TYGE W. BÖCHER & OLE B. LYSHEDE

ANATOMICAL STUDIES IN XEROPHYTIC APOPHYLLOUS PLANTS

I. MONTTEA APHYLLA, BULNESIA RETAMA AND BREDEMEYERA COLLETIOIDES

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter **16,** 3

Kommissionær: Munksgaard
København 1968

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Synopsis

Three xerophytic leafless shrubs from the Monte region of Western Argentina were studied anatomically, the idea being to compare the internal structures of different plants belonging to the same life-form. The three species, viz. *Monttea aphylla* (*Scrophulariaceae*), *Bulnesia retama* (*Zygophyllaceae*) and *Bredemeyera colletioides* (*Polygalaceae*) are referred to the terete apophylls. Anatomically the three species have some interesting common features: the lack of fibre strands, leaf traces and other vascular bundles in the cortex, the development of a many-layered palisade tissue in the cortex and a sinking down of the stomata in deep pits or pitchers. Two of the species develop a multiple epiderm while one, *Monttea aphylla*, produces a very thick cuticular coating, which represents a special type of cuticular layer, characterized by vesicles of fatty substances secreted by the epiderm cells and limited by dome-shaped lamellae containing cellulose. The cuticular coating in *Monttea* has been studied in more detail, as have the stomatal pitchers in *Bredemeyera*. Wax plays an important part in the cuticular layers in both species; in *Bredemeyera* the narrow entrances to the pitcher cavities are densely covered by hair-like wax figures. In this species the front cavities are partly covered by a thin diaphragm which is formed as a continuation of the outer ledges. Thus, there are here two cavities with narrow openings outside the stomatal apertures.

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1. Introduction

Xerophytic apophyllous plants with assimilatory stems constitute a life-form which seems to occur mainly in subtropical subdesert shrublands but extends to areas with macchia. The main idea of the present investigation is to undertake an anatomical analysis of members belonging to this life-form class. Life-forms represent final stages of convergent evolutionary lines. In many different families broom-like, "apophyllous" species have evolved. In this first contribution three species from the families *Scrophulariaceae*, *Zygophyllaceae* and *Polygalaceae* are described. They are undoubtedly secondary stem-photosynthetic species, descendants of foliate ones. While *Bulnesia retama* carries small foliate leaves on young shoots the two other species have only scale leaves. They represent therefore the final stage in the evolution from foliate plants.

Raunkiær (1916, 1934) treated leaf size classes as biological types or life-forms. He regarded the diminution in leaf size (the evaporating surface) as an important adaptation to increasing drought. He operated with six classes from "Megaphylls" to "Leptophylls". Although stem assimilatory leafless plants are not mentioned by Raunkiær, they represent the very extreme in his series and might be called "Apophylls". Apophylls are not stem-succulents, but many transitions between such succulents and apophylls exist, thus e.g. *Anabasis articulata* (cp. Fahn & Nina Dembo 1964).

As pointed out in an earlier paper (1963) life-forms are initiated by ecotypes, the ecotype being a heritable common morphological-physiological response of several species to the same environment. Ecotypes and life-forms have mostly been studied morphologically only; but the significance of their structural pattern can only be fully understood by anatomical and eco-physiological investigations. In the case of apophylls several questions arise, some of the most obvious being the following:

To what extent is the transition from foliate to apophyllous habit followed by a convergent evolution of anatomical characters and what kind of taxonomic characters (family-characters etc) are able to survive or influence a convergent evolution which tends to wipe out most vegetative or non-floral differences? If apophyllous non-succulent plants represent an ultimate or perhaps most advanced step of adaptation towards deficiency of moisture, they ought to show a higher degree of xeromorphy than other plants. Hence we may ask: Are such features, which usually are looked upon as xeromorphic, exaggerated in apophyllous species?

Fig. 1. *Bulnesia retama* (*Zygophyllaceae*) in gravelly desert east of the Andes north of Mendoza. The white area in the background is a dried up salty clay flat which sometimes receives water from a river rising in the high mountains. — T.W.B. phot. Jan. 1st, 1956.

Acknowledgements

The present study was planned and material collected by the senior author during an expedition to the arid areas of Western Argentina in 1955–56. This expedition was made possible by grants from the Fundacion Williams in Buenos Aires and the Danish State Science Foundation. The authors are indebted to Mag. scient. Ole Mattsson for much valuable advice during the microscopical work.

2. Monttea aphylla (Miers) Benth. & Hook.

Material: Prov. Mendoza, San Rafael, circ. 2 km south of the town, Altitude 900 m, near Ruta 144 (Böcher, Hjerting & Rahn No. 1125). — Prov. Neuquén, 12 km south of Buta Ranquil, near Ruta 4 (Böcher, Hjerting & Rahn No. 1561).

Monttea aphylla (*Scrophulariaceae*) is a tall shrub which can reach a height of 3–4 m and trunk-diameters of 10 cm. The branchlets end in thorns. Small leaves are found on young stems; they are shed very early. The species is distributed in the western provinces of Argentina between Tucuman (Tafi) and Rio Negro (Valcheta). According to Cabrera (1961) it is characteristic of the phytogeographical province called "Monte" (cp. Morello 1958).

Leaf anatomy

The leaves are very small; only a single leaf was available. In cross sections it was rhomboid but somewhat flattened and clearly isolateral, although rows of palisade cells radiated to all sides from the central vascular bundles. The epidermal cells had thick walls and the stomata were raised a little above the other cells. The cuticular layer was not particularly thick (see Plate VIII b) except at the leaf margins where a thick layer was formed. It is interesting to find that the character of producing a very thick cuticular layer is deeply rooted in the stems only, which are longliving and must be able to withstand very dry climatic conditions.

Stem anatomy

Epidermis

In contrast to the two following species the epidermis remains one-layered. In young stems the cells are, apart from the enormous cuticular layer, almost isodiametric and the outer walls convex (Plate VIII a), but later they enlarge tangentially and the outer non-cutinized walls become concave. The inner periclinal wall and the anticlinal ones are thick. The outer periclinal wall is usually thin and covered by a cuticle and a cuticular layer of quite unusual dimensions and properties. Cabrera (1961), who rightly states that the stem in *Monttea aphylla* is very conspicuous from an ecological point of view because of this very thick layer, uses the word cuticle for it, but in *Monttea* there is a thin normal cuticle and below, a thick coating which, although deviating in many characters, must be regarded as a special type of cuticular layers.

Cuticula

The cuticular layer is always bounded by a thin cuticle. The cuticle is continuous but may be broken if fissures are formed in the cuticular layer beneath. Its surface can be nearly smooth, but frequently it covers a system of folds and grooves (Plate VII a). It stains yellow with iodine-zinc chloride and in many cases shows a weak birefringence (Plate IV c). It fluoresces with a golden yellow shine.

Cuticular Layer

Immediately below the cuticle there is a very thin layer which resembles the layer rich in pectin described in epidermis cells of *Aloë* by Fritz (1935: 723). It appears dark in polarized light (Plate IV c) but may show up when observed with phase contrast (Plate IV b). In material treated with ruthenium-red it stains red and with periodic acid-Schiff reagent (PAS) it appears to be very slightly salmon-coloured.

Inside this very thin and homogeneous looking outer layer follows the main part of the cuticular layer. Its thickness increases considerably with the age and size of the branchlet. In very young parts it is 20–25 μ (Plate VIII a), later in about one-

year-old branchlets it is 50 μ (Plate VIIId) and finally it reaches 140–180 μ in older branchlets (Plate Ic and Fig. 4a). CABRERA measured a breadth of 140 μ in three-year-old branchlets.

Chemical properties

With Sudan IV the whole layer stains intensively red. Nile-blue gives a blue colour only. Chlor-zink-iodine stains the outer part yellow, the inner part very pale yellow.

Using Johansen's quadruple staining the inner part remains almost unstained while the outer part becomes purplish (Fig. 2b, Plate Ia–b). With this staining procedure which includes tertiary butyl alcohol the layer as a whole shrinks to about two thirds and a number of lamellae appear (Plate Ia). Very frequently the weakly stained parts resemble vesicles arising from a group of epidermal cells (Plate IIIb, Fig. 6b). Hot alcoholic alkali (5 % and 12 % KOH) dissolve the cuticular layer. Treatment with Sudan IV with NaOH leads to a strong shrinkage (Plate VIIc). 4 % NaOH (after pretreatment with ammonium oxalate) affects the layer strongly and reduces it to about half its thickness and, 17 % NaOH dissolves the layer completely. The cuticle and cutinized parts of the guard cell walls and those of the subsidiary cells (see later) also disappear after treatment with alcoholic alkali.

These chemical data suggest that the main part of the cuticular layer consists of substances of lipid character, e.g. cutin and wax.

The above-mentioned vesicles or bubbles in the inner part of the layer were examined for carbohydrate contents. Ruthenium-red stains the limiting membranes of the vesicles red in rare cases only, but they were heavily stained by methylene blue (Plate VIb). With periodic acid-Schiff (PAS) the membranes of the vesicles were clearly stained (Plate II c–d). This shows that thin dome-shaped carbohydrate lamellae enclose the contents of the vesicles.

The chemical composition was further investigated by following the extraction procedure of cell wall components worked out by JENSEN (1962). After the extractions with ammonium oxalate and/or NaOH the material was stained with PAS, Sudan IV or Nile-blue.

The treatment with ammonium oxalate alone had no or a very limitied effect as no shrinking was observed. This suggests that the cuticular-layer apart from the above-mentioned thin external membrane does not contain pectin. The same result is obtained with ruthenium-red. This dye stains the middle lamellae in the epidermis, cortex and the stele intensively red but leaves the major part of the cuticular layer unstained. As already mentioned, however, pectin was present in some of the dome-shaped vesicle membranes.

When the slides, after the extraction with ammonium oxalate, were treated with 4 % NaOH the result was a great shrinkage, a dissolution of some of the components along ± cylindrical corrosion cavities and greater distinctness of the vesicles (Plate VI). The tubular cavities are orientated perpendicular to the surface of the outer wall

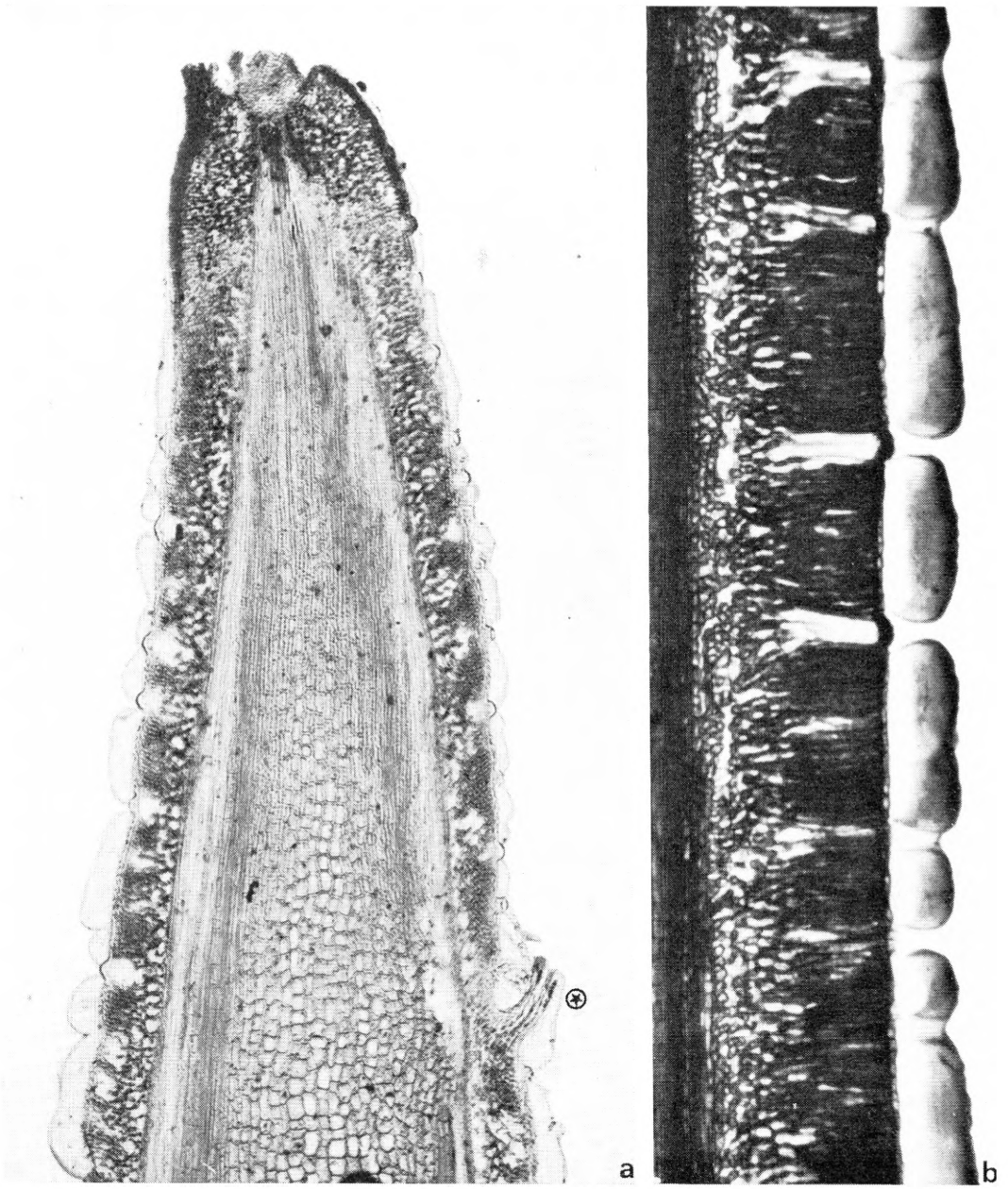

Fig. 2. *Monttea aphylla*. A longitudinal section through young stem terminating in thorn and bud-like structure, which probably is a hydathode. Dark colour on cells which sclerify. At asterisk is a rudimentary leaf. ×64. — b. Longitudinal section of branchlet showing thick cuticular coating, palisade layers and spongy parenchyma in cortex; several stomata and substomatal air spaces. ×100.

from which they arise. Only very rarely do they reach the outer surface. They develop mainly outside the anticlinal walls and preferably in the basal parts of the membranes of the dome-shaped vesicles, but sometimes the corrosion takes place in all parts of such a membrane (Plate VI d). By using phase contrast the cavities appear to be much more numerous and some of them clearly distend in the abaxial part (Plate VI d-e). The most important fact is that all have the same orientation which is perpendicular to the surface and that they show that ± pillarshaped parts of the cuticular layer are less resistant and are dissolved by 4 % NaOH. It is difficult to interpret these results. If hemicelluloses were present they would probably be dissolved by 4 % NaOH. However, the great shrinkage must be due to the disappearance of substances which are more abundant, viz. some of lipid character. Two different processes seem to take place simultaneously; a dissolution of less resistant carbo-hydrates (e.g. hemicelluloses, slimy substances) and a beginning decomposition of the fatty substances.

By addition of cuoxam (Schweitzer's reagent) the limiting vesicle membranes are dissolved. A comparison of untreated material stained with PAS and material treated with cuoxam shows that cuoxam unveils many more vesicle borders. This means that the amount of carbohydrates changes from one vesicle to another and that it is only immediately above the epidermis cells that the vesicles contain carbohydrates in such densities that they can be demonstrated with PAS. As a rule the carbohydrate lamellae, when the vesicles grow, are stretched considerably; finally they become invisible. A rare case is shown in Plate II d. Here the margin of a vesicle which had almost reached the upper surface was stained with PAS.

After treatment with ammonium oxalate succeeded by 4 % NaOH the whole cuticular layer was often loosened. This is probably due to a complete dissolution of the pectin layer which occurs in the outer wall beneath the cuticular layer. A layer of this kind was already found by Fritz (1935) and several workers later.

In *Monttea* a continuous layer of pectin, however, seems only to be present in epiderms, where the production of new material to the cuticular layer is small or has stopped. In young stems there is usually a pectin layer except outside the anticlinal walls. This fact explains why the cuticular layer is not loosened after extraction with ammonium oxalate only.

Physical properties

In the fluorescence microscope the cuticular layer appeared dark. Some areas, however, had a light bluish fluorescence suggesting the presence of wax. By adding acridine orange the walls in the epidermis, cortex and stele shone reddish. There was no trace of such a reddish fluorescence in the cuticular layer after this treatment.

Very instructive pictures were obtained by using the polarizing microscope. Observations were made on (1) slides produced from herbarium material and mounted in glycerol, (2) slides produced from alcohol material and mounted in

glycerol, (3) slides stained with PAS, Lightgreen-Safranin or Johansen's quadruple stain, all treated with xylene before mounting in canada balsam.

(1) In the slides originating from the herbarium material the whole cuticular layer was birefringent. Whole vesicles showed up brightly and had crossing black extinction lines (cp. Plate IIIa). On heating most of the birefringence disappeared and returned on cooling. Outside the anticlinal walls, however, pairs of brightly shining lines perpendicular to the surface persisted. In slides which were treated with cuoxam these lines disappeared while the generally distributed light persisted. Using the Red I plate the lines always had the opposite colour to most of the light which came from the rest of the layer. Very often there was a change in colour along a periclinal, somewhat undulating, line through the layer. When the inner part and the perpendicular lines were blue, the outer part was orange. In many cases there were areas mainly with blue lines along the outer cell walls and roundish orange areas in the exterior part of the cuticular layer corresponding to outer parts of vesicles.

(2) Slides made from branches fixed and kept in 70 % alcohol for 10 years had almost the same qualities although the general birefringence was clearly less bright.

(3) In slides which, during preparation were treated with xylene, the birefringence was usually restricted to the lines off the anticlinal walls (Plate IIIc, IVa). However, very locally, clearly birefringent areas had survived and sometimes the whole cuticular layer appeared to be slightly shining (Plate IVc).

From this the following conclusions may be drawn: The lines outside the anticlinal walls must be due to cellulose micells while the more extensive birefringence is caused by wax. The fact that some birefringence persisted after heating or after treatment with strong alcohol and xylene may perhaps be ascribed to wax which is adsorbed to cutin (cp. Roelofsen 1959: 265). It is impossible to estimate the amounts of cutin and wax, but the wax component is undoubtedly a prominent one.

In many cases there are two shining lines outside each of the anticlinal walls (Plate IVc) or one broader line which divides below into two thin ones. Obviously each cell produces a dome-shaped vesicle with cellulose arranged in the limiting lamellae or membrane and membranes from two adjacent cells may remain separate or merge. The lines taper towards the periphery, but at the same time they branch or split up into a number of very delicate shining lines. This splitting up is due to the stretching of the vesicles which are filled up with substances of lipid character. The beginning of this process can be studied in small (young or slowly enlarging) dome-shaped membranes, where the cellulose is stained with PAS. Near the anticlinal walls such domes appear rather compact although sometimes clearly lamellated, but their distal parts, which correspond to the middle parts of the original outer cell walls, are expanded and here the membrane shows a fine network of stained fibrillae (cp. Plate IIc). It is usually the middle parts of the enclosing lamellae which during the expansion are split up and appear to burst, but sometimes the stretching primarily affects the marginal parts, resulting in a displacement of small cellulose caps to the outermost part of the cuticular layer (Plate IId, IIIa arrow).

The question is, what happens to the cellulose and possible hemicelluloses which from time to time are pushed out towards the periphery. It was sometimes possible to detect some slightly birefringent pairs of lines near the surface, they were independent of the bright lines near the epidermal cells but had probably the same origin being remains of old vesicle membranes. This seems to show that with increasing distance from the cell surface the cellulose is obscured, being perhaps more and more enveloped by cutin. At the same time, however, hemicelluloses or pectin which are probably placed together with the cellulose may be decomposed. If products of such hydrolyses reached the surface through fissures in the cuticle the common occurrence of colonies of fungal cells on the surface would be more comprehensible (cp. p. 15 and Fig. 6 a).

As the production of new carbohydrate lamellae takes place with certain intervals, the matrix inside the lamellae will also be formed intermittently. Even when no limiting cellulose is detectable the matrix seems to be deposited in ± cubical or more irregular and elongated entities which have almost the same breadth as the epidermal cells beneath. This is particularly easy to see with phase contrast.

According to the chemical and physical properties of the cuticular layer previously referred to the matrix probably has lipid character and may consist of procutin and wax. Immediately outside the outer periclinal cell walls the Sudan IV reaction is as strong as at some distance from the wall. On the other hand, with the quadruple stain, the dark purplish colour indicating fatty substances only occurs in the outer area. With this method the major part of the wax is removed. The lighter area in the inner part of the cuticular layer (e.g. Plate I a, b and Fig. 2 b) may therefore contain less cutin, but possibly procutin.

That substances of lipid nature are formed as a matrix between lamellae which consist of carbohydrates is illustrated by Fig. 3 c–d. In Fig. 3 c the majority of an epidermis cell is filled with a substance which stains red with Sudan IV as the cuticular layer above. In Fig. 3 d a small part of another cell behaves in the same way. In both cases the areas which stain in the same way as the cuticular layer are limited towards the layer and towards the living part of the cell by firm cell walls. For some reason the cells in question stopped or slowed down the production of material for the cuticular layer. Hence, the outer membrane did not bulge and split up but remained firm or returned to a firm stage and developed into a wall. The inner membrane also developed into a wall, which now limits the cell from the cuticular island.

The formation of the thick cuticular layer in *Monttea aphylla* has the character of a secretion. In the young epidermis almost all cells are involved except the guard cells (Plate VIII a, c, d), and perhaps the subsidiary cells. The cells adjacent to the guard cells in young branchlets are less active and in older branchlets there are 3–4 cells on each side of the stomatal pore which do not have a thick cuticular coating (Plate VIII e, f). As a result of this differential activity in the epidermis, the stomata and the surrounding cells will be placed in the bottom of cavities formed by the thick cuticular coating produced by the other epidermal cells.

