be related to the thickness of the pieces of bark, V.schweinfurthii containing numerous pieces thicker than any found in V.africana.

The similarity of the two species was further emphasised by the closeness of the dimensions of the various structures and cell inclusions present, as given in Table 16, and by determination of the various numerical values discussed in Part VII.

Table 16 : Dimensions of microscopical structures in the stem barks of V.schweinfurthii (VS) and V.africana (VA).

Structure		Size (μ)	
		V S	V A
Cork	R	8 - 16 - 28 - 56	4 - 16 - 24 - 36
	T	14 - 27 - 45 - 70	10 - 27 - 45 - 66
	H	10 - 21 - 39 - 62	8 - 23 - 37 - 60
Phellogen	R	10	7 - 10 - 21
	T	28	14 - 24 - 45
	H	28	10 - 28 - 45
Phelloderm solereids	R	14 - 26 - 54 - 128	16 - 29 - 51 - 80
	T	16 - 34 - 62 - 110	16 - 40 - 80 - 134
	H	18 - 30 - 50 - 80	16 - 30 - 54 - 98
	B	4 - 5 - 15 - 28	6 - 10 - 18 - 32
Fibres	H	2000-13500-22000	2000-14000-20000
	D	10 - 21 - 39 - 62	8 - 26 - 54 - 80
	B	4 - 8 - 16 - 30	4 - 9 - 23 - 40
Latex vessels	D	20 - 25 - 60	21 - 26 - 48
Sieve tubes	D	11 - 20 - 37	10 - 18 - 28

Table 16 - continued.

Structure		Size (μ)	
		V S	V A
Phloem sclereids	R	14 - 36 - 76 - 156	18 - 34 - 74 - 156
	T	18 - 34 - 78 - 156	20 - 32 - 68 - 134
	H	18 - 37 - 115 - 300	20 - 35 - 81 - 182
	B	6 - 12 - 24 - 48	6 - 12 - 24 - 40
Medullary rays			
Type A	R	20 - 36 - 60 - 94	22 - 34 - 50 - 74
	T	6 - 13 - 23 - 40	8 - 18 - 30 - 46
	H	10 - 14 - 22 - 30	12 - 18 - 26 - 48
Type B	R	8 - 18 - 34 - 56	14 - 24 - 36 - 56
	T	14 - 26 - 50 - 86	16 - 27 - 45 - 82
	H	18 - 32 - 56 - 90	24 - 35 - 65 - 124
Starch			
Simple	D	2 - 4 - 8 - 18	2 - 4 - 8 - 14
Compound D max.		8 - 12 - 14	9 - 12 - 15
Calcium oxalate			
Type A		6 - 13 - 31 - 52	6 - 14 - 26 - 42
Type B	length	32 - 59 - 165 - 356	20 - 92 - 172 - 278
	breadth	2 - 9 - 27 - 58	4 - 9 - 19 - 30

Comparative taxonomy of the species *V.schweinfurthii* and *V.africana*

The information presented in this thesis, that is in both the general review of the genus *Voacanga* and the results of original experiment, indicates a close relationship between the species *V.schweinfurthii* and *V.africana*, and poses the question of whether the differences between the various plants of the two species are sufficiently significant to warrant maintenance of such discrete taxonomic separation. If taxonomic classification is regarded as being based on the "maximum correlation of attributes"[137], that is not restricted merely to morphology but including other aspects of a plant's character, for example its chemical constituents, it might be considered more appropriate to class all the plants described under these two species as a single species.

Both species were first described by Stapf[6,7], and the main morphological differences were in the types of inflorescence, colours of corolla and sizes of flower. Yet Stapf's list of plants attributed to *V.africana* included two with larger flowers than allowed by his description of the species and one, considered to be either the species *V.angolensis* or *V.africana*, with a yellow corolla. These two characters indicated that the plants might be more appropriately designated *V.schweinfurthii*. Stapf considered these plants to be varieties of *V.africana*, but in a later consideration of the genus[4], he retained *V.angolensis* as

a distinct species, differentiated from V.schweinfurthii solely by the presence of some pubescence on the young branches and leaves. Later, Pichon[5] placed V.angolensis within the species V.schweinfurthii.

Other examples of confusion between the two species included the description of V.schweinfurthii var. parviflora by Schumann[70], a 'small-flowered' variety, considered as V.africana by both Stapf[4] and Pichon[5], and of the plants designated V.schweinfurthii by De Wildeman and Durand[58], of which at least one was identified as V.africana by Stapf[4].