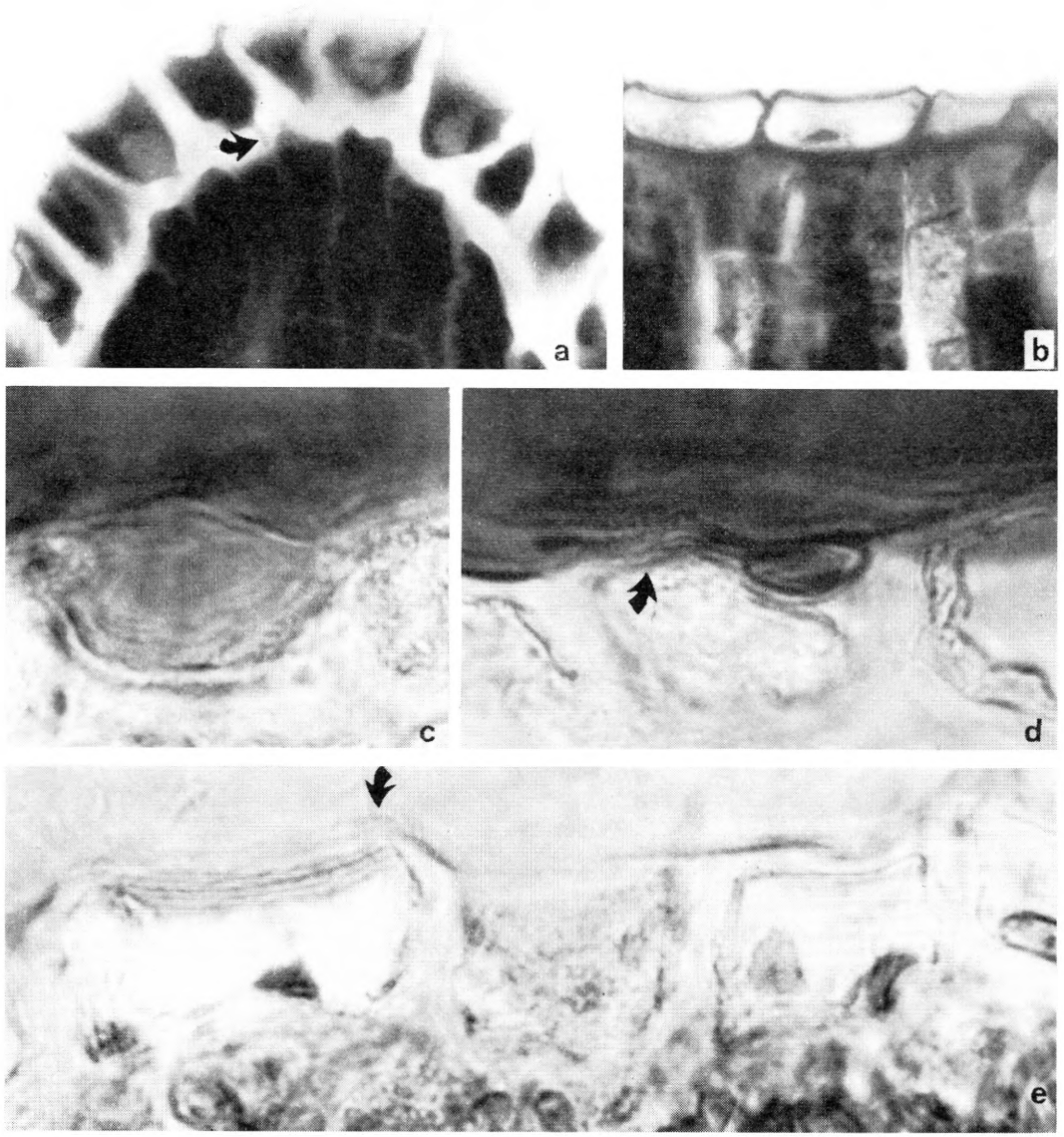

Fig. 3. *Monttea aphylla*. a. Young very active epidermal cells forming a ridge. The non-cutinized outer walls hardly visible, but a few thin lamellae. At the arrow is a wide primordial pit through the inner wall. ×1376. — b. Older, not very active epiderm cells showing complete non-cutinized outer walls and the grooves outside the anticlinal walls. ×800.

c–d. Insular areas of epiderm cells filled with matrix which is stained with Sudan IV. In c a major part, in d a minor part of the original cell was demarcated by walls towards the rest of the cell and towards the cuticular layer. At the arrow where the outer cell wall is very thin a number of short lines issuing from the surface. ×1950.

e. Part of epiderm showing cells at different stages and degree of secretory activity. From the left: Cell with six lamellae in outerwall (two nuclei), cell where outer wall is nearly decomposed, a single lamella can be traced, cell with two nuclei and solid outer wall. At arrow delicate short lines perpendicular to the surface. Nile blue staining of cuticular coating. ×1950.

In young branchlets the secreting activity of the epidermal cells is reflected in the shape and behaviour of the non-cutinized part of the outer walls. In such branchlets the areas between stomatal cavities bulge and form ridges (Fig. 3a and Plate VIIId) which are later smoothed out. With increasing distance from the cavities the cuticular coating becomes thicker and at the same time the epidermal cells undergo considerable changes: The outer walls become blurred and seem to disappear, at the same time the cytoplasma appears to bulge inwards (Fig. 3a). The cells look as if they were surrounded on three sides by thick walls, whereas the outer wall was replaced by the enormous cuticular layer common to all the cells. The anticlinal walls seem to be projected in the cuticular layer and fork (Plate VIIId, arrow), but this forking is merely due to the fact that those parts of the outer walls which border the anticlinal ones are often maintained whereas the middle parts are more or less decomposed.

By adding ruthenium-red it became evident that the thick inner periclinal and the anticlinal walls stained intensively red. Non-decomposed parts of the outer walls were also stained (Plate IIIf). However, using high magnification and phase contrast it is possible to trace some kind of wall substance in the middle parts. In many cells a very thin translucent lamella was seen bridging the gap between areas with firm wall substance (Plate IIIe, f). Sometimes there was clearly a number of thin lamellae (Fig. 3e). In other cases the translucent lamella appeared to be traversed by very delicate pores and in oblique views it seemed to be densely perforated. Some pictures lead to the assumption that the openings were like small protruding tubes or that perhaps some material passed through (see e.g. Fig. 3d and e at arrows, Plate IIe, f).

According to our observations it seems possible to distinguish between three steps in structure which correspond to three grades in excretory activity.

(1) Low activity. External wall distinct, occasional occurrence of delicate pores.
(2) Medium activity. Middle part of external wall indistinct, sometimes present as a very thin translucent lamella with numerous delicate pores.
(3) High activity. Majority of external wall decomposed, split up into a network of cellulose fibrillae.

In this connection some granular structures deserve to be mentioned. In phase contrast many small dark granules occur in two ways. They may either follow the boundaries of the vesicles (Plate Va), or they occupy areas off the anticlinal walls (Plate Vb). Near these walls they run perpendicular to the surface but when they approach the outer part of the coating they usually spread out as in a fountain. In slides mounted in water some larger translucent granules had the same positions (Plate IIIf). Although the origin of these granules is obscure it seems obvious that they represent particles which are placed on the surface of enlarging dome-shaped vesicles; if such vesicles do not merge, we get the linear arrangement and the "fountains" of granules near the surface. A possibility which cannot be excluded is

that the granules are small remnants of the decomposed outer walls which in this way glide aside when the vesicles grow. However, they are not birefringent and thus hardly composed of cellulose.

Evidently older branches have less active epidermal cells. But the cells are never at the same stage. Particularly active cells are found inserted between some in which the excretion is probably slowed down to a minimum or has stopped. In Plate II a, b the middle cells are probably actively producing a number of thin lamellae. Less active cells possess outer walls with a characteristic shape. Outside the anticlinal walls there is usually a groove (Fig. 3 b). Sometimes, however, this groove is bordered by ridges. Each cell may bear a ridge of wall substance along the groove, which is cut twice into transverse sections (Plate II b). In polarized light the shining lines radiate from the corners along the grooves or the ridges. The domeshaped cellulose lamellae all issue from the corners of the ridges (Plate II d). The ridges, therefore, are interpreted as basal parts of a number of domes the upper part of which during expansion of the encircled matrix were distended, split up and burst.

Stomatal apparatus

The surface of the thick cuticular layer is not smooth. A system of very low grooves gives the surface a labyrinthic or network-like appearance. The entrances of the cuticular cavities around the stomata are elliptic (Plate VII a). By lower focussing these cavities in some cases become narrower and longer, but often they enlarge and elongate (Plate VII a, b on the right). The walls of the cuticular cavities are delicately striated (Plate VII b). Usually each of them at the bottom contains one stoma only, but not infrequently two stomata occur (Fig. 4 a).

The guard cells have their longitudinal axes parallel to the axis of the stem (Fig. 2), but the major axis of the elliptic entrances is perpendicular to the axis of the stem (Plate VII a). The stomatal apparatus, as a whole, is raised above the surface of the non-cutinized outer walls of the neighbouring epidermal cells. This elevation is due to the growth of the subsidiary cells which turn outwards (Plate VIII e, f). The bending takes place in three small cells following after the cells which are adjacent to the guard cells. In older stems none of these cells take part in the production of the thick cuticular layer. Sometimes this cessation may be infectious so that even some more adjacent cells stop or slow down production. The thick surrounding layer may in such cases form a roof which covers an expanded part of the cavities containing stomatal openings. The subsidiary cells next to the guard cells attain a dark blue-violet colour in slides trated with Johansen's quadruple stain. The other epidermal cells remain green (cp. Plate I b).

As in *Bredemeyera colletioides* (p. 30) the outer ledges are very conspicuous. They form an arched roof over the big stomatal front cavity, only leaving a narrow fissure open. By closure of the stomatal pore the two ledges approach one another very much. The walls of the front cavity are covered by the cuticle and are cutinized.

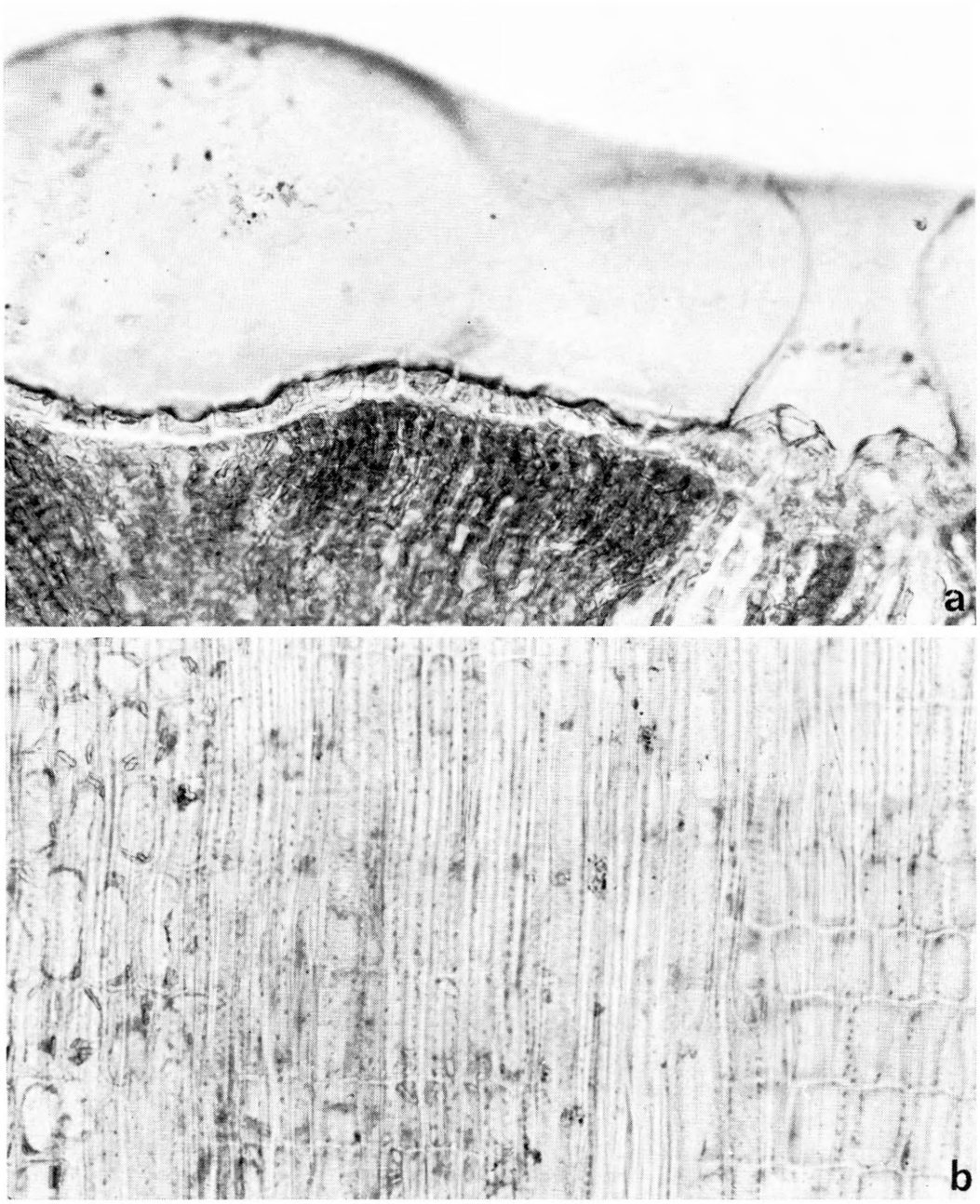

Fig. 4. *Monttea aphylla*. a. Cross section of epiderm and outer part of palisade tissue in cortex. Note two stomata in one cavity in the thick cuticular coating. — b. Radial section through wood, showing high ray cells, on the left with crystals. ×320.

The same is the case with the walls which form the outer limitation of the substomatal air chambers, except in the areas just inside the pore where the inner walls of the guard cells are particularly thick (Plate VIIIf). The same wall areas which are stained with Sudan IV, however, also stain weakly with Safranin and may therefore also contain lignin. Wax is present in the cutinized outer walls of all subsidiary cells and the guard cells apart from the outer ledges. These walls show very bright birefringence while there is no double refraction in the walls of the front cavity and the substomatal air chamber.

After treatment with hot alcoholic alkali the guard cells lose wax, cutin and pectic substances. This causes a complete change in their appearance. The rather thick outer wall shrinks so much that only a thin wall of cellulose is left, forming part of an arc of a circle and terminating towards the aperture with a very small tip which is the only thing left of the outer ledge. Also the projecting wall areas where the guard cells are closest together (cp. Plate VIII) disappear, with the exception of one lamella bordering the cell lumen and one near the aperture. The best preserved wall sections are those between the cell lumina and the substomatal chamber. Here the thick wall contains a number of cellulose lamellae which by using phase contrast and high magnification stand out very clearly, the pictures reminiscent of those found by means of the electron microscope (cp. Roelofsen 1959: 243–244). Obviously the guard cells of *Monttea* contain very much of pectic substances, this especially applies to the inner walls, which by using the quadruple stain attain a dark violet colour (Plate Ib, IIId). Whether the above-mentioned collapse of the outer walls in the guard cells of *Monttea* is connected with the existence of many small lacunae is doubtful. Such cavities were found near the outer ledges in *Helleborus* by Huber et al. (1956).

The outer and inner thick walls of the guard cells increase their thickness as they grow older. The increase in thickness may be about 5 μ or 20 %.

In most parts of the material which after collecting in nature was fixed in alcohol, the cuticular cavities and sometimes even the stomatal front cavities were infected by an imperfect fungus with brownish cells (Fig. 6a). It occupied the entrances and sometimes single cells in the front cavities acted as a kind of plug (Plate Ic). Such fungal plugs probably reduce stomatal transpiration and the fungal cells receive some moisture escaping from the apertures. However, this partnership, in spite, perhaps, of being mutually advantageous, is hardly more than an accidental symbiosis. The fungus was absent from some part of our material thus all the younger branchlets, and no fungal infection was mentioned by Cabrera (1961) and may have been absent from his material.

Thorns

The branchlets of *Monttea aphylla* are terete, usually tapering and terminating in a thorn. In Fig. 2a a beginning sclerification of the tip is seen, at the same time, however, there is a structure on the very tip which probably may serve as a hydathode or water gland. It resembles a bud and may be a transformed terminal bud. The

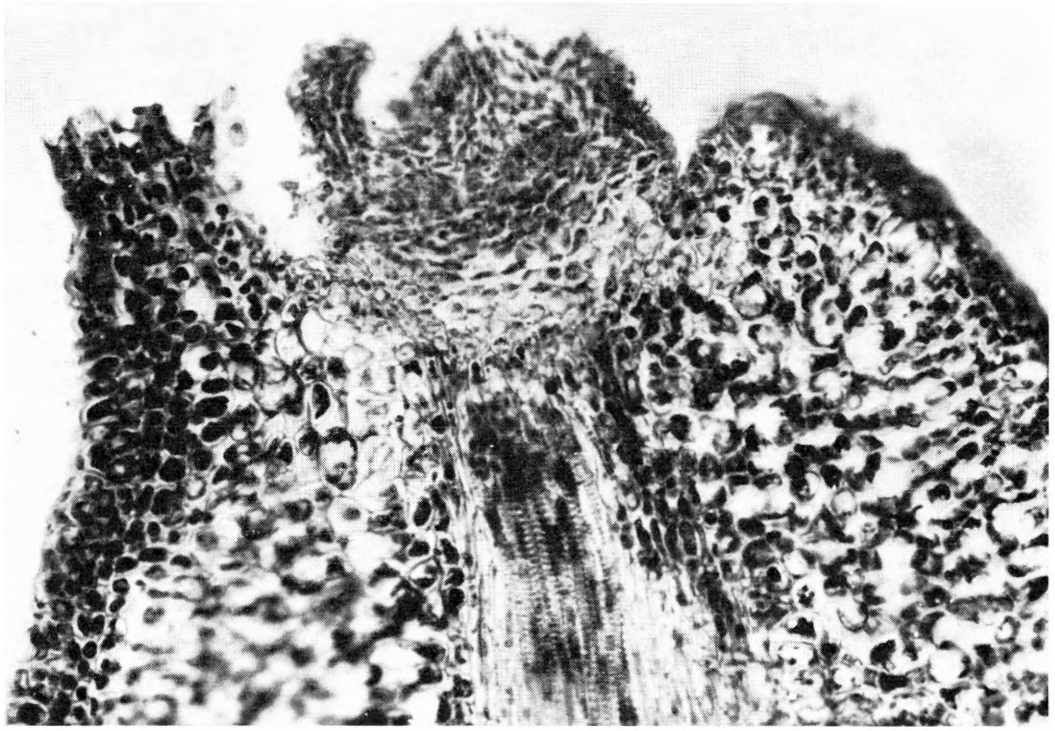

Fig. 5. *Monttea aphylla*. End of thorn showing bud which possibly functions as a hydathode and is supported by a reduced leaf (on the left). On the right the tip of the branch (cp. Fig. 2a). Below the bud a strand of tracheids. ×320.

cells are small with unlignified walls and very dense, they may serve as an epithem. A strand of many tracheids ends abruptly just below the small-celled tissue (Fig. 5). If the interpretation is correct we must assume that the function as water gland is restricted to the period of growth of the branchlet. Later the bud-like structure may be shed. In fact most thorns in our material have small circular scars in their tips.

Increase of girth

As the branchlet has a secondary growth the tissues in the cortex and the epidermis must adapt themselves to this growth. In the epidermis the cells enlarge tangentially, but also anticlinal divisions occur. Particularly this may be the case outside such areas where the cortex undergoes a dilatation. Here, a fan-shaped arrangement of the rows of palisade cells is common (Figs. 4a, 6b), and many epiderm cells seem actively to take part in the production of the cuticular layer. Another increase in girth is obtained by initiation of periderm areas, which seem to be able to expand rapidly. This type of diameter growth is presumably the most important one in older branches.

Periderm

The first periderm commonly originates in a similar way as in *Bulnesia retama* (p. 27) as local cork formation resembling a lenticel. In any case only localized phellogens occur which bend inward and soon become rather deeply situated. On the other hand some periderm areas in old branchlets grow very large, the result being a localized rhytidome formation.

The initiation of the phellem areas most frequently takes place in an unusual way. Epidermal cells situated below the thick cuticular layer and often in areas of girth increase, undergo periclinal divisions and initiate a phellogen (Fig. 6a). Next the cuticular layer may break and the phellem expand in the crack (Fig. 7c). During this process the expanding phellem tissues merge with the cuticular layers which surround them and which they penetrate (Fig. 7c, 9b). The first cells which are found in abaxial direction, however, may sometimes at the beginning be living and even contain chloroplasts (Plate Vc). Later such cells die as their walls become suberized and finally they are pushed out by the normal phellem which is formed beneath. In many cases, however, there are no ruptures in the cuticular coating. What happens is a sideways and inward growth of the periderm areas which finally reach the substomatal air chambers and inwards may even reach the phloem. The substomatal spaces may be filled with phellem cells but in some cases it looks as if cells which are perhaps a kind of phelloderm grow into the substomatal air chambers and other intercellular spaces in the cortex and fill them (Fig. 7a, white arrow). Very often the cuticular coatings outside the phellem are maintained, but at the same time the original narrow cavities are widened as a result of the sideways growth of the periderm, the result being large phellem areas between islands of cuticular substance (Fig. 7a). However, in cases where a large and vigorous periderm is formed the thick cuticular islands scale off or are bent outwards.

Several successive periderms may arise and a rhytidome be formed. Such areas are found in branches the greater part of which are covered by the thick coating. The innermost phellogens arise in the phloem thus cutting off extra xylary fibre cells and sclerified inner cortex cells. Fig. 8 may show a special case. Here the branch apparently has been wounded and the rhytidome accumulation is very strong on both sides of the wound which reaches more than half way into the xylem. Perhaps this case may be explained as a wound periderm formation. However, in some cases the periderm areas seem to crack and no signs of injury of the branch is found.

A vigorous rhytidome accumulation covering large parts of the older branches replaces the transpiring green cortex and may thereby bring about a reduction of the transpiring part of the plant body.

Cortex

According to CABRERA (1961) the cortical parenchyma is nearly 100 μ thick in branches which are 2 mm in diameter. It consists of ten layers of cells. The two or

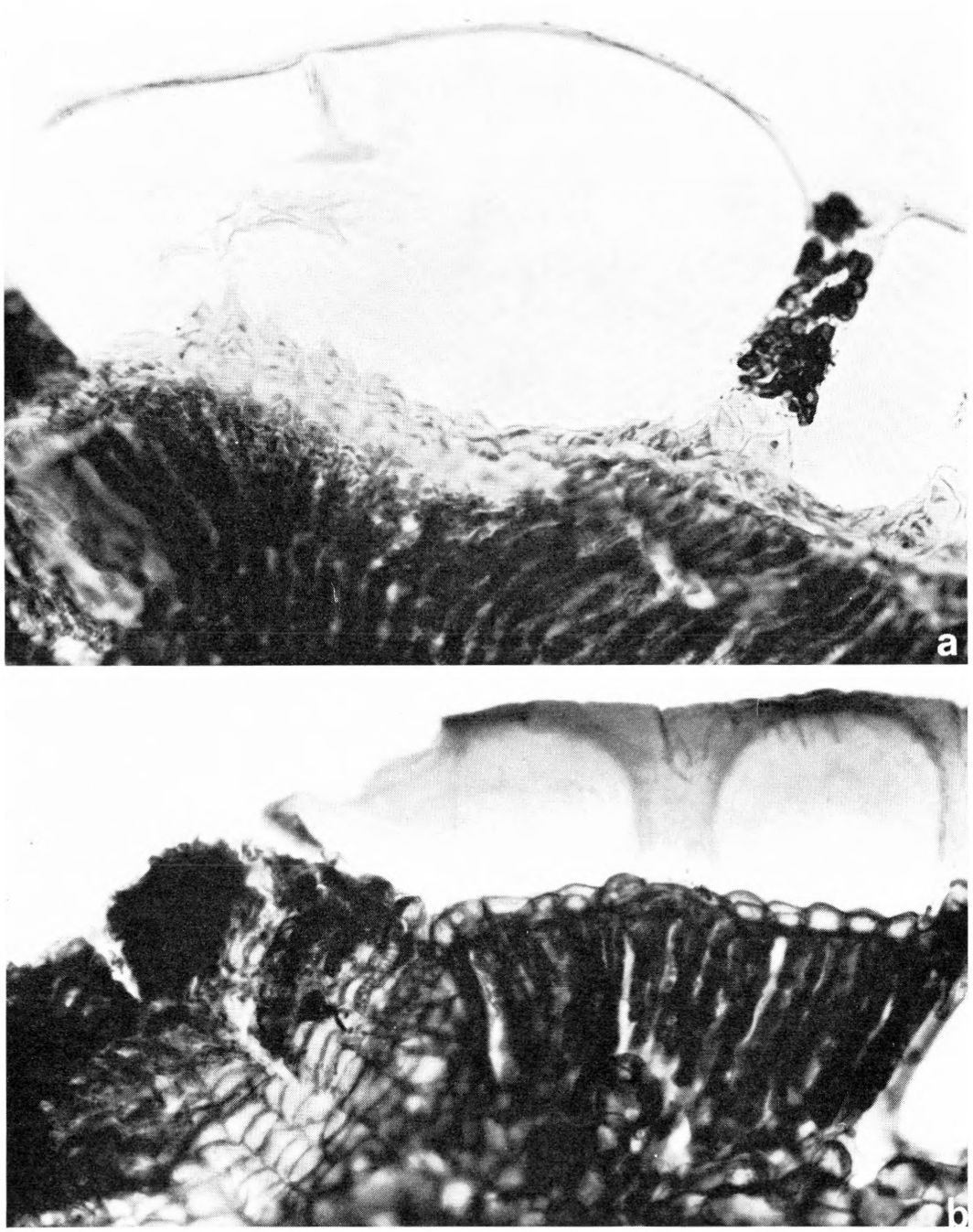

Fig. 6. *Monttea aphylla*. a. Periclinal divisions in epiderm cells initiate phellem formation and cause the cuticular layer to crack. One stomatal cavity in the cuticular layer filled with cells from an imperfect fungus. Fan-shaped arrangement of palisade tissue. — b. A phellem area (on the left) has filled out a crack in the cuticular layer which is found undisturbed side by side with the phellem. Two vesicles clearly shown by lighter colour (quadruple staining). ×320.

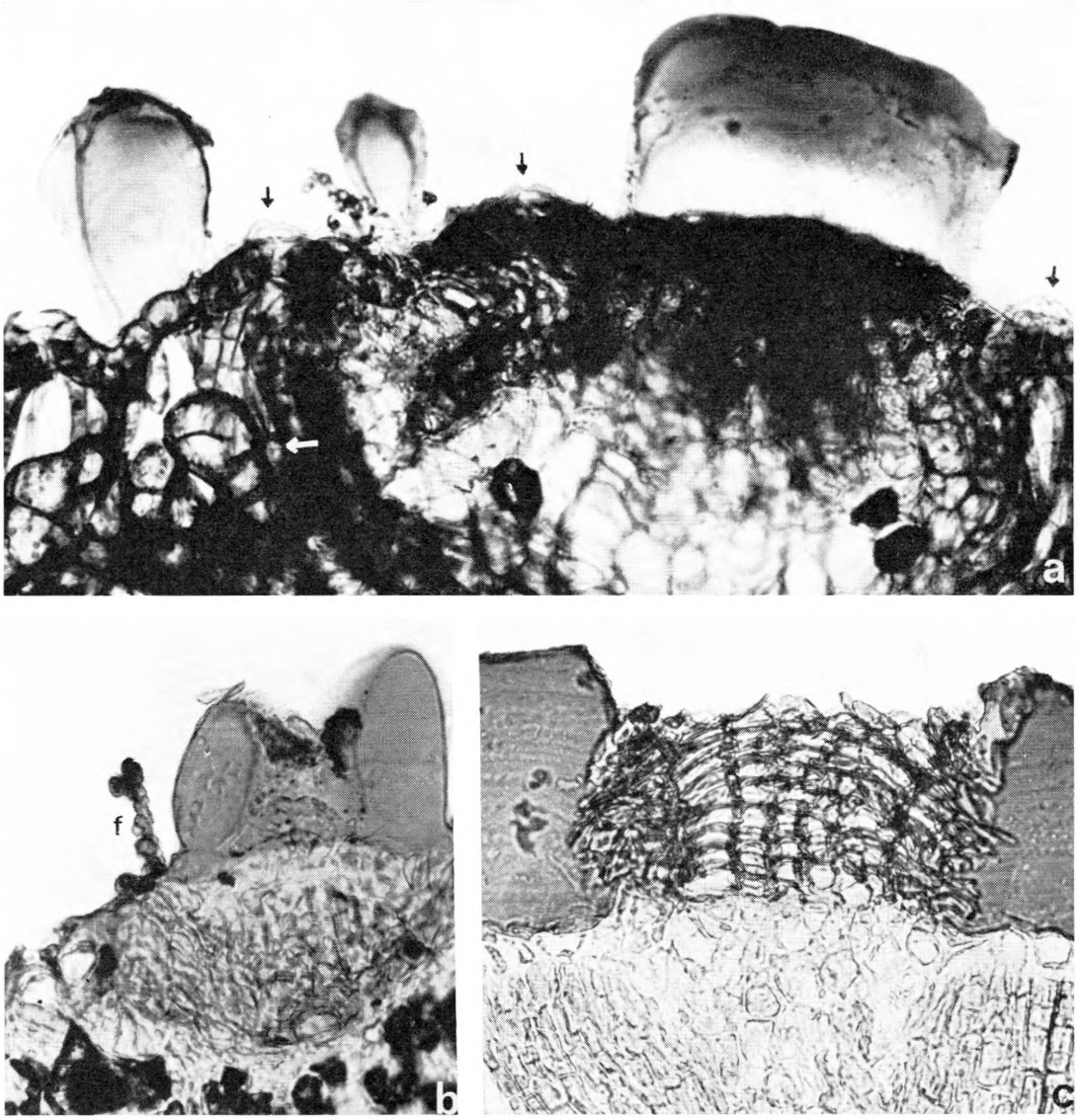

Fig. 7. *Monttea aphylla*. a. Three remaining fragments of cuticular coating separated from one another by developing phellem. Irregular cell masses perhaps phelloderm (white arrow) grow into substomatal air chambers, guard cells (on the extreme left) or outer ledges of guard cells are still seen at three points (black arrows). Quadruple staining. — b. Phellem formation beneath cuticular layer. Small row of fungal cells (f) and on the left two guard cells and rest of substomatal chamber (black). Sudan IV. — c. Phellem formation in crack in cuticular coating. Below a kind of phelloderm filling space in palisade tissue. Sudan IV stains cuticular layer and phellem. ×320.

three external layers have elongated and very compact cells, the internal layers have isodiametric cells with intercellular spaces.

This is in accordance with our observations although in our material it is possible

to follow a development from a young stage where the cortex is 100–150 μ thick (cp. Fig. 2a) to older stages where a thickness of 250–280 μ can be measured. This growth is caused by radial cell elongation of the outer layers, which develop into a palisade tissue and an increase in number of cell layers. The internal layers get very wide intercellular spaces and develop into a kind of spongy parenchyma. The cells here contain not so many chloroplasts but they accumulate much more starch than the palisade cells do.

Stele

The stele has a clear demarcation towards the cortex, a cylindrical belt consisting of 2–5 layers of fibres. CABRERA (1961) mentions that the pericycle has several layers of cells and some bundles of sclerenchymatous fibres and that the phloem is continuous, 60–70 μ thick. In our material there is mostly a fairly continuous cylinder of fibres. In one-year-old branchlets, however, there are broader interruptions of parenchymatous living cells. In older branchlets such interruptions are usually one cell broad. They form connections between the photosynthetic tissues and the phloem.

The secondary xylem was first studied by CHRISTIANI (1948) who compared *M. aphylla* and *M. schikendantzii*, both West Argentinian species, the latter being taller and foliate. In spite of this he found such great similarities between the structure of the xylem that he was unable to distinguish the wood of the two species clearly enough. However, according to Plate I, Figs. 1–2 in CHRISTIANI's paper the vessels are significantly wider in *M. schikendantzii*, the foliate species. Here the average vessel diameter is 24 μ (tangential direction) and 31 μ (radial direction) while in *M. aphylla* the corresponding values according to CHRISTIANI are 20 μ and 23 μ.

As appears from the cross section shown in Fig. 8 the wood in *M. aphylla* is very uniform. There are no wide vessels. Growth rings may sometimes be difficult to detect. The difference between late and early wood is usually very small but the wood is locally slightly ring-porous.

The number of xylem rays is great, every third or fourth cell row being a ray. The rays are 1–2(–3) cells broad and often very high. Most of them are primary. CHRISTIANI measured the height expressed in cells to 9–20 (–32–40) but we have occasionally counted about 80 cells. The height of the ray cells is greater than the breadth, those found near the phloem are regularly provided with crystals (Fig. 4b), the same being the case with the cells in the phloem rays. The axial parenchyma is very poorly developed if present at all.

The primary xylem vessels are helical. Those in the secondary xylem have a very delicate spiral striation in their walls. CHRISTIANI found particularly large quantities of small pores. The perforation is simple. The majority of the cells in the areas between the rays are fibres with thick walls.

The pith is broad (Fig. 8). Its cells bordering the xylem have thick lignified walls.

Fig. 8. *Monttea aphylla*. Rhytidome formation with deep crack in the phellem reaching far into the xylem. The undisturbed original surface with cuticular layer, epiderm, cortex with palisade tissue is seen on the left. Xylem without wide vessels. ×64.

3. Bulnesia retama (Gill. & Hook.) Griseb.

Material: a. Prov. San Juan, between Uspallata and Barreal (Böcher, Hjerting & Rahn No. 2228, Fig. 9b), b. Prov. San Juan (Hawkes, Hjerting & Rahn No. 3330), c. Prov. Mendoza 25–30 km north of Mendoza (Böcher, Hjerting & Rahn No. 2100, Fig. 1).

Bulnesia retama (Zygophyllaceae) is a 1–3 m tall shrub and a characteristic element in the Monte vegetation of Western Argentina. It is distributed between lat. 27° and 35° south and between parallels 65° and 70°W. According to Record & Hess (1943: 555) it sometimes develops into a short and stout tree, which may be about 5–8 m high. The slender branchlets, leafless most of the time, are crowded together in broom-like masses (Fig. 1 and Plate XIX in Morello (1958).

Young plants and branchlets bear compound leaves. These are in the young branchlets very small and with isolateral structure. In the young plants, however, the leaves are larger and dorsiventral. The stems are greyish-green as a result of a covering of unicellular hairs which bend near the base and become upright (Fig. 9a, 12c). In older branchlets the hairs are shed.

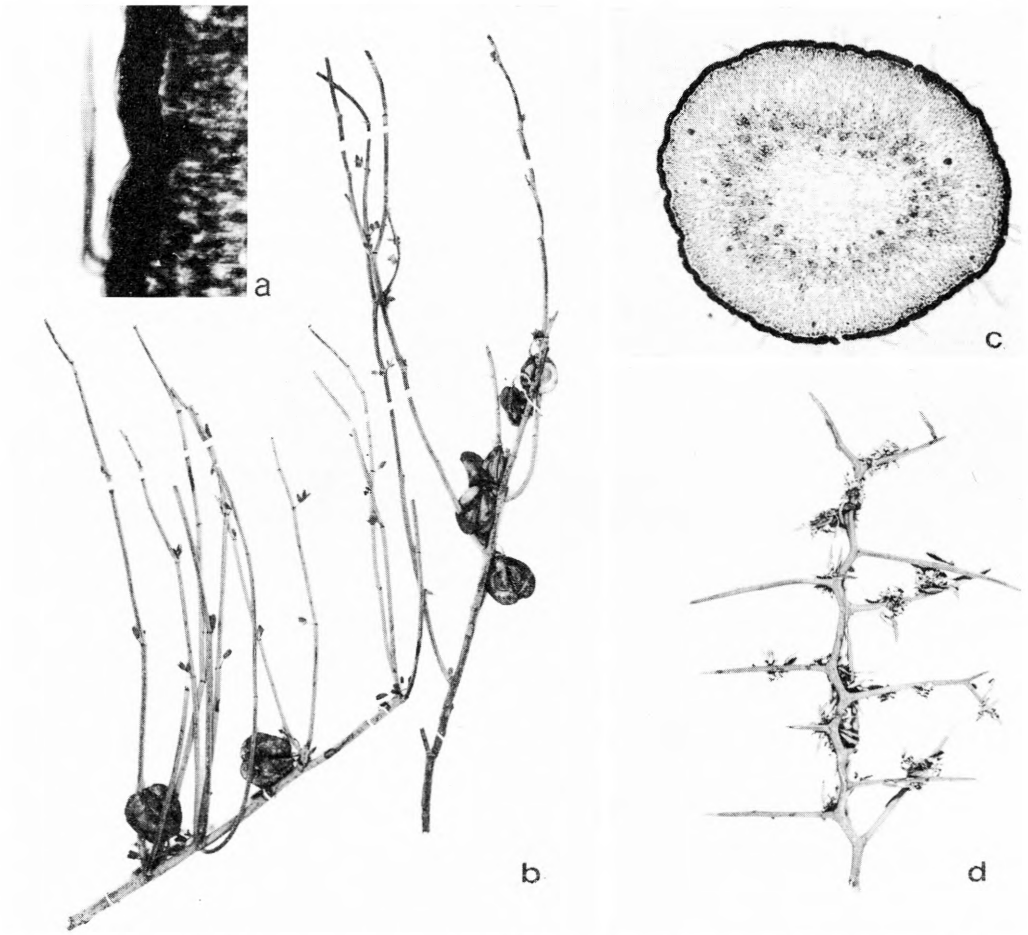

Fig. 9. a–c. *Bulnesia retama*, d. *Bredemeyera colletioides*. a. Hair and dark multiple epiderm. b. Young branchlet with small leaves, flowers and fruits (Böcher, Hjerting, Rahn No. 2100). c. Cross section of young branchlet showing cutinized epiderm (dark) and many non-cutinized hairs. — d. Flowering and fruiting specimen (Böcher, Hjerting & Rahn, No. 2228). a ×100, b and d ×1/3, c ×50.

Epidermis

The protoderm cells are almost isodiametric and polygonal in surface view. Very early the cells divide, resulting in the formation of a multiple epidermis consisting of an outer layer of radially elongated cells and 1–2 inner layers of shorter cells. The original polygonal cell pattern is outlined in the thick cuticular layer. From paradermal views (Fig. 10b) it appears that each protoderm cell is divided by anticlinal walls into 2–4 outer cells which are radially elongated, and by a periclinal wall cutting off a cell which often is further divided by another periclinal wall, the result being one to two cell layers resembling a hypoderm (Fig. 10c, Plate IXa). In not too young branchlets the cells in these layers are in the process of getting rather thick

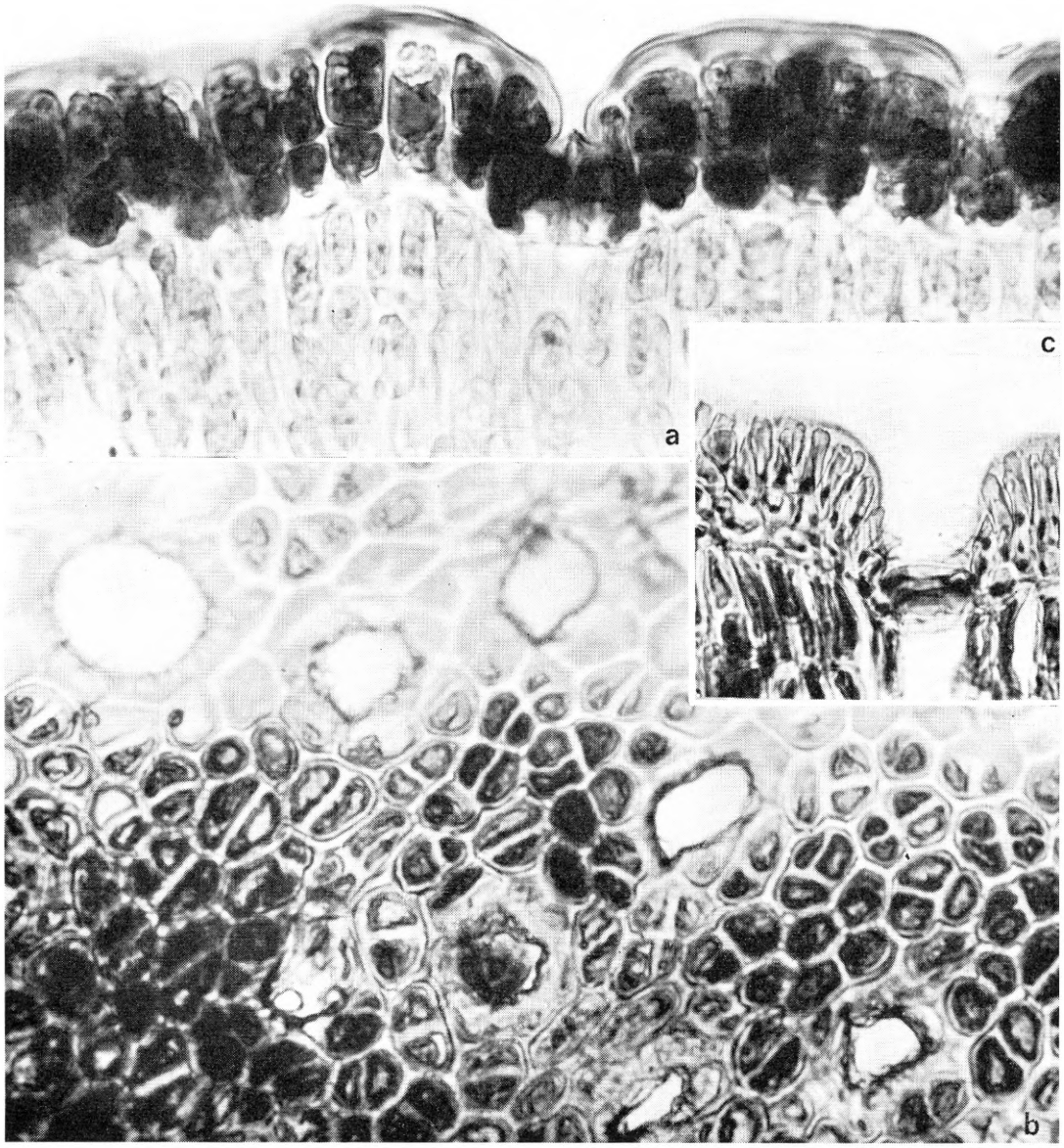

Fig. 10. *Bulnesia retama*. a. Cross section of stem. Young stage in the development of the multiple epiderm, showing periclinal walls and dark substances in the cells. One stomatal pit and one hair-pit, staining with Sudan IV. – b. Paradermal section through cutinized layer (upper part) and epidermal cells (lower part), showing several stomatal pits (roundish holes) and families of two to four cells each group originating from the same protoderm cell. — c. Longitudinal section of mature multiple epiderm in which the outer cells are divided by anticlinal walls resulting in the formation of a palisade-epiderm. a ×625, b ×800, c×320.

walls. The stomata have sunk below the surface. During the development of the multiple epidermis the stomatal pits get deeper. The cuticle extends half way between the guard cells and can be traced in the substomatal air chambers. The unicellular hairs issue from deep pits. They are without cuticle and very narrow at the base, which borders the outermost cortical palisade cells.

Young cells in the multiple epiderm contain dark substances the chemical nature of which remained unclarified but which might be phlobaphens (Fig. 10a).

In the younger branchlets the cutinization only affects the outer thick walls and wedges in the outer parts of the anticlinal walls. Later the walls in the outer hypoderm-like layer are also cutinized.

In polarized light the cutinized walls show birefringence but this double refraction disappears in slides where wax has been removed. In such slides the hairs appear bright while all the multiple epiderm cells are dark (Fig. 12b–c).

Wax crystallites are sometimes extruded. They occur in small colonies and never seem to cover larger areas (Fig. 12b).

The outer thick walls are often traversed by numerous thin strands, which issue from the cell lumina and taper towards the surface becoming undoubtedly sub-lightmicroscopical at their distal ends. In a few cases, however, it was possible to trace them from tops of cells with tapering lumina right up to the cuticle. In protoderm cells these delicate structures appear to be half as long as in the elongated cells which develop from the protoderm cells (Plate Xa–c). The position and structure suggest that they are ectodesms (see p. 38) and perhaps wax channels involved with interposition of wax in the thick outer walls and local extrusion of wax through the cuticle. The guard cells are provided with conspicuous outer ledges (Plate IXa) and in older guard cells it is possible to observe some short inner ledges (Plate IXb). The outer and inner guard cell walls are very thick. After treatment with hot alkali the ledges disappear and the thick walls appear clearly composed by 3–4 (outer thick wall) or about 8 (inner thick wall) cellulose-lamellae, the interjacent pectic substances being dissolved.

The later development of the epidermis is closely connected with the diameter growth of the branchlet. With increasing girth the elongated cells become somewhat broader and shorter and the stomatal pits and hair pits are widened; the former which in the young epidermis are nearly cylindrical (Fig. 10c, Plate IXa) become bowl-shaped (Fig. 11a, Plate IXb).

The most striking feature, perhaps, is the way in which the girth is increased. The elongated outermost cells are divided by walls which are periclinal or oblique. In the latter case a cell is formed which is able to grow in between the other cells which constitute the girth (Plate Xc on the right).

A subsequent stage may be the formation of fissures in the cuticle and the cutinized cell walls outside such enlarging cells and a filling up with new small cells inside the places of rupture. This process is illustrated in Fig. 11b–e and Plate Xd.