It appears that the character considered of major diagnostic significance was the size of the flower. This was clearly stated by Pichon[5] who dismissed such characters as type of inflorescence and colour of corolla as being too variable, and considered the size of the entire calyx and of the corolla tube, but not of the corolla lobe, to be more reliable systematic characters. On this basis, Pichon still considered V.schweinfurthii and V.africana distinct species and sufficiently so to describe, for the first time, varieties of each of these species, and yet in his key to the classification of the genus, the values for the lengths of calyx of the two species showed some overlap, and only the ranges of lengths of corolla tube were quite separate. This is a rather slight morphological distinction.

The similarities of the stem barks of the two species have already been discussed. Anatomically their differences were so slight that it

was probable they had arisen simply from the method of collection. That their macroscopical and microscopical characters were so similar was not surprising, but the absence of any significant structural difference, the remarkable similarities in size and distribution of the microscopical structures, emphasised by the studies on numerical values, suggested a particularly close relationship. Also, the alkaloidal composition of the two barks was very similar, possibly identical.

The taxonomic importance of the similarities between the stem barks of V.schweinfurthii and V.africana is difficult to assess. Similar anatomical and chemical studies, the latter being extended to include compounds other than alkaloids, of the other morphological parts of the plants would shed more light on their interrelationship. Nevertheless, the evidence presented would support the merging of the two species.

Evidence has thus been presented to indicate that the various plants described as belonging to the two species V.schweinfurthii and V.africana might be more aptly classed in a single species, the differences in the dimensions of the floral parts perhaps being sufficient to warrant division of this species into large and small-flowered varieties. It has also been noted that the geographical distribution of the plants of the two species, from those listed by Pichon[5], indicated that V.schweinfurthii occurred most commonly in a somewhat restricted region in the Congo basin which has an exceptionally high rainfall, whereas the more widely distributed V.africana was found primarily in hotter, drier areas, although a

few of the plants described occurred in the same area as V. schweinfurthii. It is thus possible that the small differences in the size of the flowers of the two species may be related to the ecology of the environment, or they may be regarded as ~~two clines~~ clinal variation of a single species.

Chemo-taxonomy of the genus Voacanga

Traditional systematic taxonomy was founded solely on morphology but modern schemes of taxonomy take into account information in general on any particular plant, including its chemical constituents. The knowledge of the constituents of a plant may be used as a method of classification in two distinct ways. It may be used as the sole basis of a classification conceived for some specific purpose, that is an artificial classification, or it may be combined with other information, particularly morphological, in a general correlation of attributes, as in a natural classification. This natural classification based on overall resemblance is of more general taxonomic value.

Briefly, this modern approach may be said to be based on the reasoning that the nature of a plant is determined essentially by its cytogenetic character and this controls the biochemistry of the plant, and hence its biosynthetic systems and their end-products, and its general morphology. From the practical point of view, investigation of the biosynthetic products and examination of the general morphology of the plant are fairly readily achieved. One obvious advantage of chemical data over many points of morphological differentiation, is that they are purely objective, whereas, for example, the description of the shape of a particular morphological structure is largely subjective.

Clearly, the more comprehensive the knowledge of the constituents

of a plant, the more accurately it reflects the overall nature of the biochemistry of the plant, but in practice, chemical investigations are usually restricted to one, or a few, types of compounds. This approach means that only compounds of restricted occurrence must be considered, the presence of, for example, simple, common sugars would obviously have no taxonomic significance. Thus it is the occurrence of complex secondary products which are of value in chemo-taxonomy, such as flavonoids, glycosides and alkaloids; even within these groups some relatively simple members may be only precursors of the more complex compounds present and thus be more widely distributed than the end-products, and, consequently, of slight taxonomic value. An example of a relatively simple alkaloid of very wide distribution in the plant kingdom is nicotine, and hence its presence in a particular plant is of little taxonomic value. However, it is possible that when sufficient information on its occurrence has accumulated, its role in biosynthetic systems may become clear and it may then prove of taxonomic significance, most likely at a level considerably higher than the species level.

The Voacanga alkaloids are complex indole compounds and hence may be considered to have taxonomic significance. Such compounds appear to be largely restricted to the families Leguminosae, Loganiaceae, Apocynaceae and Rubiaceae[138,139]. Although only a limited amount of information on the occurrence of alkaloids in the genus Voacanga is available (See Part II), this may be considered in relation to the morphological systems of

classification of the genus.