The subdivision of the original radially elongated outer cells leads to the for-

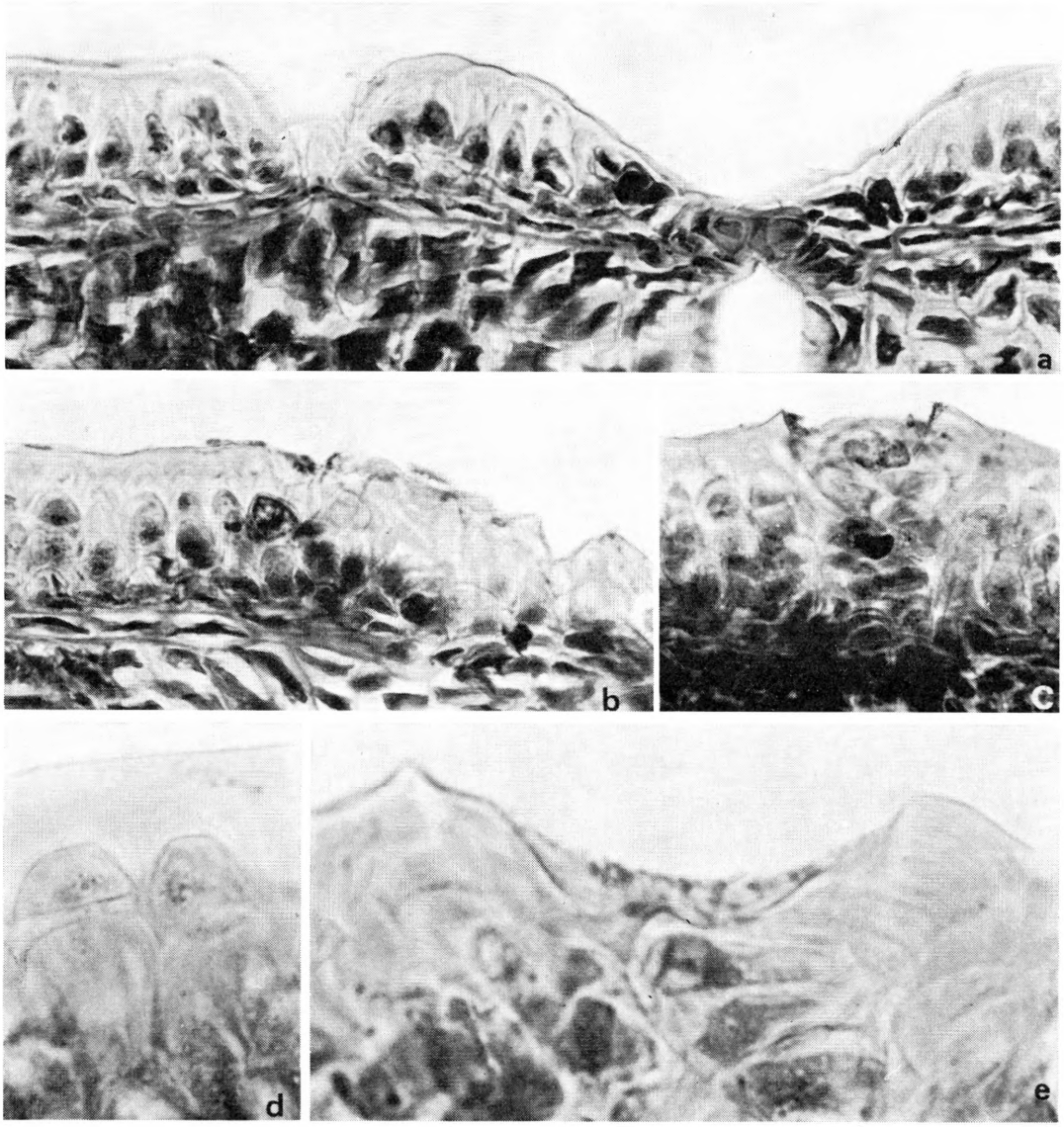

Fig. 11. *Bulnesia retama*. Cross sections of parts of older branches. a. One hair pit (on the left) and one stomatal pit which by the diameter growth has been considerably widened and now are flat bowlshaped. Cortex cells much broader, no longer more a palisade tissue. — b. Two ruptures and many dead cells in outermost layer. — c. Filling up with small new cells inside place of rupture. — d. Small dead or dying cells in outermost layer. — e. Rupture and new small cells beneath. In the depression possibly wax. a–c ×320, d–e ×800.

mation of cell rows in which the distal cells usually become short and ± hemispherical (Fig. 11 d); they seem to produce much cutinized wall substance and at the same time to withdraw, leaving a number of strata in the outer walls (Plate X e). Many of the

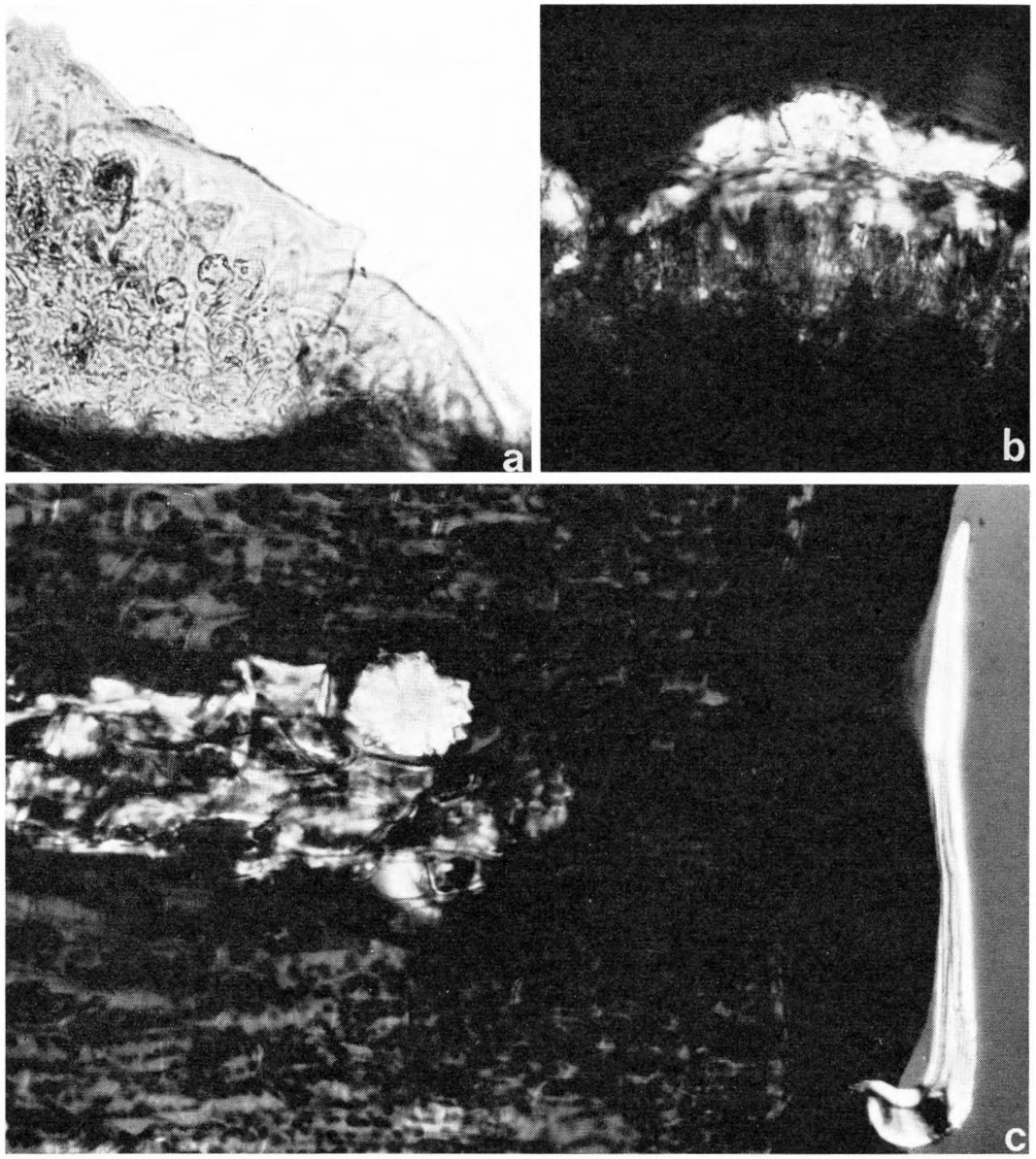

Fig. 12. *Bulnesia retama.* — a. An irregular phellem formed below multiple epiderm which ruptures and peels off. On the extreme right the phellem cells are uncovered. — b. Herbarium material. Cross section of multiple epiderm in polarized light. Wax deposits outside epiderm and cutinized parts of outer cell walls are birefringent. — c. Material after removal of wax in polarized light. Crystal druse and cells which are transformed into sclereids show up. In the epidermis all cell walls are dark except the hair. a and c ×320, b ×512.

small distal cells finally die and may, together with large parts of the cutinized outer walls, peel off. In herbarium sheets this peeling is evident and some microscopical

observations highly favour the view that at least small parts of the outer walls are also shed under natural conditions.

Periderm

In older parts of the branches scattered brownish cork areas are found, surrounded by more extensive green areas which are covered by the multiple epidermis. In young branchlets the formation of phellem is restricted to the immediate surroundings of lenticels. Later a rather loose irregular phellem tissue spreads from the lenticel-areas. Evidently the inner cells in the multiple epidermis work as a kind of phellogen and produce many cells with suberized walls. The phellem which is produced cuts off the multiple epidermis which sooner or later scales off (Fig. 12a). It seems as if the inner epidermal cells possess some cambial qualities. Usually however, they only give rise to few living cells which either substitute peripheral degenerating cells or serve the dilatation growth by being inserted between other cells in the girth.

Cortex

Almost isodiametric cells in the youngest parts stretch radially and may also divide, the result being the formation of a palisade tissue which in somewhat older stems consists of 5–6 cell layers. Between the cell rows there are very narrow intercellular spaces, only inside the stomata do they extend into larger chambers (Figs. 10c, 11a, Plate IX). Inside the typical palisade cells which are rich in chloroplasts long chlorenchyma cells occur as well as groups of elongate large parenchyma cells without chloroplasts often together with one or a few idioblasts containing crystal druses (Fig. 12c). A sclerification of the large cells takes place at an early stage, the result being the formation of radially stretched groups of sclerenchyma which include one or some few cells with druses. In old stems the sclerenchymateous groups seem to expand to the outer part of the cortex, there replacing green cells. The sclerenchyma cells attain great sizes and their walls show very many clear strata.

The sclerification can be traced in young stems by using polarized light. At an early stage the cell walls show up brightly under a polarizing microscope (Fig. 12c). Later they react with lignin – stains.

Stele

Extraxylary (pericyclic) fibre strands occur in connection with primary vascular bundles. Very early the parenchymatous cells between the fibre strands are transformed into sclereids, the result being the formation of a kind of connective tissue consisting mainly of sclereids with scattered fibres. This layer of thick-walled cells may be 5–8 cells thick (Fig. 13).

The wood anatomy of the genus *Bulnesia* was studied previously by Burgenstein (1912) and Cozzo (1948). The latter author compares five species of the genus *Bulnesia, B. retama* included. The microphotos published in Plates I–II in Cozzo's paper indicate that *B. retama* has comparatively few but much higher xylem rays.

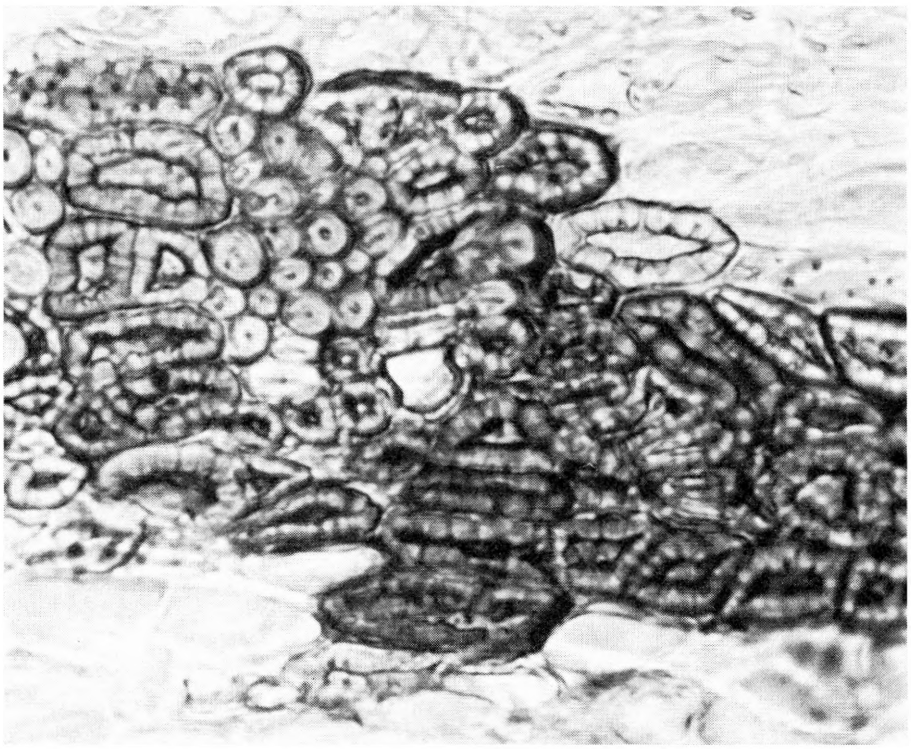

Fig. 13. *Bulnesia retama*. Cross section of branchlet. Mixed tissue composed by phloem fibres and connecting sclereids forming a separating layer between the cortex and the conducting elements in the phloem. ×630.

At the first annual ring they are 2–3, sometimes 4–6 cells broad. The vessels are diffusely arranged but in narrow annual rings they tend to be placed circularly. In our material (Fig. 14) this is also the case. The arrangement of the groups of vessels, however, is very peculiar. The groups are not confined to single annual rings, but may continue through two (or three) rings. The narrow groups follow the direction of the rays or they cross the rays obliquely. In the stem seen on Fig. 14 they tend to curve to one side.

The proportion of libriform fibres in *B. retama* is very high. According to Cozzo's photographs the proportion is much lower in the foliate species and here the tracheary cells are also arranged more circularly and follow the annual rings.

In *B. retama* there are, according to Cozzo, on an average 38 vessels pr. mm^2 whereas in the other species the mean numbers pr. mm^2 are 53, 77, 104, and 142. This reduction of tracheary elements in the xylem of *B. retama* may have some connection with the apophyllous life-form of this species. It is curious to find a many layered cortical palisade tissue and no cortical bundles. Water conduction from the vessels to the palisade cells and food-conduction from these cells to the phloem, therefore, must go in the cortex from cell to cell. The pericyclic sclerenchymatous layer

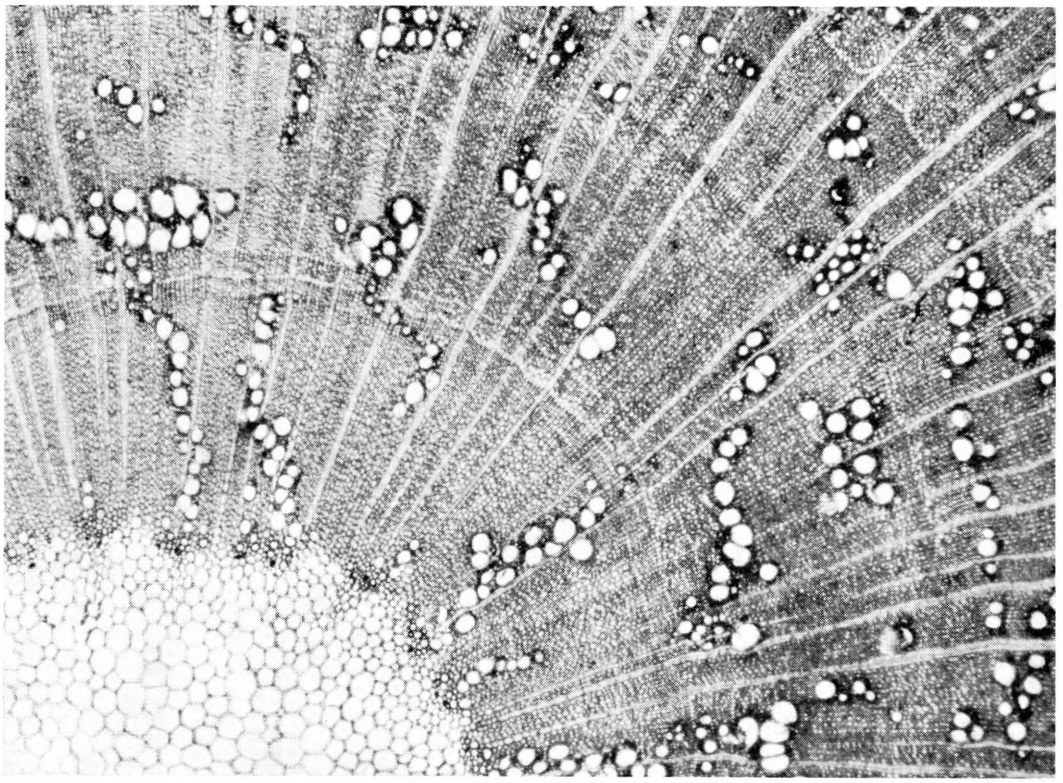

Fig. 14. *Bulnesia retama.* Cross section of xylem showing irregular groups of vessels, some growth layers, on the left some indistinct narrow ones suggest periods of stagnation. ×50.

(Fig. 13) is not completely continuous. It contains scattered passage-ways of non-lignified cells, arranged in one row only. While water may pass thick and lignified cell walls, the translocation of food-stuffs to the phloem, probably proceeds through living cells forming pathways through the sclerenchyma cp. further p. 35.

4. Bredemeyera colletioides (Phil.) Chod.

Material: Prov. San Juan, Pachaco 89 km west of the town San Juan. Altitude 1200 m (Böcher, Hjerting & Rahn No. 2228).

Bredemeyera colletioides (*Polygalaceae*) is a shrub which can reach a height of 1.8 m with trunks which may reach 4–5 cm in diameter. The relative main axes zig-zag, and at each turn a perpendicular branchlet issues (Fig. 9d). At the angle between the axes and the branchlets a deep and very narrow fissure is found on the upper and lower side of the branchlet. The fissure is covered by the epidermis which is folded inwards. Sometimes the two fissures merge into one which can be traced

all the way round the base of the branchlet. The branchlets terminate in spines and bear lateral inflorescences. Before the petals are shed the capsules elongate and almost ripen. They contain two seeds which are covered by a wisp of long hairs. The species occurs in the western provinces of Argentina from Tucuman to northern Neuquén, having its main distribution in areas dominated by Monte vegetation. It is also known from Chile.

Epidermis

As in the preceding species a multiple epidermis develops and the guard cells sink below the surface of the epidermis. SCHWABE (1947) interprets the innermost layer as a hypodermis, but the ontogeny shows that a separate hypodermal layer does not exist. The protoderm cells are radially elongated with a thick outer wall (Fig. 15). The guard-cell mother cells are differentiated at an early stage by being broader and shorter than the other epidermal cells. The cells adjacent to the guard-cell mother cells (the subsidiary cells) become particularly long and expand in their outer part, the result being a sinking down of the mother cell in a small depression which is surrounded by the expanded portions of the adjacent cells (Fig. 15a–b). After the division into guard cells (Fig. 15c–d) the latter sink further down as a result of periclinal divisions in the other epidermal cells and radial growth of the new cells (Fig. 15e–g). The cells in the multiple epidermis remain arranged in radial rows. While, however, normal epidermal cells form rows of 2(–4) cells, the rows formed by the adjacent subsidiary cells contain 5(4–7) cells. As the guard cells are always connected with the innermost cells in these rows they are finally placed very far from the surface at the bottom of a deep pitcher. The pitcher with the guard cells forms a most interesting type of stomatal apparatus. This was already emphasized by SCHWABE (1947: 66). The outermost cells in the rows forming the pitcher elongate and expand, thereby forming an entrance which is narrowed and becomes protruded as a mouth (Fig. 16, 17b). As the normal epidermal cells form rows of a few cells only, the bottoms of the stomatal pitchers sink down and become surrounded by cortical palisade cells. Inside the guard cells there are large substomatal air-chambers (Fig. 17b, Plate XIb).

A striking feature is the frequent displacement of the narrow entrance in relation to the position of the stomatal pore. In very many cases the pitchers curve. Therefore, in such cases, the entrance to the pitchers cannot be seen in sections where the guard cells are cut transversely (Plate XIb, XVb).

Another very interesting feature is the structure of the outer ledge of the guard cells. This ledge has a rather normal appearance in young guard cells (Fig. 15), but in final stages it forms a thin diaphragm which covers the majority of the front cavity (Plate XIb, d, Plate XVd–e, Plate XVIa–c).

In young stomata the ledges are clearly formed by the guard cells only, however, the subsidiary cells may possibly in ripe stomata contribute to the final development of the ledges into the diaphragm, see e.g. Plate XVIb).

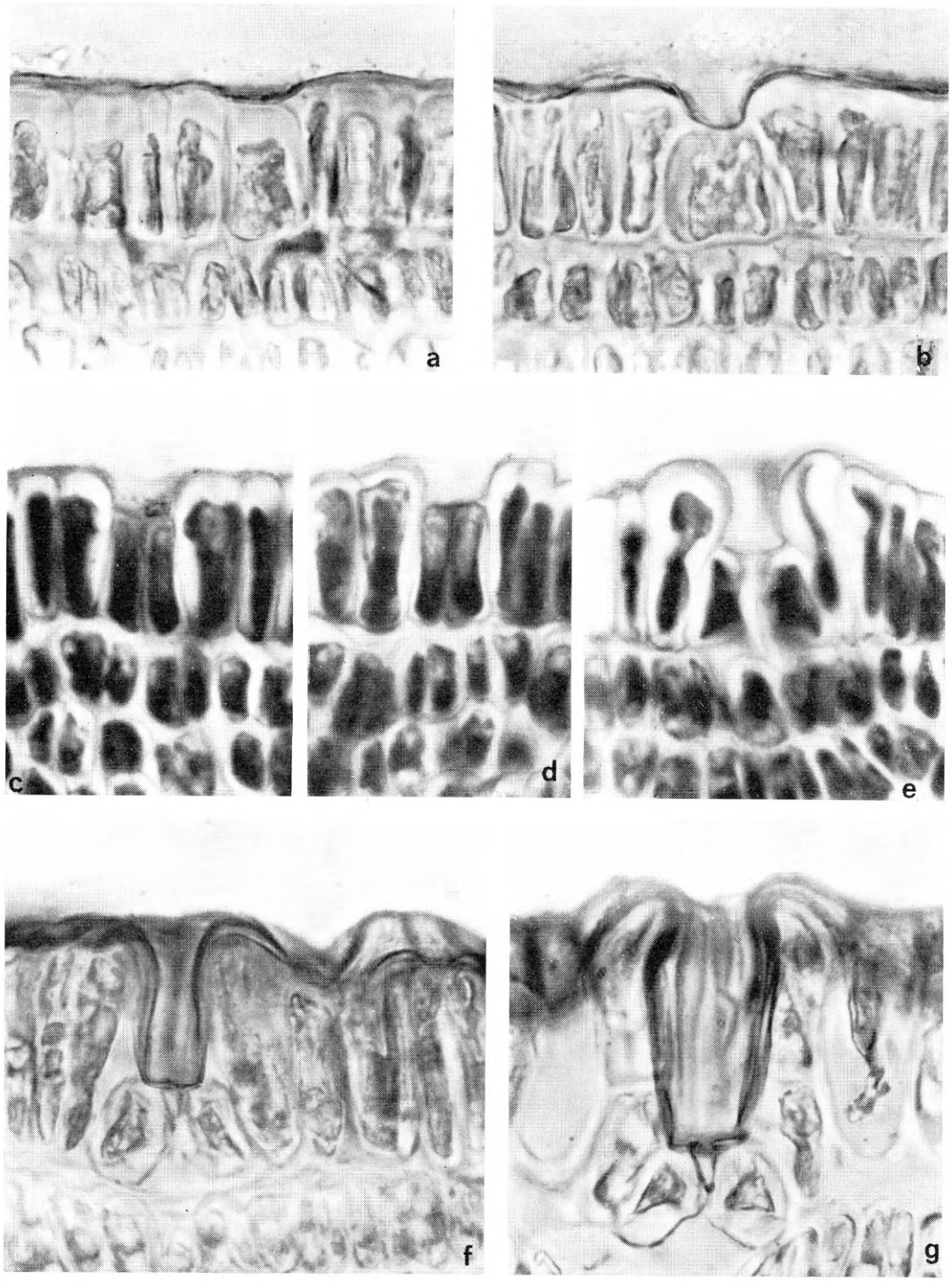

Fig. 15. *Bredemeyera colletioides*. Development of multiple epiderm and stomatal pitchers. a–b, f–g stained with Sudan IV, c–e with Lightgreen-Safranin. — a. Protoderm undivided, shallow depression at guard-cell mother cell. — b. Depression at guard-cell mother cell deeper, the two adjacent cells overtopping and partly overarching mother cell. — c–d. Mother cell divided. — e. Wall thickening and stretching of upper part of subsidiary cells, guard cells shorter and broader; initiation of outer ledges. — f. Stomatal pitcher formed, maximum stretching of epiderm cells. — g. Division of elongated cells, pitcher mouth and front cavity developing. ×880.

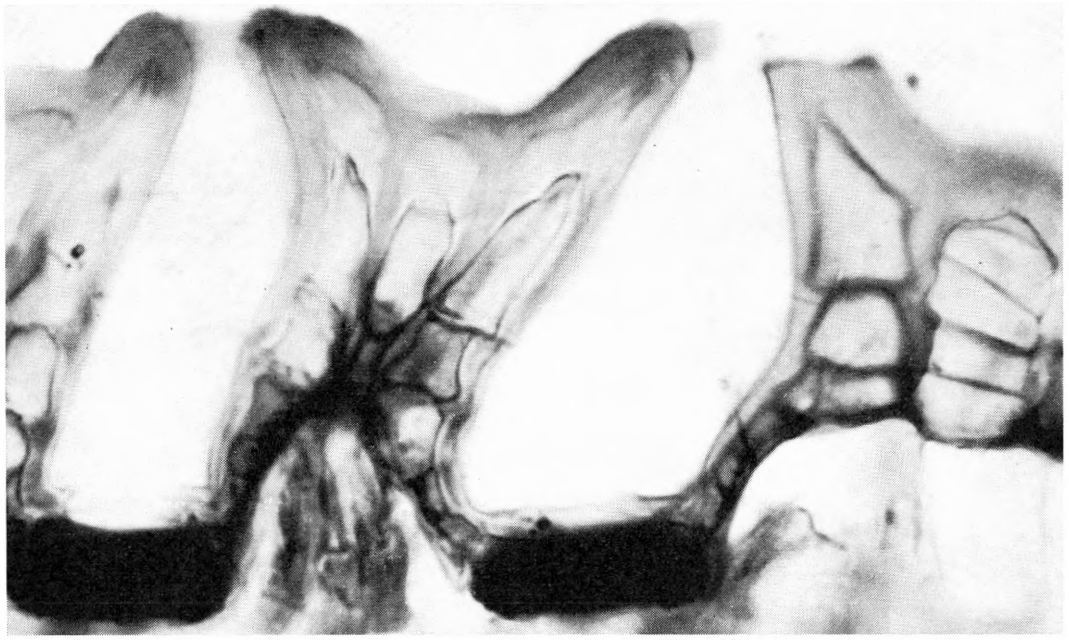

Fig. 16. *Bredemeyera colletioides*. Longitudinal section through two mature stomatal pitchers, that on the right slightly curved. Row of six subsidiary cells forming the pitcher. 2–4 cells in the rows between the pitchers. ×800.

In mature stomata the diaphragm always has a central opening, which is circular or elliptic (Plate XIII–XIV). But the margin of the opening is sometimes somewhat fibrous or is provided with some small, warty wax excrescences.

The outer and inner walls of the guard cells are very thick, but in the area where the pore narrows most they carry ridges, which in transverse sections appear as two opposite noses. These ridges are situated just outside or a little inside the place where the cytoplasmatic parts come closest to the pore.

Beneath the cuticle the walls of the cells in the multiple epidermis are in the process of becoming cutinized. The mature multiple epidermis appears, with Sudan IV red in all layers, even the inner walls bordering the cortex have a thin lamella which is red. The thick walls covering the stomatal pitchers are cutinized as are the ledges (diaphragm) and the outer walls of the guard cells adjacent to the pore (Plate XVIb–c). The walls inside the pore are covered by a cuticle and appear sometimes to be crispate (Plate XVIa).

A similar folding of the inner walls and cuticle was beautifully demonstrated in guard cells of *Helleborus* by Huber et al. (1956) using the electron microscope. According to Huber et al. this folding cannot be a result of shrivelling. However, in *Bredemeyera* it was found only in material which had been fixed, imbedded and stained. Perhaps such shrivelling is therefore due to a dehydration of some of the

wall compounds, e.g. pectic substances, see p. 15. In material treated with hot alkali the thick inner and outer walls appear clearly stratified. Using phase contrast it is possible to distinguish about eight lamellae of cellulose on each side of the cell lumen which after this treatment swells and becomes elliptic when viewed transversely. The interjacent spaces in such walls appear empty but were probably originally filled with pectic substances. The outer ledges and the diaphragms as well as the cutinized parts of the guard cell walls disappear after treatment with hot alkali.

Of particular interest is the observation made by VOLKENS (1887) and FAHN & NINA DEMBO that the walls of the guard cells in desert plants continue to thicken after maturation. The cell lumen is finally reduced to such an extent that it does not seem possible that turgor pressure is capable of opening the stomatal apertures. This character is thought to be of adaptive value in the dry summer of the desert.

A comparison of young and old guard cells in *Bredemeyera colletioides* (Fig. 15g and Plate XVIa–c) clearly shows a reduced cell lumen in the mature cells. Furthermore the cutinized ridge at the pore is absent or very small in young guard cells. It therefore seems probable that the movements of the guard cells in older parts of the branchlets are reduced or sometimes even stopped.

The thick outer epidermal walls are very interesting; they appear stratified and are clearly traversed by very delicate channels or ectodesms (Plate XVa–b). Sometimes the cytoplasm of the outer cells taper or have a few narrow protrusions which continue in a fine channel in the wall (Plate XVa, lefthand cell). In most cases, however, the channels first become visible at some distance from the cell lumen where they radiate against the outer surface (Plate XVb). The system of channels in the walls is particularly clear in the long protruding outer walls at the entrance to the stomatal pitchers. Transverse sections of the entrance show that the tips of the outermost subsidiary cells all have about five channels which merge, forming a small nodule from which a number of very fine plasmatic strands continue in the direction towards the surface, see Fig. 18. The area in the outer walls of the normal epiderm cells where such strands occur (Plate XVa–b) is much wider. Corresponding to the nodule, there are here, at certain levels in the wall, plasmatic layers from which the delicate strands continue and which are mutually connected and connected with the cell lumen by fewer and perhaps slightly broader strands. The system of plasmatic strands, which are easy to observe at high magnification in the light microscope and particularly in the protruding walls at the pitcher entrances, represent presumably main channels from which much finer ± submicroscopical strands issue. This view was corroborated by a number of observations where diaphragming and the use of different filters made it possible to see a very fine, dense striation issuing from the main channels (see Plate XIIb–c).

Observations in the polarizing microscope added a number of important facts. If herbarium material was cut and the material mounted in glycerol the cutinized parts of the multiple epiderm appeared to be birefringent (Plate XIc). Furthermore, hair-like birefringent structures were found in great quantities on the walls of the

stomatal pitchers (Plate XIc, XIIe). Also the thin diaphragms formed as continuations of the outer ledges were birefringent (Plate XId). Finally a particularly bright shine occurred in the area of the middle lamella in some particularly broad cutinized wedges in the outer anticlinal walls (Plate XIe).

Using the red I plate the thick non-cutinized walls of the guard cells and the walls of the inner epiderm cells on both sides of the pitchers appeared blue, while the diaphragms and the thick cutinized outer walls of the outer epiderm cells were orange.

The bright birefringence of the non-cutinized parts of the guard cell walls was maintained in material treated with hot alkali and where the cutinized parts were dissolved. It also persisted in material which was fixed and during staining and mounting treated with tertiary butyl alcohol and xylene. On the other hand birefringence in the thin diaphragms disappeared in the fixed and stained slides. The inner parts of the walls in the outer epiderm layer and the parts of the walls bordering the cortex also showed birefringence. This persisting double refraction is probably due to cellulose, while most of the birefringence which disappears is caused by wax.

Wax seems to be interposed in the cutinized walls and extruded. On the surface of ordinary epiderm cells it occurs as small scale-like bodies outside the cuticle and perhaps also in the cuticle, but the wax-covering is hardly complete.

The hair-like wax protuberances (wax-hairs) form particularly dense coverings at the entrances to the stomatal pitchers. In most cases the wax-hairs from both sides meet and are wrapped up. Some very long ones placed on the border between the entrance and the pitcher cavity form a dense interlacing pattern, which must be able to slow down the air movement and have other functions as a kind of a closure of the pitcher (Plate XIIc–e).

The density of wax-hairs decreases with the distance from the entrance. In some cases, small hairs or warty bodies of wax even seem to reach the diaphragm and the front cavities.

The coincidental occurrence of many and long wax-hairs and the nodules in the cell walls behind is striking and suggests that the above-mentioned channel system is of importance for the displacement of wax precursors. In this connection it is interesting that the larger channels or nodules showed some birefringence (Plate XIc, XIIe).

The middle lamellae or central areas in some of the broadest outer anticlinal walls show up particularly brightly. However, the shining middle parts were not continuous, being traversed by thin dark strands which presumably are plasmodesms. In phase contrast the same walls were also delicately cross-striped. Outside such walls there were, on the surface, often small collections of bodies with the same degree of birefringence and dimensions corresponding to the breadth of the birefringent wall area (Plate XIe). As the birefringence also in this case suggests wax deposition the occurrence outside such walls of bodies with a similar type of double refraction seems to be of some interest. Perhaps wax precursions follow plasmodesms through the

cutinized anticlinal walls and ectodesms in the outer ones so that wax depositing occurs where these wax channels end, viz. in the middle lamellae and on the surface. However, if the middle lamellae are particularly broad (perhaps expanded) and filled up with wax, some of the wax may be extruded through fissures outside the anticlinal walls.

While it is clear that many anticlinal divisions occur in the young epidermis which consists of one cell layer only (Fig. 15), there is no evidence of any mitotic activity in older stems, where the epidermis is multiple and cutinized. How an increase in the girth takes place in old stems is therefore the question. Some facts, however, suggest that some late anticlinal divisions occur. Some of the multiple epidermal rows of 2–3 cells are particularly narrow and placed two side by side. The two peripheral sister cells have, in the cuticular layer, first their own strata, but the outer strata are common to both. Sometimes single, undivided, narrow cells are inserted between rows of 2–3 broad cells. These single ones taper towards the periphery. If they were able to increase their breadth and to widen abaxially, this would also contribute to the diameter growth.

There are many points of resemblance between *Bredemeyera colletioides* and *Anabasis articulata* (*Chenopodiaceae*) described by Fahn & Nina Dembo (1964). The latter species deviates by having a thick water-storing parenchyma, but it has extremely reduced leaves and is clearly stem-photosynthetic. *Anabasis articulata* has also a multiple epidermis and the multiseriate subsidiary cells produce a deep cavity above the guard cells. The cells bordering the entrance to the cavity, together with their papillae, make the outer portion of the cavity narrower.

Cortex

In young branchlets the cortex consists of 5–6 layers of photosynthetic cells (Fig. 17a). Later a differentiation takes place. The outer 3–4 (5) layers develop into palisade cells, those between the palisade cells and the pericycle into chlorenchyma and scattered cells which contain druse crystals (Fig. 17b, Plate XVId). Except inside the guard cells the intercellular spaces are narrow. In the innermost cortical layer the cells are enlarged tangentially and form an almost continuous layer. Each cell may have connections to two rows of palisade cells and may serve as collecting cells for food-materials produced by the palisade cells.

Stele

Young stems contain 2–3 layers of cells which have no chloroplasts and develop into an extraxylary cylinder of fibres which is probably pericyclic. As in *Bulnesia* there are passage areas of living cells connecting the innermost cortical layer with the phloem. Such passages are often found outside phloem rays (Plate XVId).

As already mentioned by Schwabe (1947) there are no wide vessels in the xylem. In the protoxylem the vessels have helical thickenings. The vessels of the secondary xylem have simple perforations.

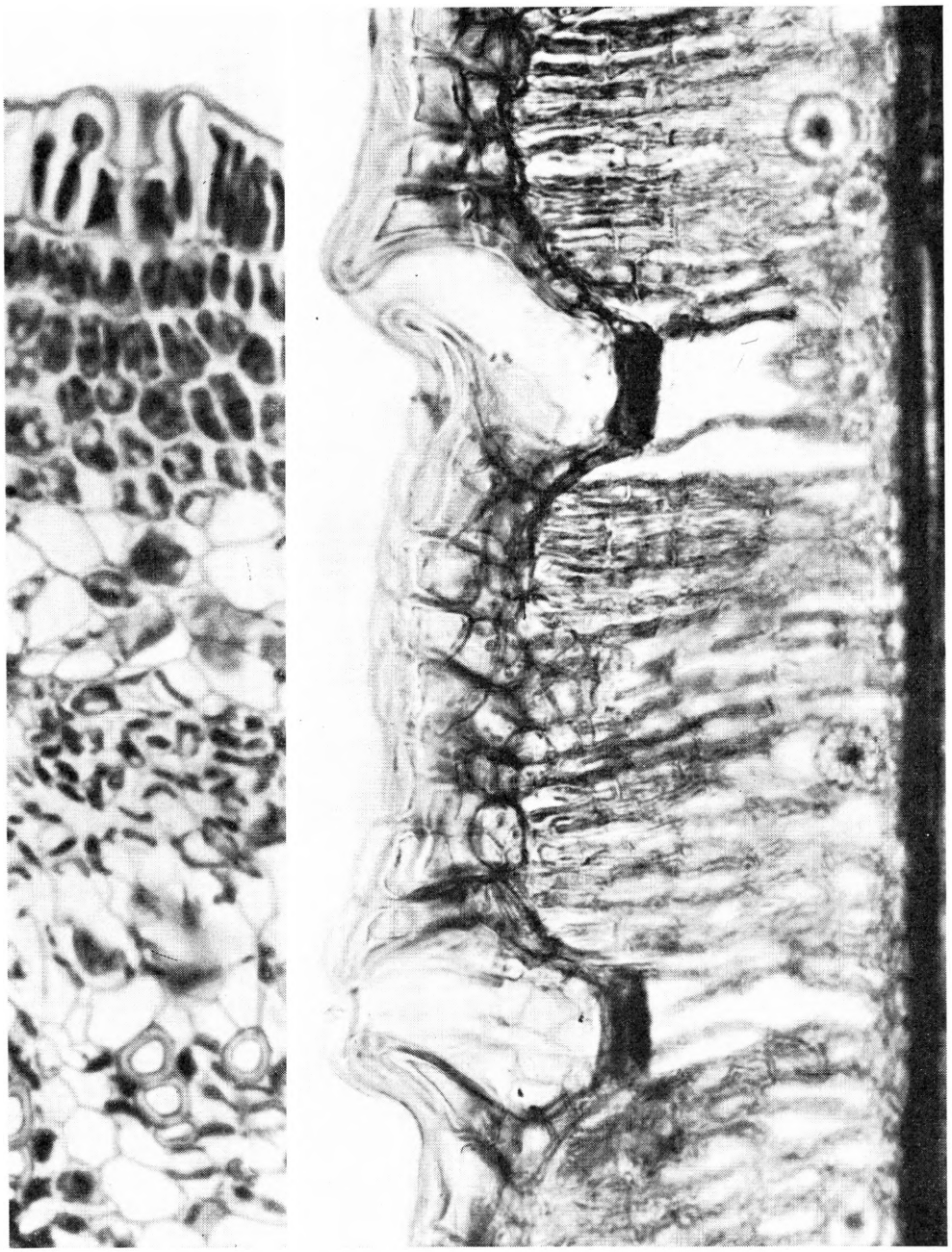

Fig. 17. *Bredemeyera colletioides*. On the left: Part of cross section of young stem with epidermis, 4–5 rows of palisade initials with many anticlinal divisions, large cells which develop into fibres, protophloem, initials of metaxylem and protoxylem. ×470.
On the right: Longitudinal section of older stem with mature multiple epiderm, palisade cells in cortex, crystal druses and fibres (black). Two stomatal pitchers cut, the uppermost one curving very much. ×320.

The axial xylem parenchyma is poorly developed but mainly paratracheal. In the centre of the branchlets the parenchyma of the pith gets thick walls. The xylem rays are 1–(2) cells broad, cp. Plate XVI d.

5. Discussion

Structure of outer epidermal wall

Frey-Wyssling (1959: 56) and Frey-Wyssling & Mühlethaler (1965: 313) discuss the nature of the cuticular layer and conclude that it is situated outside the primary wall; it is not a part of the wall but a secretion between the cuticle and the primary wall. Of particular interest is the opinion that the cuticular layer can be compared with the polysaccharides deposited as a mucilage layer on the epiderm of aquatic plants, which on terrestrial plants, however, are strongly mixed with or replaced by cutin substances.

The studies in *Monttea aphylla* referred to above corroborate with this view, but in *Monttea* the cuticular layer has reached an unusually great thickness. This makes it possible to see details which are not known from cuticular layers in other plants; but just because of the exceptional dimensions of the layer it is hardly justifiable or at least very difficult on the basis of observations in *Monttea* to make any generalization with regard to the structure of cuticular layers in xerophytes.

There is a strong resemblance between mucilaginous epidermis walls and the epidermis walls found in *Monttea*. The cellulose mucilage formed in seed coats (e.g. in *Lepidium sativum*) is birefringent and gives a picture in a polarizing microscope which is highly reminiscent of our pictures of walls in which wax has been removed (e.g. Plate IV a). The radiating shining lines in the *Lepidium* mucilage according to Frey-Wyssling (1959: 155) are due to the layering of the cellulose fibrils, which are turned during the swelling and become orientated perpendicular to the surface of the seed.

Another important similarity is the presence of wall lamellae which are separated by matrix which expands. In walls with cellulose mucilages the system matrix + cellulose fibrils as stated by Frey-Wyssling & Mühlethaler (1965) can, according to the quantity, capacity for hydration and state of hydration, produce soft slimes, form mucilages and walls with different degrees of plasticity and elasticity.

The process which in *Monttea* resembles a swelling is not immediately comprehensible and ought to be studied in more detail. The tubular corrosion cavities which mainly were found outside the transverse walls may be due to the presence of hemicellulose or pectin. But these substances seem to occur together with cellulose and not to constitute a matrix between the carbohydrate lamellae. The expansion in *Monttea* may be caused by a migration of large quantities of substances found together with those having lipid character or by the liquid wax and cutin precursours themselves. The inward bulging of the protoplasts in young very active cells (Fig. 3 a), the

occasional occurrence of Sudan IV-positive islands in the epiderm (Fig. 3c–d), the clear lamellation and the perforation of the outer walls indicate that the cell walls in the active phase are split up into thin porous lamellae, the intervening spaces being filled with matrix, and that the amount of matrix sometimes is so great that the surface of the protoplasts become concave.

If the outer walls are not exposed to higher pressure from a larger amount of expanding material they may be maintained, the very delicate pores or channels giving enough passages for the secretion. In this connection it is interesting to refer to the investigations of LAMBERTZ (1954: 164) who did not find such microchannels or ectodesms in the outer walls of secretory cells. Such walls, he says, are extremely thin and it is therefore difficult to distinguish small pores for possible ectodesms from granular coagulated cytoplasma. Perhaps the explanation is that such delicate structures are only present as long as the wall lamellae are not decomposed as a result of stretching.

In *Bulnesia* and *Bredemeyera* delicate channels in the outer walls are easy to demonstrate (Plate X, XV). As mentioned these structures are probably not always identical with true ectodesms (see SCHUMACHER 1942, 1957, LAMBERTZ 1954, SCHNEPF 1959, SCOTT et al. 1958), they are wider, being probably often main channels from which ectodesms issue. The channels seem to deviate from true ectodesms in many ways. First, the fine channels found by us were very conspicuous after fixation with alcohol only. SCHUMACHER and LAMBERTZ had to use Gilson fixation and staining with pyoktanin to make them observable. Second, according to LAMBERTZ, it was not possible to detect ectodesms in heavily cutinized walls, where they were considered to be obscured by the cutinization process. In our material there seem to be few or no clearly observable microchannels in the heavily cutinized outer wall layers. On the other hand the channels pass cutinized inner wall layers and go right out to the outer layers and may continue as very thin strands, but in any case they seem to stop before the cuticle. This is accordance with the results of SCOTT & al. (1958), SCHIEFERSTEIN & LOOMIS (1959), and HALL (1967).

The plasmatic nodules or layers observed in the outer walls of *Bredemeyera* (Plate XIVe) are of particular interest. It is here sometimes difficult to distinguish or demarcate a cuticle from the cuticular layer, but it is evident that the wall layers placed slightly outside the nodules or plasmatic layer stain more intensively purple with the quadruple staining whereas the inner parts in the multiple epidermis are paler. The delicate plasmodesmatal structures which radiate from the nodules or plasmatic layers towards the periphery stop or become submicroscopical where the layering in the wall is getting indistinct and the purplish colour becomes deeper.

The nodule shown in Fig. 18c appears empty while perhaps some plasmatic substance is left in the channels. It is striking here that these wider channels found by us in *Bredemeyera* appear to be double. By successful fixation and addition of oxalic acid or nitric acid it was possible for LAMBERTZ to demonstrate that the true ectodesms were built as two parallel threads. Later this double structure also seems to be observable in the electron microscope (SCHUMACHER 1957, SCHNEPF 1959: fig. 9–10).