The four major alkaloids of V.africana, voacamine[9], vobtusine[9], voacangine[8] and voacorine[12], also occur in V.schweinfurthii and V.bracteata[20], and, with the exception of voacorine, in V.thouarsii var. obtusa[9,10]. According to the classification of the genus by Pichon[5], the first three of these species occur in the section Streptostephanus, along with the species V.micrantha and V.psilocalyx, whose chemistry has not been studied. However, V. thouarsii var. obtusa is placed in the section Orchipeda.

According to the earlier classification of Schumann[3], although the species V.thouarsii again appears in the section Orchipeda, the species V.obtusa K.Schum., based on the plant later described as the variety V.thouarsii var. obtusa by Pichon, is placed in the section Streptostephanus. A chemo-taxonomic classification would suggest the retention of V.obtusa as a separate species dissociated from V.thouarsii, and thus all the species within the section Streptostephanus, whose chemistry has been studied, would contain the alkaloids voacamine, vobtusine, voacangine. and (possibly) voacorine. Alternatively, if further study of the alkaloids of V.thouarsii var. obtusa confirmed the absence of voacorine, this may be of taxonomic significance.

Conflicting reports on the alkaloidal content of the other variety of V.thouarsii nominated by Pichon, V.thouarsii var. dregei, have been presented. According to Schuler et al.[18], this variety contains two of

the alkaloids present in plants belonging to the section Streptostephanus, namely voacangine and vobtusine. This would suggest a close relationship between this variety and V.thouarsii var. obtusa. However, Neuss and Cone[19] later failed to find these alkaloids in V.thouarsii var. dregei, but isolated instead an alkaloid not present in any of the plants belonging to the section Streptostephanus, namely dregamine. If the more recent results are accepted as accurate, this could be taken as confirmation of the maintenance of V.obtusa as a species, and the presence of dregamine may be a character of the section Orchipeda. Unfortunately no information is available on the chemical constituents of the other species in this section.

The only other species of the genus which has been studied chemically is V.chalotiana[21]. This species is classified quite separately by Pichon as the sole member of the section Chalotia, and it is significant that it contains the alkaloid voachalotine, as yet reported in no other plant.

Several of the Voacanga alkaloids occur in other genera[1,37,91-95], and it is noteworthy that these are all closely related taxonomically within the family Apocynaceae.

It is thus seen that the information available is not yet sufficient to permit any firm conclusions as to the chemo-taxonomy of the genus Voacanga, though the evidence which has been presented would seem, in general, to confirm the existing morphological classification.

PART IX

SUMMARY AND CONCLUSIONS

1. A general review of the genus Voacanga is presented, comprising the history and development of the taxonomy of the genus, a detailed survey of the alkaloids present in several members of the genus, and an account of the pharmacological actions of these alkaloids.

2. The alkaloidal composition of the stem bark of Voacanga schweinfurthii Stapf has been studied in detail and shown to consist of about ten alkaloids.

Of these alkaloids, the three major ones, voacamine, vobtusine and voacorine, have been isolated and characterised; two others have been shown to be present in small amounts, voacangine and an unidentified alkaloid; and the presence of traces of several other alkaloids, probably at least five, has been indicated.

3. A comprehensive study of the anatomical structure of the stem bark of V. schweinfurthii, in both the whole and powdered conditions, has been made; this structure has been described and illustrated and shown to be typical of the barks of plants of the family Apocynaceae.

4. A similar study of the anatomy of the stem bark of Voacanga africana Stapf has been carried out and shown to differ from that of the bark of V. schweinfurthii in only a few insignificant details.

5. Attempts have been made to devise suitable numerical values,

based on sclereids, fibres, and crystals, to differentiate the barks of V.schweinfurthii and V.africana, but these values have served only to emphasise the structural similarities of the barks of the two species.

6. From the slight differences in the general morphology and in the structures and alkaloidal compositions of the stem barks of the two species V.schweinfurthii and V.africana, the possibility of merging these into a single species, is discussed. It is considered that insufficient information has been accumulated to permit any firm conclusion, but that which has been presented would support such a move.

7. Consideration of the chemo-taxonomy of the genus Voacanga has been made and it is suggested that the three major sections of the genus, according to accepted morphological classifications, may be characterised by the presence of specific alkaloids.

On this basis, Voacanga obtusa K.Schum. should be retained as a separate species, in the section Streptostephanus, and not designated a variety of Voacanga thouarsii Roem. and Schult., which belongs to the section Orchipeda.

Chemical studies must be extended to other species of the genus, and to morphological structures other than the stem bark, before the relationship between the chemical constituents and taxonomy can be fully assessed.

PART X

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