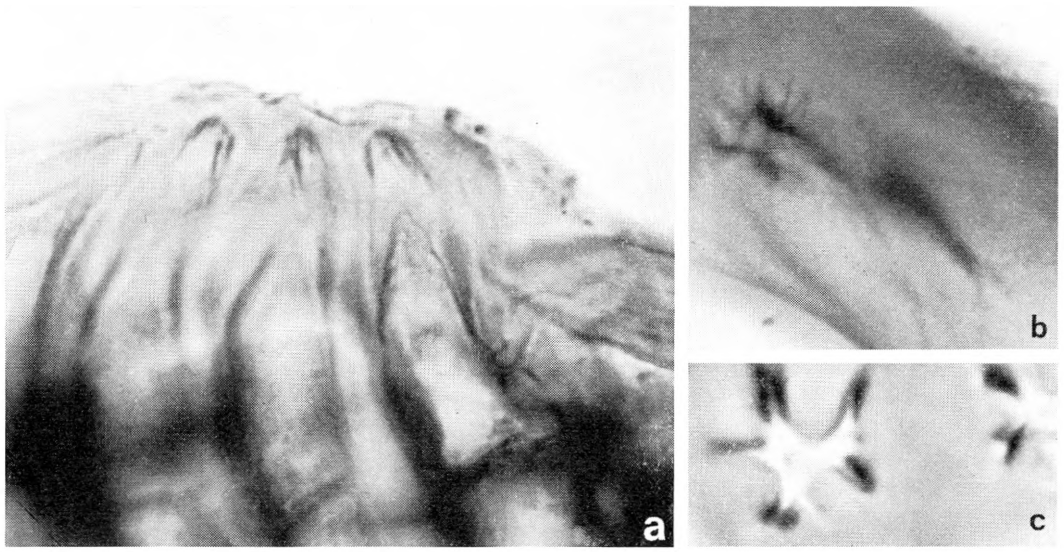

Fig. 18. *Bredemeyera colletioides*. a. Cells forming the entrance to the pitcher showing tapering cell lumina and strands leading to apical nodule. — b. Apical nodule from which a number of very fine strands radiate towards the surface at the entrance. — c. Cross section of apical nodule showing twin strands leading to the cell lumen. a ×800, b–c ×3200.

Lambertz demonstrated that ectodesms were best developed in young cells. In *Bulnesia* and *Bredemeyera* it is evident that the protoplasmic part of the outer epiderm cells is reduced with increasing age of the epiderm and recedes as new wall layers are added by apposition. During this process fewer and perhaps sometimes no microchannels are formed. In the latter situation the cytoplasmatic connections from the cell to the plasmatic nodules and layers in the outer wall would be broken. In any case the nodules or plasmatic layers are probably remains of a peripheral part of the cytoplasm with structures resembling ectodesms, a part which for some reason was cut off by the next secondary wall layers (cp. Plate XV). This favours the view that the plasmodesmatal structures in thick outer walls are of particular importance in the young stage or during the apposition growth.

Schieferstein & Loomis (1959) and Hall (1967) discuss the presence in the outer walls of microchannels through which wax might migrate before it was extruded through the cuticle.

In *Bulnesia* and *Bredemeyera* we may perhaps be allowed to suppose that the channels mainly function during the period of enlargement of the outer surface, where new cutin has to be inserted in the expanding cuticle and a polymerization of cutin and deposition of wax must take place in the deeper wall layers when the cutinization of the multiple epiderm takes place. On the other hand, the possibility exists that e.g. the production of wax hairs in stomatal pitchers could be increased during periods with particularly dry conditions, resulting in a partial closure of the

opening and a cutting down of the transpiration. If so, we may assume a function of the channels also in later stages.

The process of cutinization and wax depositing is probably slow when compared with the secretion which we assume takes place in *Monttea aphylla*. In this species there are no microchannels in the cuticular layer. The whole organization of the outer walls in this species is strikingly different from that of the two other species in which channels in the outer walls are found. Nevertheless we find it justifiable to speak about cuticular layers in all three species. This attitude was also influenced by the paper of Sitte & Renner (1963), who in the upper leaf surface of *Ficus elastica* found a deviating type of cuticular layer where cellulose is only found in the inner lamellae. The two authors therefore rightly modify the definition of cuticular layer so that all cutin-containing layers should be classified as cuticular layers, regardless of whether they contain cellulose or not. According to our observations it might further be justified to include under the definition such cuticular layers as are intensively secreted and have the cellulose arranged as thin dome-shaped lamellae.

Life-form

Usually comparative anatomy is closely connected with taxonomy. Ideas about the interrelation of various taxa and evolutionary lines within certain groups are often supported through comparative anatomical studies. But comparative methods have also proved to be very useful in ecological anatomical investigations. In our case we find some very striking similarities between the three apophyllous species. Mainly, on the basis of their anatomy it is possible to refer them to the same type. If apophylls constitute a life-form, our three species belong to a subtype of this life-form. Let us call this subtype terete apophylls.

Slade (1951) has, for the New Zealand brooms (e.g. *Carmichaelia*, *Corallospartium*, *Notospartium*, *Chordospartium*), described two important morphological evolutionary lines, both arising from the terete subgenus *Kirkiella*. One morphological trend is a flattening in the stems, another a development towards a shallow grooving and further to a deep furrowing of the stems. Nothing is said about the possible ecological background for these two lines, but it seems likely that different selective forces have affected a terete ancestral type and that in some kind of environment a backward evolution took place towards a flattened leaf-like structure, while in other environments the evolution became a progressive furrowing and sinking down of the stomata in the furrows.

These points of view might be generalized. As stems usually are ± round, the ancestors for apophyllous species in the various taxonomic groups had probably more or less terete stems. They were also probably foliate but with the character of shedding their leaves early or at least at the height of the summer. While some of the morphological evolutionary lines led to deviations from terete structure, this was maintained in others. Adaptation to very dry conditions may lead to the furrowed

type in some groups but not in others. According to SLADE (1952), the occurrence of cortical fibre bands is of fundamental importance for the trend leading to furrowed stems. Even species with smooth stems have alternating longitudinal areas with and without stomata. The epidermal strips devoid of stomata are underlaid with bands of cortical fibres. Successive developmental stages show that the long stomatal areas are the forerunners of stomatal furrows. In this connection it is interesting to note that none of the three terete xeromorphic apophyllous species dealt with in the present paper have any fibre bands in the cortex. In *Bulnesia retama* some cortex cells develop into groups of sclereids but there are no subepidermal fibres which would impede development of stomata in the epidermis outside.

The type with furrowed stems is developed in several families. There is a striking similarity between stems of the Leguminous *Corallospartium crassicaule* from New Zealand, the Mediterranean *Retama raetam* (EVENARI 1938, Fig. 6) and the Verbenaceous *Neosparton aphyllum* from South America (see Fig. in CABRERA 1961). Here the sinking of the stomata is connected with the furrowing. Obviously the sinking down of the stomatal apertures is a character which is of great adaptive value. In the three species which we have studied, the formation of large chambers outside the front cavities is due to two widely different processes: (1) a development of a multiple epidermis (2) a secretion of a very thick cuticular layer. In all three species there are two front cavities outside the aperture and this double front cavity system is, in the case of *Bredemeyera colletioides*, very conspicuous. The complicated stomatal pitchers with their wax hairs and the diaphragms with their wax covering in this species would probably never have come to existence if the protection by sinking down of the stomata was not of fundamental importance for the water economy of the plants and therefore of great adaptive importance.

Species of the family *Restionaceae* (CUTLER 1966) although being herbs, have xeromorphic photosynthetic stems and may belong to the terete type or to an independent type. The stomata are here in some species sunken but in many species not. However, here the substomatal air chambers have a special coating of protective, thick-walled, modified palisade cells. The protective cells form the wall in a short tube or cavity which becomes closed at the inner end, but it is open to the green cortex cells by special pores. The protective cells have a cuticle on the surface exposed to the atmosphere. In this way the substomatal cavities here are transformed to structures which protect against water-loss.

A number of other similarities between the three species presented here may be mentioned. Thus, in the xylem there are relatively few and narrow vessels and a high proportion of libriform fibres. Other, and probably more significant similarities, are found in the cortex.

In all three species external cortex cells are elongate and rich in chloroplasts and form a many layered typical palisade tissue.

No leaf trace bundles are found. All conduction of water as well as food translocation proceeds from cell to cell in the photosynthetic tissues. Soluble carbohydrates

reach the phloem through narrow passages in the extraxylary fibre- or fibre-sclereid cylinder which occurs outside the phloem in all three species.

The cortex in the *Restionaceae* has no traces either. Here the palisade cells are separated from the sclerenchyma cylinder with the bundles by a cylinder of parenchymatous cells. Only the smallest peripheral bundles approach the parenchymatous cylinder and may have connection with the chlorenchyma (cp. Figs. 1–3 in CUTLER 1966).

There is a striking difference between this organization and that found in some other stem assimilating plants in which a cortical venation system is developed. In the articulated succulent *Chenopodiaceae* (FAHN & ARZEE 1959) a cortical network is connected with leaf strands or with leaf strands and stelar strands. In the non-succulent Leguminous brooms of New Zealand the cortical venation pattern is derived from a leaf trace system. Leaf traces may here occupy the cortical ridges and are here separated from the photosynthetic cortex cells by one layer of colourless cells which may act as a physiological sheath or endodermis (see in SLADE 1952, Fig. 3).

The most important dissimilarities concern, as already mentioned, the development of the cuticular complex and the occurrence of periclinal divisions in a protoderm leading to a multiple and highly cutinized epidermis in *Bulnesia* and *Bredemeyera*. Trichomes were only found in *Bulnesia retama*. As they are unicellular and non-cutinized (Fig. 12c) they may perhaps be concerned with water absorption from the atmosphere (cp. the classical studies by VOLKENS 1887: 31–33). On the other hand trichomes may just represent family characters which are kept because they are neutral, having no negative adaptive value. Quite similar hairs were found in *Nitraria retusa* (also to *Zygophyllaceae*) by VOLKENS and are not considered by him to be water absorbing.

If we finally ask whether the three species studied, as regards xeromorphic anatomical characters, are particularly well-equipped or possess xeromorphic features which are more specialized or advanced than in most other xerophytes, the answer should probably be positive. The cuticular complexes found are particularly thick, the stomatal apertures have double front cavities, the green assimilatory cells are all arranged with very short distances to the tracheary elements in the stele and the intercellular spaces are narrow. However, it is not our intention here to penetrate into the physiological side of the problems. Xeromorphic characters ought always to be looked upon as genetically fixed ecotypical characters which mainly occur and are particularly well-developed in plants which are connected with dry habitats. This definition is neutral. A xeromorphic character might as well reduce the loss of water as play a part in insulating the green cells from excessive heat or reduce the light intensity. But frequently it will serve several different functions at the same time.

However, the eco-physiological side ought certainly not to be forgotten. It is definitely more difficult to tackle but important results might be reached if teamwork was organized between biochemists, physiologists, anatomists and ecologists.

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Literature cited.

Burgenstein, A., 1912: Anatomische Untersuchungen argentinischer Hölzer des k.k. naturhistorisches Hofmuseums in Wien. — Ann. des k.k. naturh. Hofmus. **26**: 1–36.

Böcher, T. W., 1963: The study of ecotypical variation in relation to experimental morphology. — Regnum Vegetabile **27**: 10–16.

Böcher, T. W., Hjerting, P. & Rahn, K., 1963: Botanical studies in the Atuel Valley Area, Mendoza Province, Argentina. — Da. Bot. Ark. **22**, nr. 1.

Cabrera, A. L., 1961: Anatomy of some xerophilous plants of Patagonia. — Plant-Water Relationships in Arid and Semi-Arid Conditions. — Proc. Madrid Symposium (Unesco): 235–239.

Christiani, L. Q., 1948: Anatomia del leño secundario de las especies Argentinas del genero *Monttea*. — Com. Inst. Nacional Invest. Cienc. Nat. — Bot. **1**: 1–6.

Cozzo, D., 1948: Anatomia del leño secundario de las especies Argentinas de la tribu *"Zygophylleae"*. — Rev. Inst. Nacional. Invest. Cienc. Nat. Bot. **1**: 57–85.

Cutler, D. F., 1966: Anatomy and taxonomy of the *Restionaceae*. — Notes from the Jodrell Lab. Kew Gardens — IV: 1–25.

Evenari, M., 1938: The physiological anatomy of the transpiratory organs and the conducting systems of certain plants typical of the wilderness of Judaea. — Journ. Linn. Soc. London. Bot. **51**: 389–407.

Fahn, A. & Tova Arzee, 1959: Vascularization of articulated *Chenopodiaceae* and the nature of their fleshy cortex. — Amer. Journ. Bot. **46**: 330–338.

Fahn, A. & Nina Dembo, 1964: Structure and development of the epidermis in articulated *Chenopodiaceae*. — Israel Journ. Bot. **13**: 177–192.

Frey-Wyssling, A., 1959: Die Pflanzliche Zellwand. — Springer, Berlin-Heidelberg-Göttingen.

Frey-Wyssling, A. & Mühletahler, K., 1965: Ultrastructural Plant Cytology. — Elsevier, Amsterdam-London-New York.

Fritz, F., 1935: Über die Kutikula von Aloë- und Gasteria-arten. — Jahrb. wiss. Bot. **81**: 718–745.

Hall, O. M., 1967: Wax microchannels in the epidermis of White Clover. — Science **158**: 505–506.

Huber, B., Kinder, E., Obermüller, E. & Ziegenspeck, H., 1956: Spaltöffnungs-Dünnschnitte im Elektronenmikroskop. — Protoplasma **46**: 380–393.

Jensen, William A., 1962: Botanical histochemistry. — Freman & Co.

Lambertz, P., 1954: Untersuchungen über das Vorkommen von Plasmodesmen in den Epidermisaussenwänden. — Planta **44**: 147–190.

Morello, I. 1958. La provincia fitogeografica del Monte. — Opera Lilloana II, Tucumar.

Raunkiær, C., 1916: The use of leaf size in biological plant geography. — The life forms etc. p. 368–378, l. f.

— 1934: The life forms of plants and statistical plant geography. — Oxford.

Record, Samuel J., & Hess Rob. W., 1943: Timbers of the New World. — Yale University Press.

Roelofsen, P. A., 1959: The Plant Cell-Wall. — Encyclopedia of Plant Anatomy III, 4.
Schieferstein, R. H. & Loomis, W. E., 1959: Development of the cuticular layers in Angiosperm leaves. — Amer. Jour. Bot. **46**: 625–635.
Schumacher, W., 1942: Über plasmodesmenartige Strukturen in Epidermisaussenwänden. — Jahrb. wiss. Bot. **90**: 530–545.
— 1957: Über Ektodesmen und Plasmodesmen. — Ber. Deutsch. Bot. Ges. **70**: 335–342.
Schwabe, H., 1947: Estudio anatomico de las especies Argentinas del género *Bredemeyera* (*Polygalacéas*). — Bol. Soc. Argent. Bot., **2**: 65–72.
Scott, F. M., Hammer, K. C., Baker, E. & Bowler, E., 1958: Electron microscope studies of the epidermis of *Allium cepa*. — Amer. Jour. Bot. **45**: 449–460.
Sitte, P. & Renner, R., 1963: Untersuchungen an cuticularen Zellwandschichten. — Planta **60**: 19–40.
Slade, B. F., 1952: Cladode anatomy and leaf trace systems in the New Zealand Brooms. — Trans. Royal Soc. New Zealand **80**: 81–96.
Volkens, G., 1887: Die Flora der ægyptisch-arabischen Wüste auf Grundlage anatomisch-physiologischer Forschungen. — Berlin.

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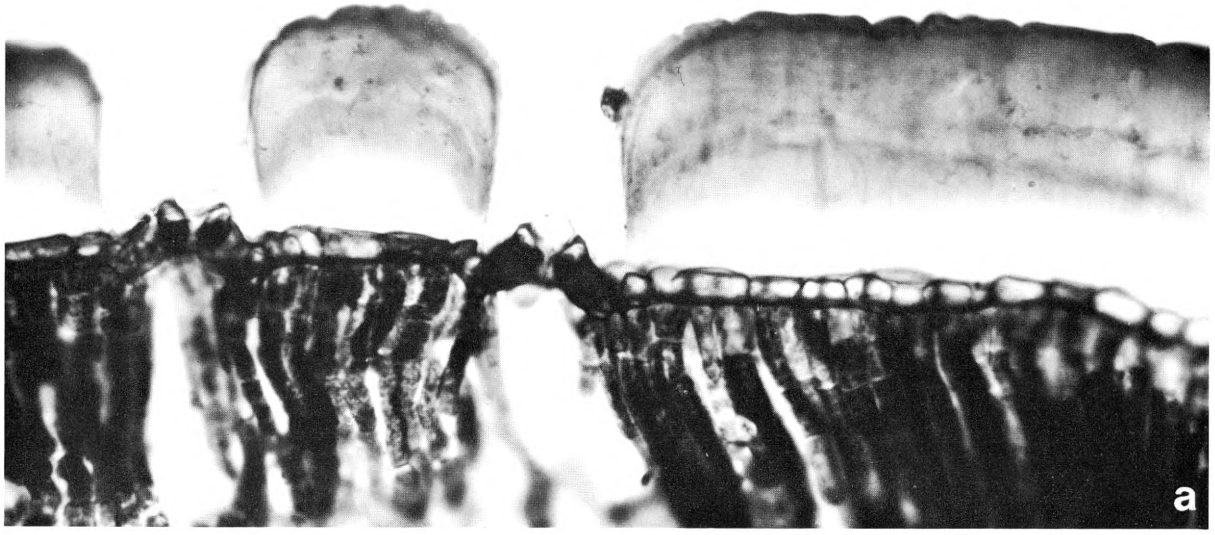
Færdig fra trykkeriet den 31. oktober 1968.

PLATES

PLATE I *Monttea aphylla*

a and c transverse sections, b longitudinal section of epidermis and cortex. a–b Johansen's quadruple staining, c Lightgreen-Safranin staining. a–c × 320.

a. Two stomatal cavities. Cuticular coating with 1–2 clear layers both parallel with the surface which has a number of shallow furrows.
b. One stomatal cavity, showing four differently coloured parts of the guard cell. The outer layer which corresponds to the cuticularized outer ledge is light purplish, next follows a green line (outer thick part of the wall), a light green area (cell lumen + wall) and finally a dark bluish violet part, which is the very thick inner wall of the guard cell. The other epidermis cells are green, the outer part of the cuticular coating dark purplish and the inner part light purplish. – In the cortex: layers of palisade cells and a spongy parenchyma, finally a dense parenchymatous layer and extraxylary fiber.
c. The same, but fibres cut transversely. Pear-shaped substomatal air chamber and plugs in the cuticular cavities formed by an imperfect fungus.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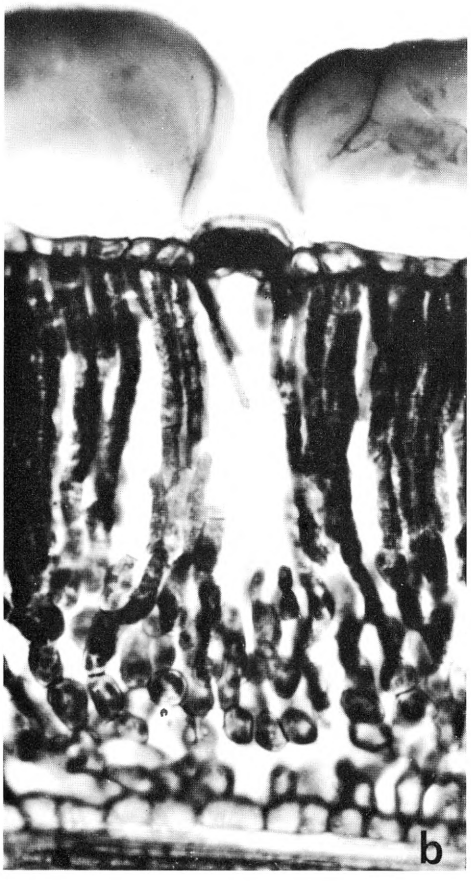
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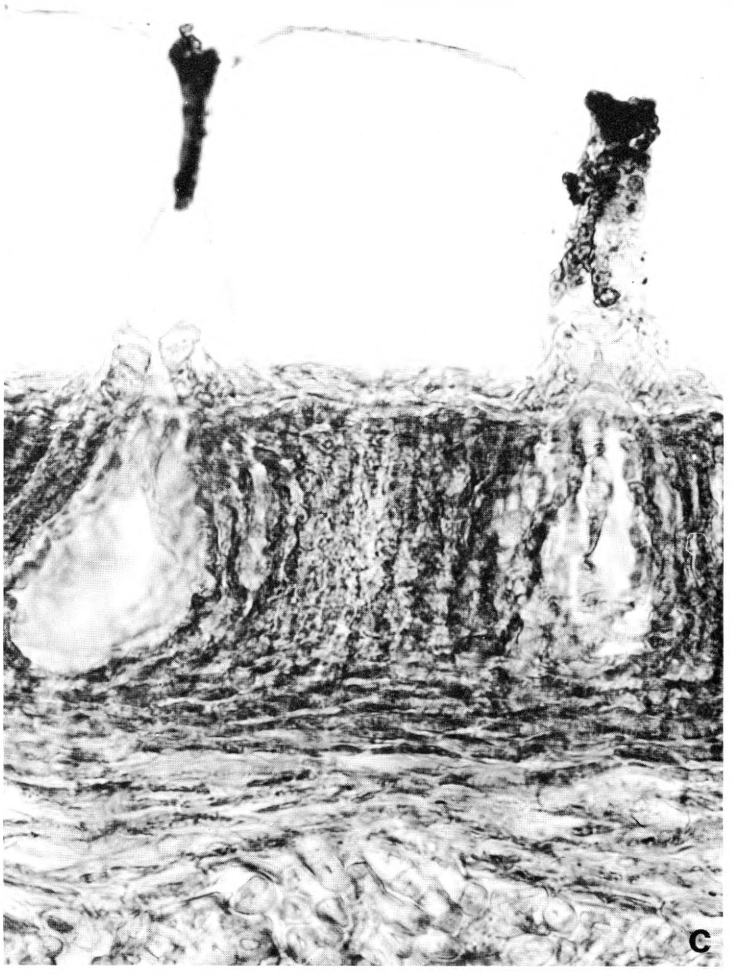
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PLATE II *Monttea aphylla*

a–f transverse sections of epidermal cells, in d including whole cuticular coating. Stainings with Sudan IV (a–b), PAS (periodine–Schiff, c–d), Nile-blue (e–f). a–c, e–f × 1950, d × 800.

a. Cells in active stage. Middle part of outer wall with several thin lamellae. Peripheral parts not split into lamellae and joining anticlinal walls which seem to fork.
b. Middle cell active with lamellated outer wall. On each side cells with firm walls and ridges as relics of basal parts of dome-shaped cellulose lamellae.
c. Dome-shaped vesicle limited by thin cellulose lamellae, two are clearly seen on the right; on the left the lamellae are ± dissolved.
d. The same as c, but a large vesicle has almost reached the surface of the cuticular layer and its outer edges stained with PAS.
e–f. Active epiderm cells, the outer walls viewed slightly obliquely; in e the surface appears as a network from which a number of short dark threads radiate. Thick anticlinal wall with "forking". – f. Same as e but pores in network smaller and may be shaped as small protruding tubules, leftmost cell with five distinct lamellae.

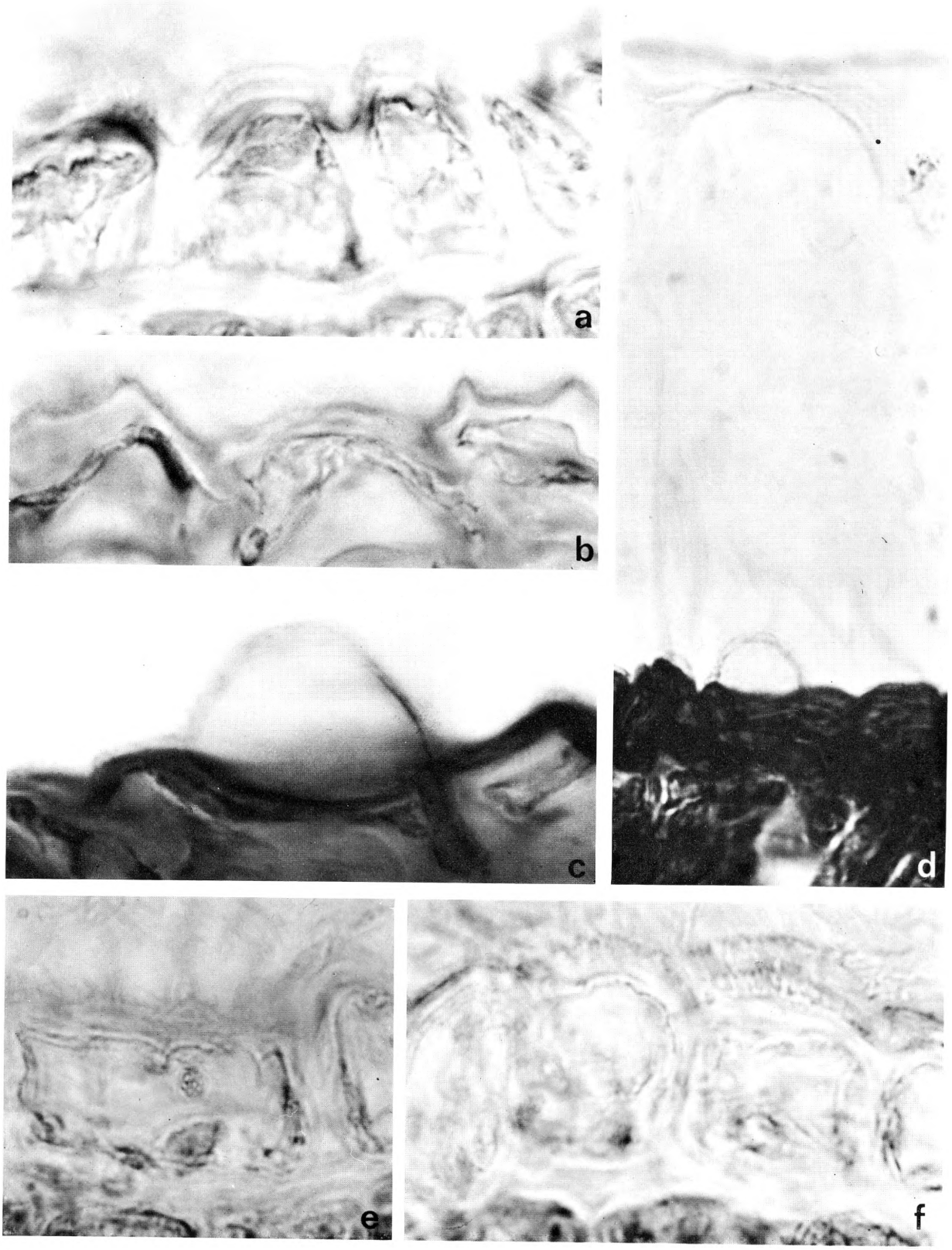
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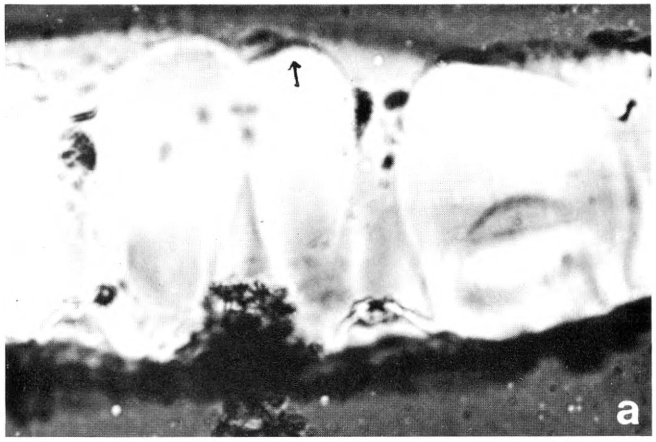
PLATE III *Monttea aphylla*

a–f transverse sections of epidermal cells, in d and f only inner part of the cuticular coating visible. a and c in polarized light, a stained with PAS, b–d Johansen's quadruple staining, e staining with Nile-blue, e–f staining with Ruthenium red. – a–b × 320. c–d × 800, e–f × 1950.

a. Birefringence of whole vesicles probably due to wax. Outer edge stained with PAS (arrow), a very fine radial (anticlinal) striation to be seen dimly.
b. Similar area showing paler vesicles and dark purplish outer part of cuticular coating. Note: cell of an imperfect fungus in the front cavity of the stoma.
c. The usual aspect of birefringence after removal of wax, showing two shining lines outside each anticlinal wall. Most active cells have concave walls towards cuticular covering.
d. Stomatal apparatus and cavity in cuticular layer with small group of fungal cells (on the right). Note dark staining of thick walls in guard cells and subsidiary cells.
e. Actively secreting cell; on the right a thick anticlinal wall and adjacent parts of outer periclinal walls. The very thin middle part of the outer wall has a number of small pores and outside the middle area some structures in the cuticular layer which possibly has been secreted by the cell.
f. One actively excreting cell the outer wall of which is very blurred and seems to be decomposed into small translucent bodies. Outside righthand anticlinal wall (dark red with Ruthenium red) small bodies and vesicles arranged perpendicular to the surface (cp. Plate V, b).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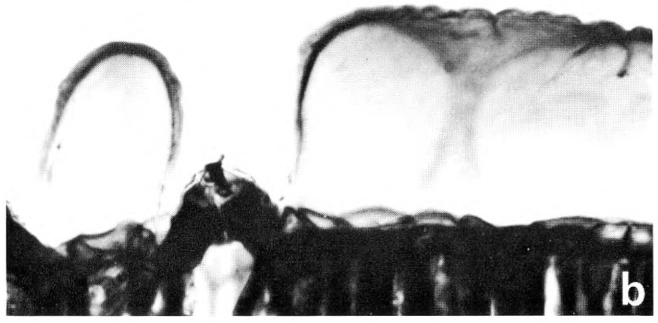
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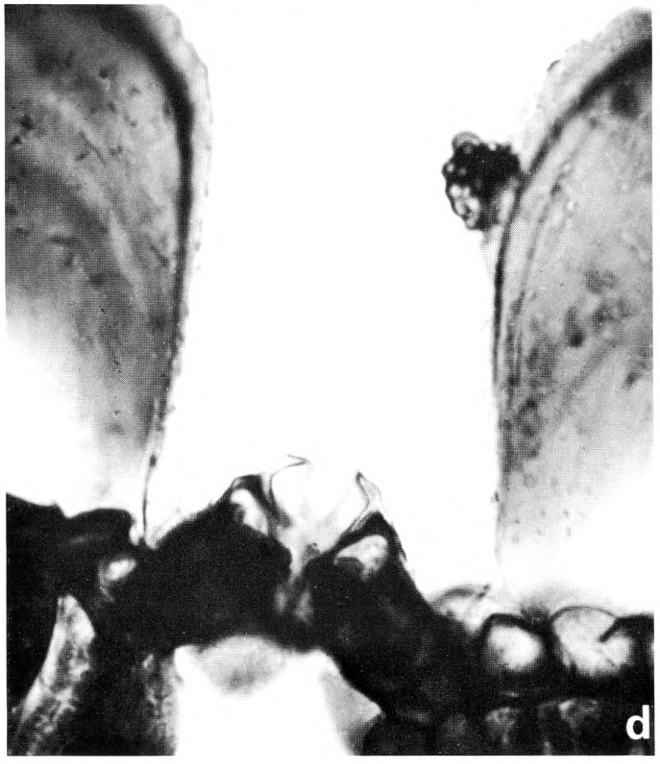
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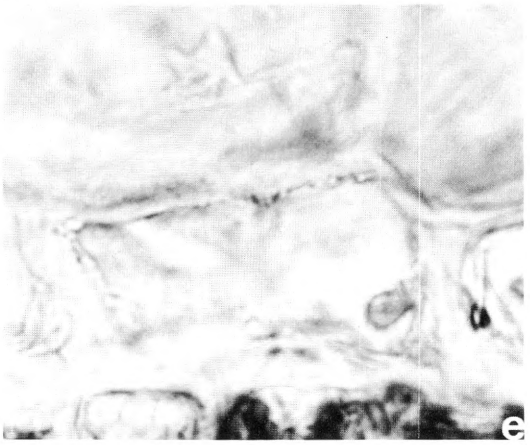
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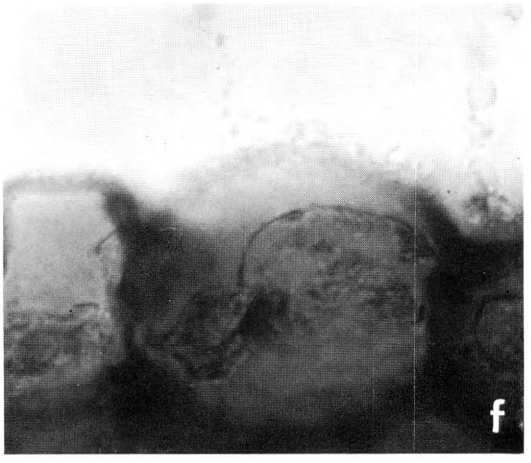
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PLATE IV *Monttea aphylla*

a–c transverse sections of epidermal cells with cuticular coating as shown in polarized light (a and c) and with phase-contrast (b). b–c the same area. ×320.

a. Arched area of cuticular coating between two stomatal cavities, one of which is seen on the right. The bright perpendicular lines represent two merging vesicle boundaries of two adjacent cells.

Immediately above the anticlinal walls the lines divide into two which issue from the corners along the grooves (cf. Fig. 3b).

b–c. Similar area as in Fig. a as observed with phase contrast (b) and in polarized light (c). With phase contrast the outermost layer is bright, while it appears dark in all other cases. There are pairs of dark lines in the peripheral part (Fig. b). These lines are weakly birefringent (Fig. c), whereas the inner lines show up brightly and are clearly paired (arrow).

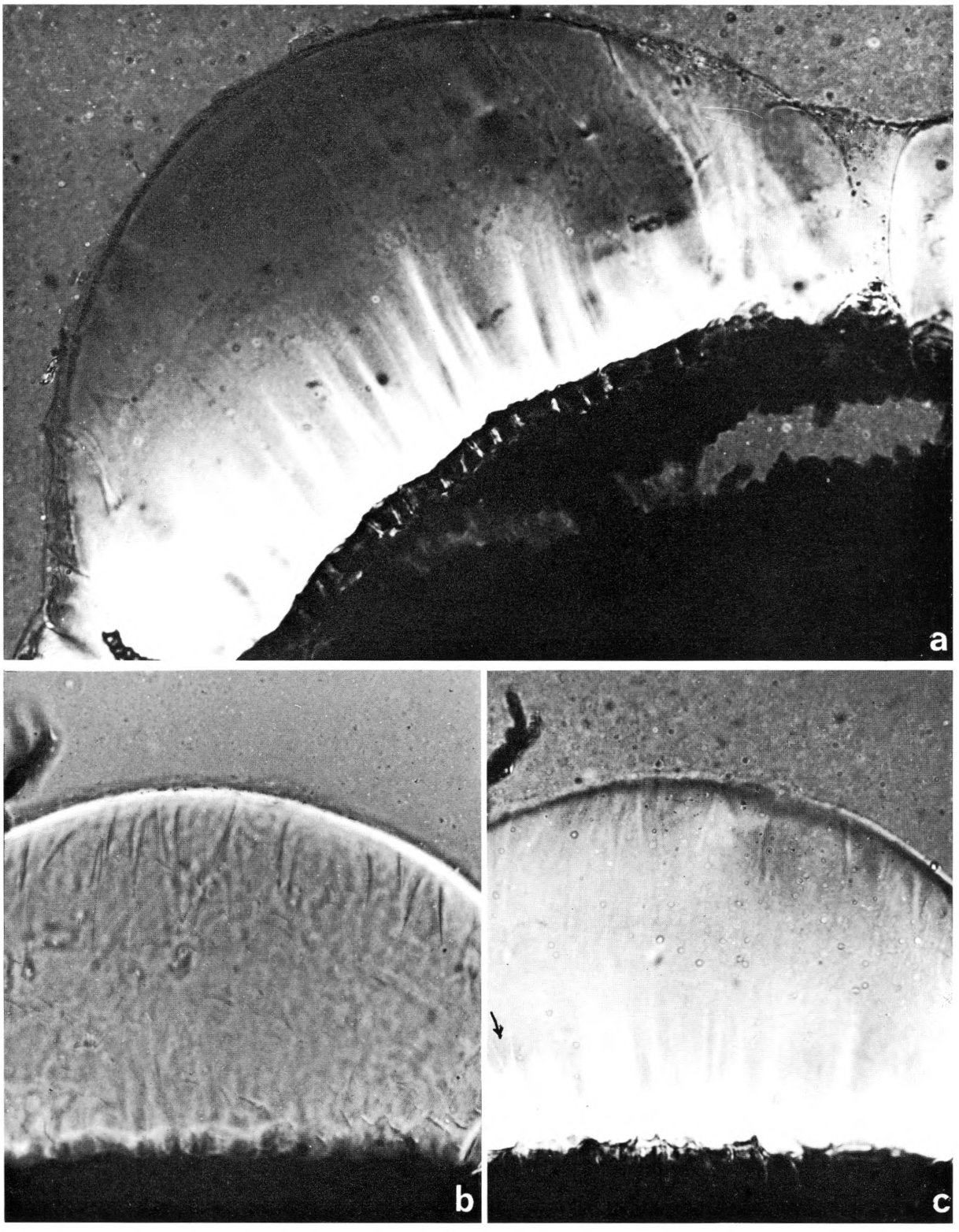
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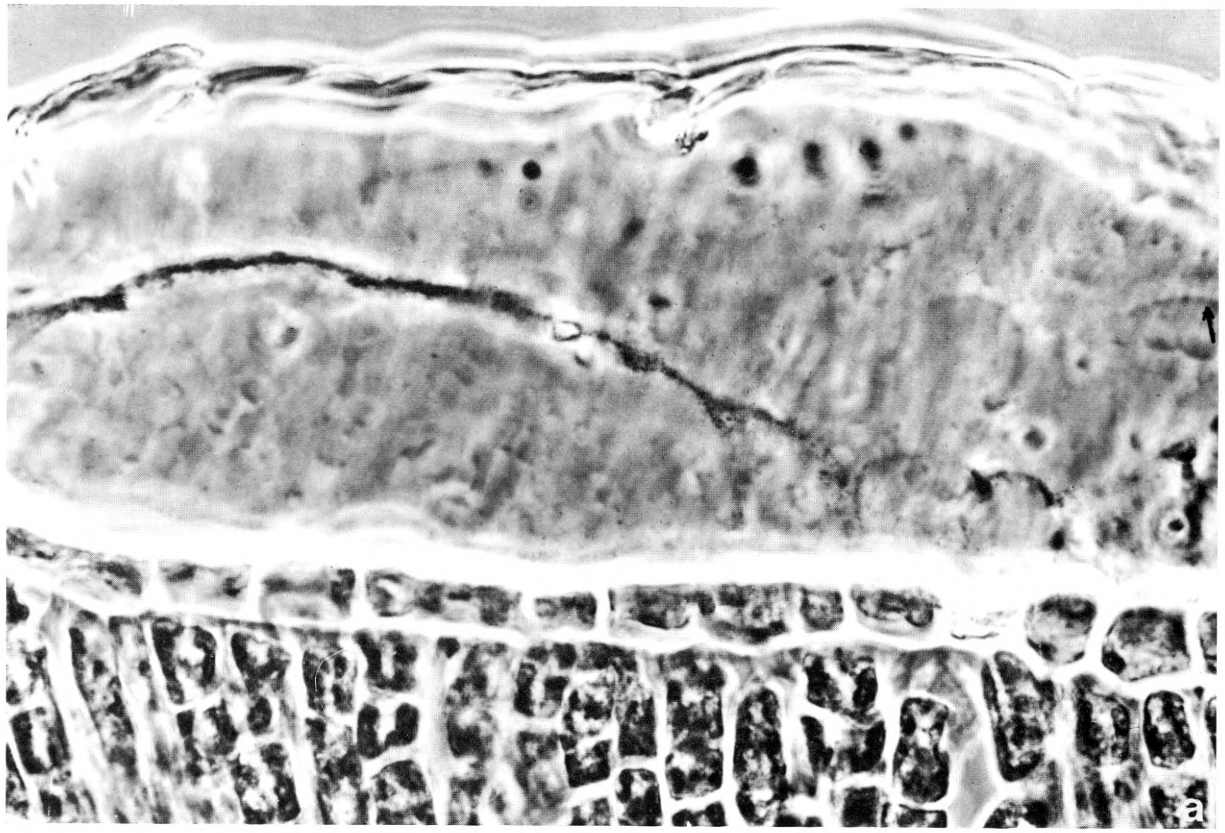
PLATE V *Montiea aphylla*

a-c transverse sections of epidermis and outer part of cortex. Phase contrast and staining with Lightgreen-Safranin. ×800 (a–b), ×625 (c).

a. Contours of vesicles provided with great quantities of small granules. Large vesical on the left and three smaller ones on the right. Older vesical boundaries on the right just visible (arrow).
b. Line with small granules outside anticlinal wall. The line corresponds to a border area between two vesicles which here nearly have reached a low depression in the surface.
c. Area near cuticular stomatal cavity (the stoma is just outside the picture on the right but the subsidiary cells are seen, cp. Plate VIII e–f). Some of the epidermis cells have been divided by periclinal walls and a number of cells with chloroplasts have been formed and fill out a depression in the cuticular layer. The picture illustrates how the initiation of the phellogens takes place.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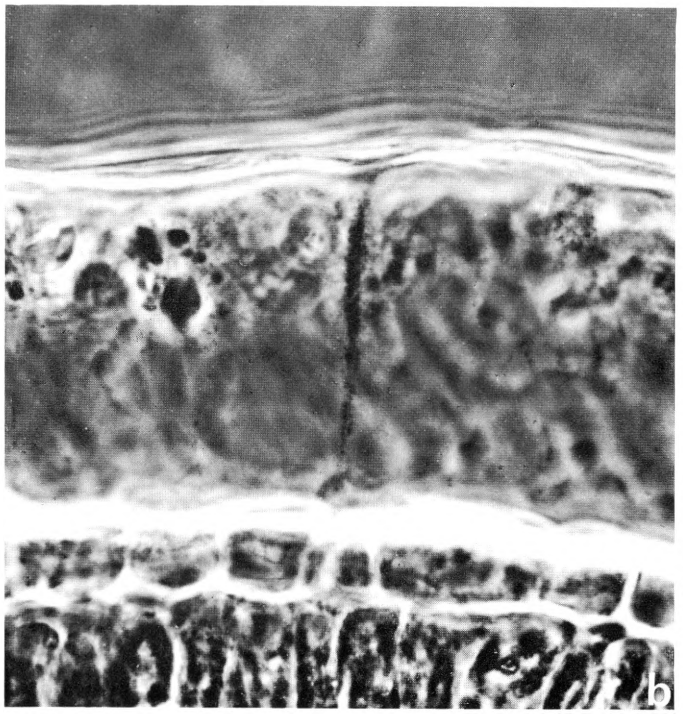
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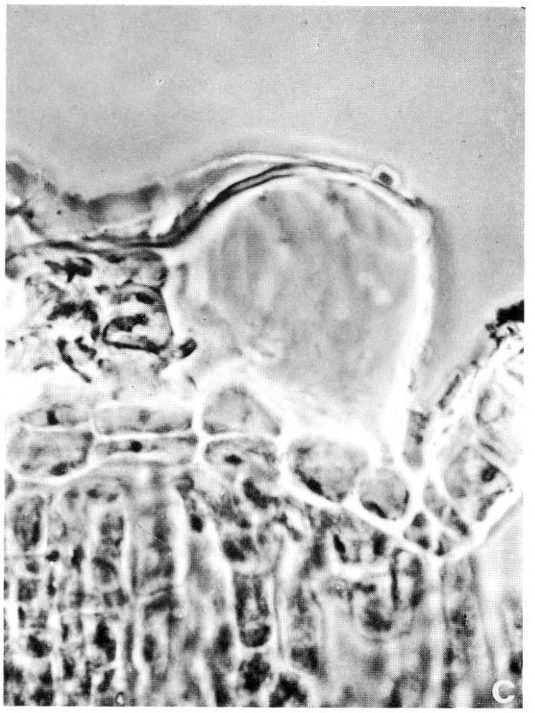
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PLATE VI *Monttea aphylla*

a–e transverse sections of cuticular coating, b after staining with methylene blue, a, c–e after treatment with ammonium oxalate followed by 4% NaOH. a and c stained with Nile-blue, d–e with PAS. c–e phase contrast. a×264, b–c ×320, d–e ×800.

a. Corrosion channels in continuation of anticlinal walls; sometimes two parallel channels situated in two adjacent dome-shaped vesicle-membranes (arrows).
b. Dome-shaped vesicle membranes stained with methylene blue. Most of them represent one cell only, on the extreme right, however, one semicircle covers at least two cells.
c. The contents of the vesicles stain dark blue while the corrosion channels appear bright and shining. The cuticular coating loosens from the epidermis (except on the right).
d. Corrosion channels dark, very numerous, many situated in and upon dome-shaped vesicle membrane. Cuticular coating completely loosened from epidermis.
e. Corrosion channels very numerous, the longer ones expand abaxially.

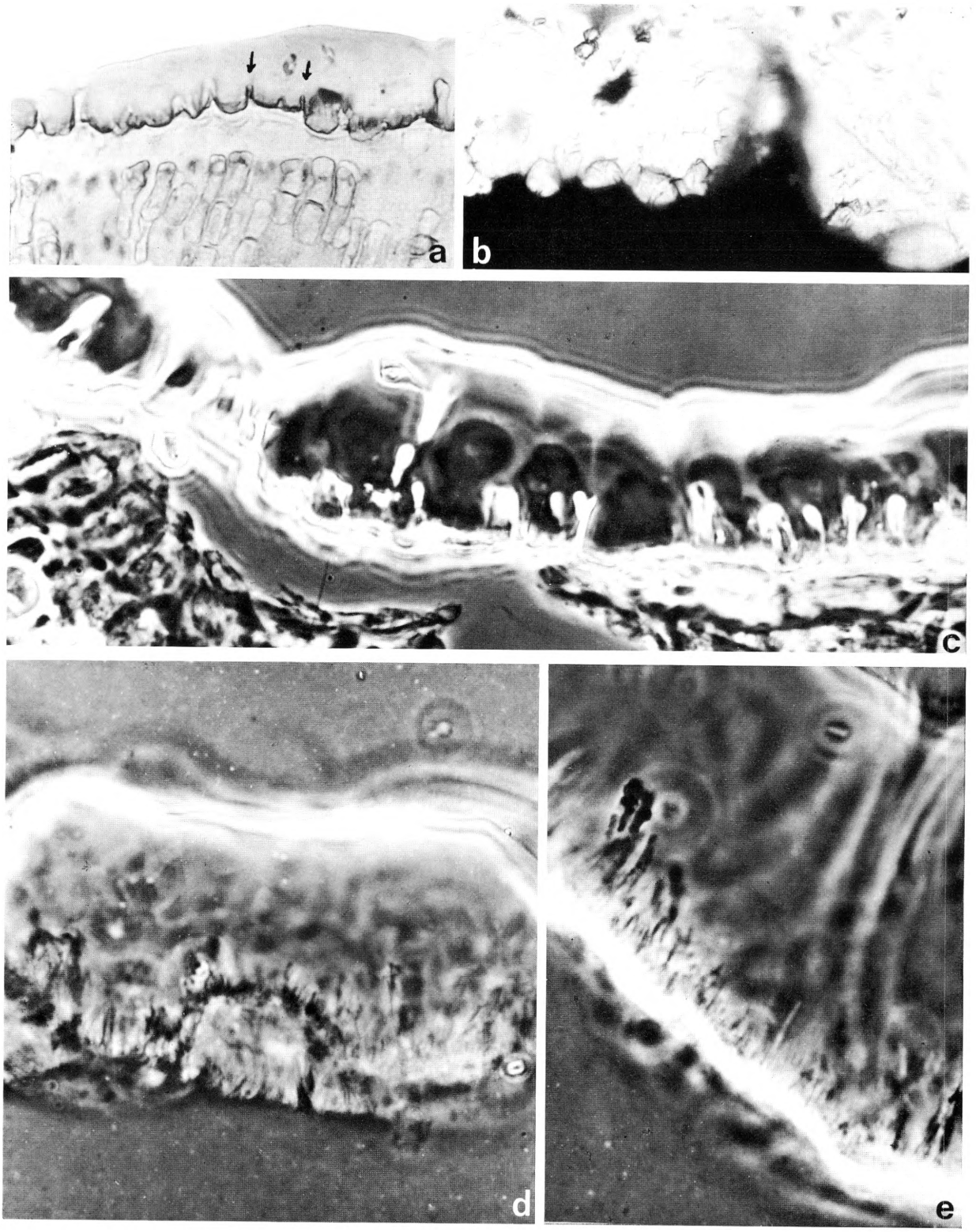
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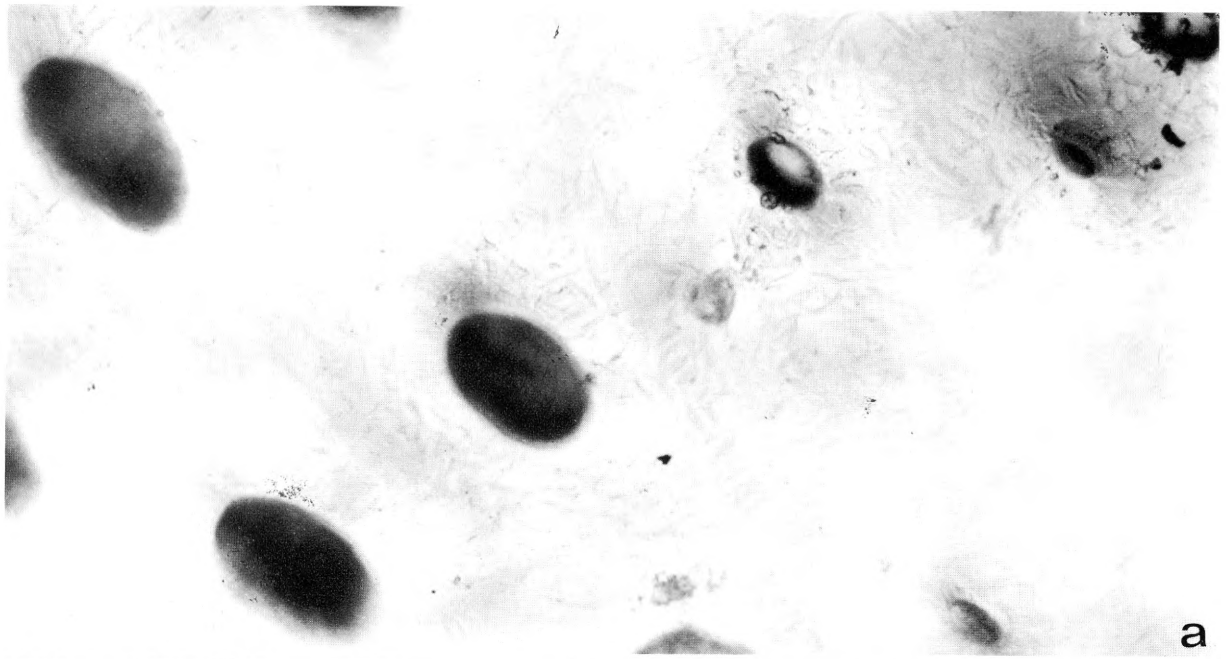
PLATE VII *Monttea aphylla*

a–c ×320.

a. Cuticular coating seen from outside. The cuticle is provided with a system of shallow grooves. The elliptic entrances to the stomatal cavities are very different in size but all orientated with their major axis perpendicular to the axis of the stem.
b. The same but paradermal view at lower focussing. The cuticle lining the stomatal cavities is finely striated. The contours of epidermal cells beneath the cuticular coating are seen.
c. Transverse section of epidermis stained with Sudan IV and NaOH. Cuticular coating shrivelled, cuticle of guard cells and subsidiary cells maintained. Note gaps in cuticle immediately inside stomatal pore.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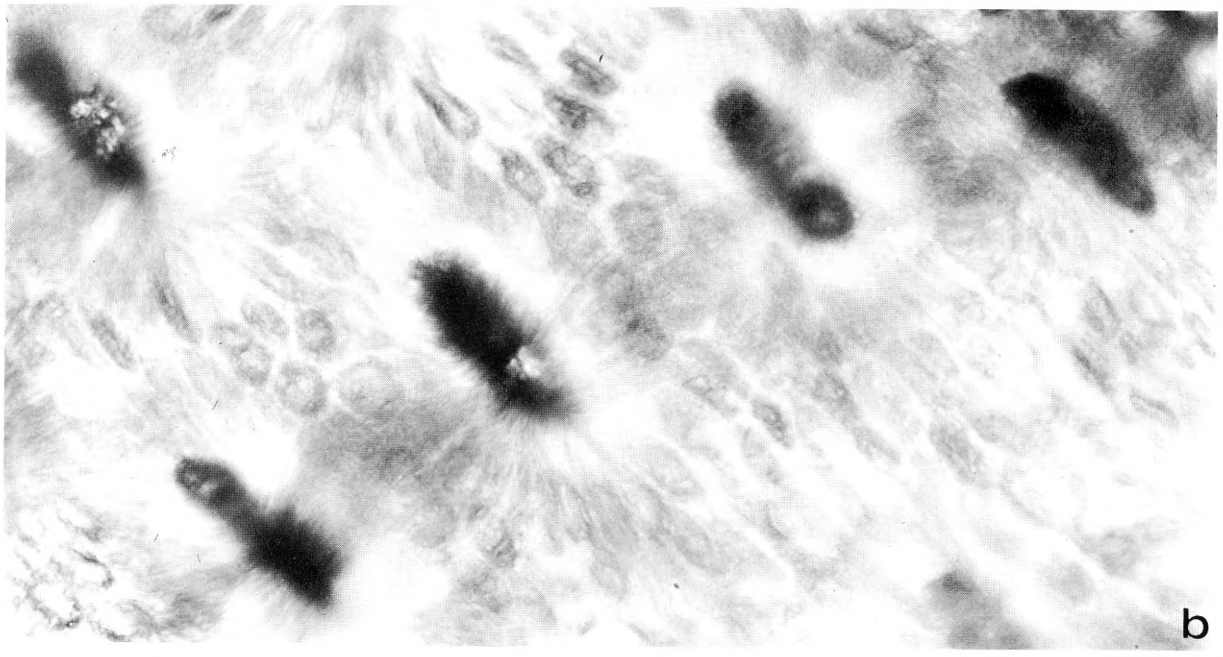
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PLATE VIII *Monttea aphylla*

a longitudinal b–f transversal sections through epidermis with stomata. a–e stained with Lightgreen-Safranin, f. with Sudan IV (+NaOH). a–f ×800.

a. Very young stage near tip of branchlet, note short epidermal cells and small cuticular coating.
b. Epidermis in leaf.
c–d. Young branch, in d very active excreting cells forming a thick coating. The stem forms here a ridge which by later growth is smoothed out. At arrow "fork" formed of remains of the outer wall. At the asterisk two thin lamellae covering two less active cells.
e. Stomatal apparatus.
f. Stomatal apparatus; cuticle stained with Sudan IV. Note outer ledges partly dissolved by NaOH, cp Plate VIIc.

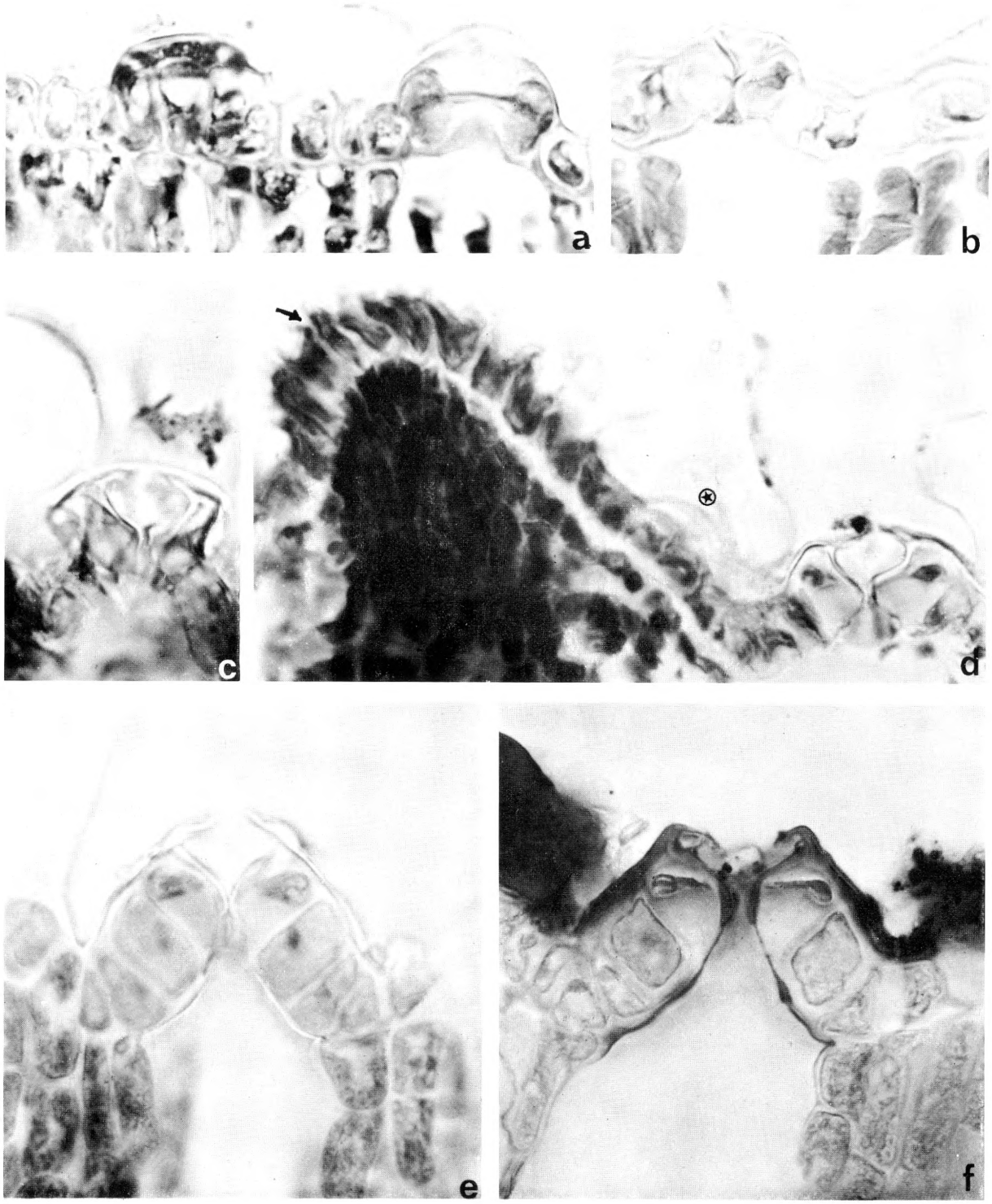
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PLATE IX *Bulnesia retama*

Transverse sections of branchlet at stomatal pits. ×800.

a. Young branchlet. In the multiple epiderm there is an outermost layer of narrow elongate cells forming a palisade-epiderm. Below one layer resembling a hypoderm. Front cavity limited by rather long outer ledges.
b. Older branch. Cells in outer palisade epiderm divided by periclinal walls. Outermost cells withdrawn with pointed tips towards thick stratified cuticular layer, from which wedges are pushed deeply between the cells. Pit above stoma widened, guard cells also with inner ledges. Palisade cells in cortex broader at their abaxial ends or divided by periclinal walls.

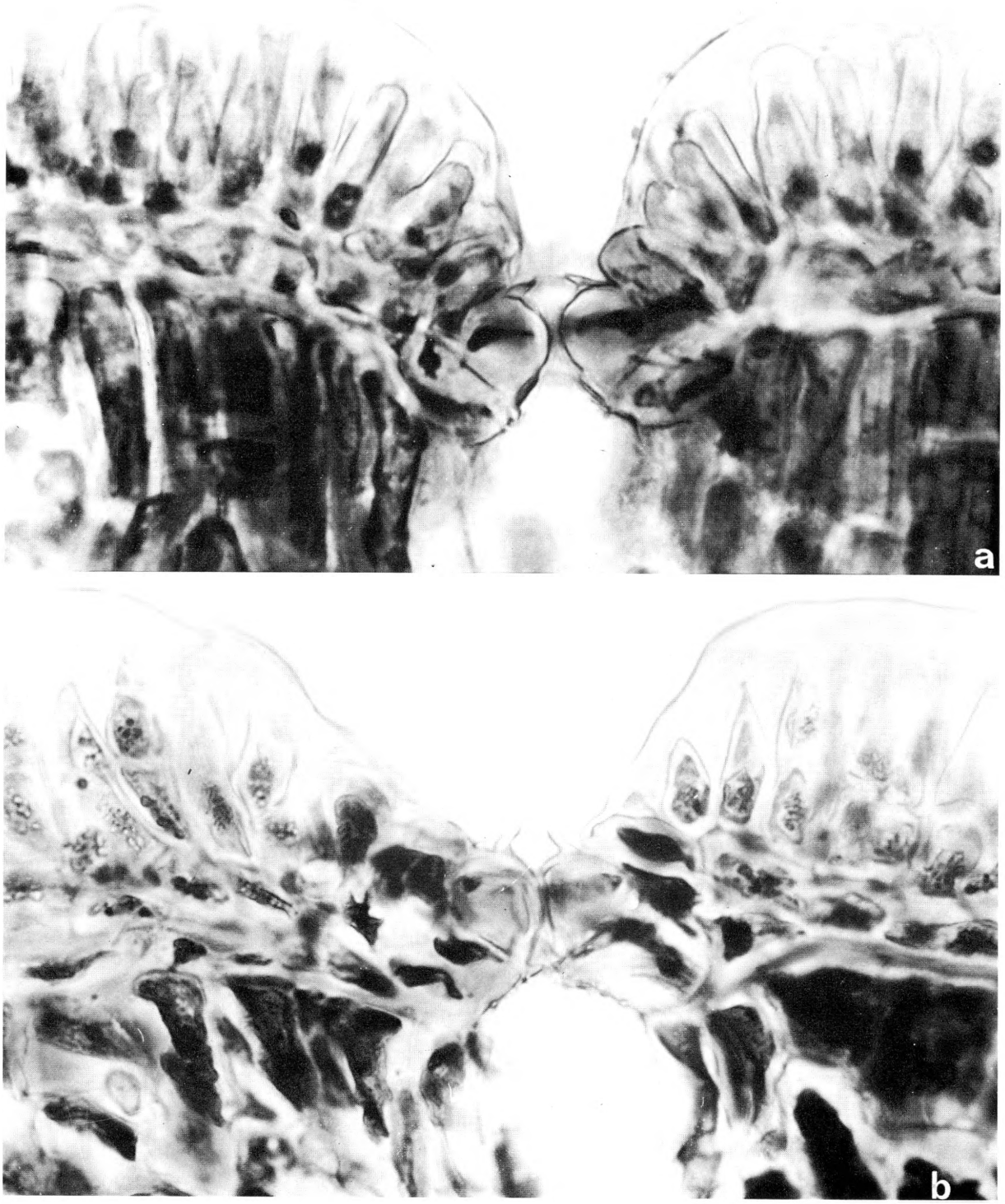
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PLATE X *Bulnesia retama*

a–d. Behaviour of outermost epiderm cells. a, protoderm cells, still undivided, b–c, young cells in outermost layer of multiple epiderm, d, old stage showing ruptures in thick cuticular layer, e, cell row; old stage. a stained with Lightgreen-Safranin, b–c, Johansen's quadruple staining. a–d × 1950, e × 1024.

a. Many short plasmatic threads (ectodesms?) issuing from outer part of protoderm cell. Probably remain of wax deposit outside the cuticle.
b. Two pairs of sister cells, each pair originating from one protoderm cell. Cuticular layers broader, ectodesmatal structures longer. Cuticle distinct.
c. The same as b but showing whole cells and on the right one cell divided by an oblique wall, the lower cell probably being able to grow up against the surface, thereby increasing the girth.
d. After rupture of thick cuticular layer, one cell (probably of the type shown on the right in Fig. c) reaching the place of rupture and starting here to form new outer wall layers. Empty cells on both sides. The increase in thickness of the cuticular layer is considerable (in a about 8 μ, in b 15–20 μ, in d a little more).
e. Cell row in multiple epiderm, the outer cell small. In the cuticular area outside there are four distinct layers. In the top of two of the layers marks after a few strands.

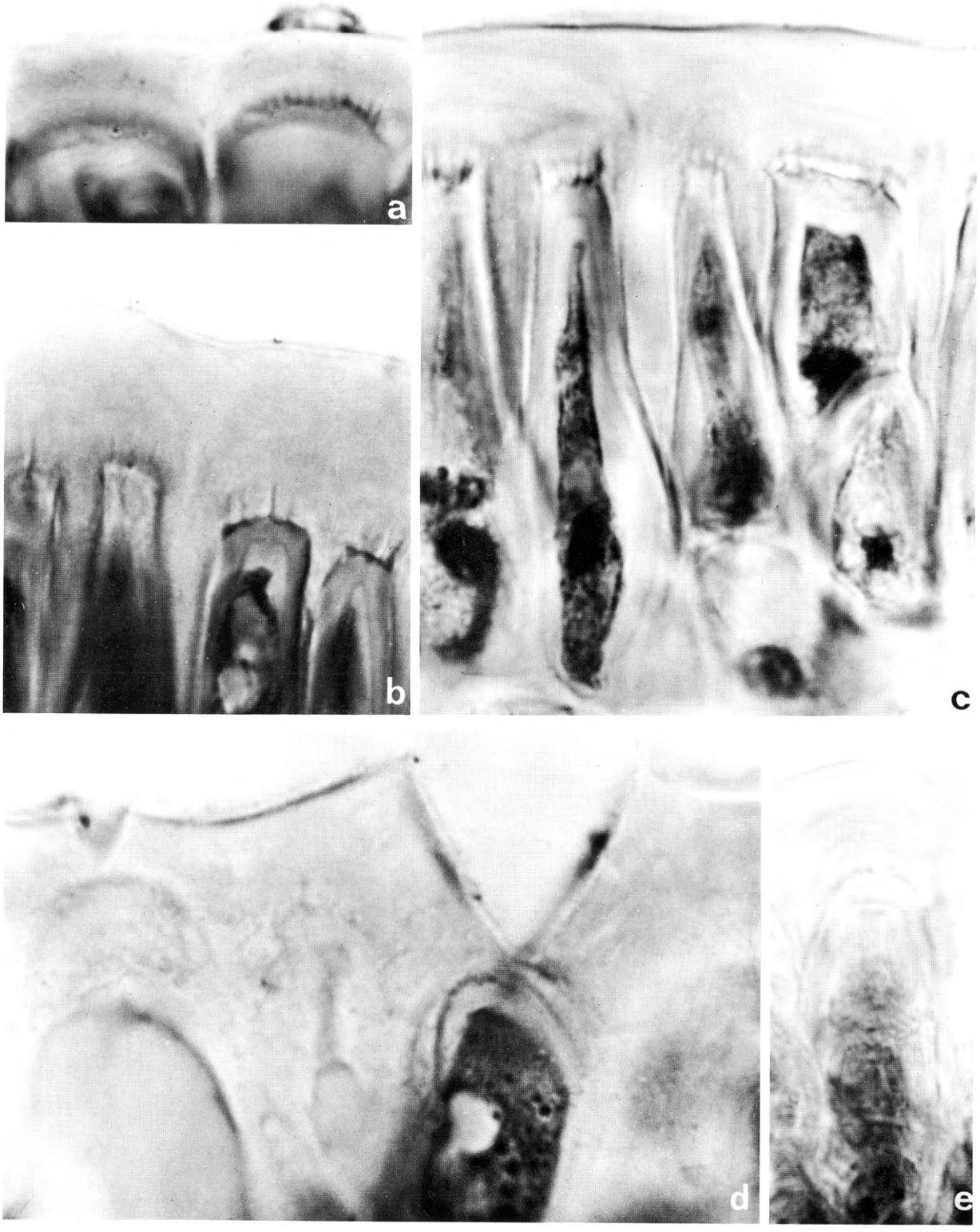
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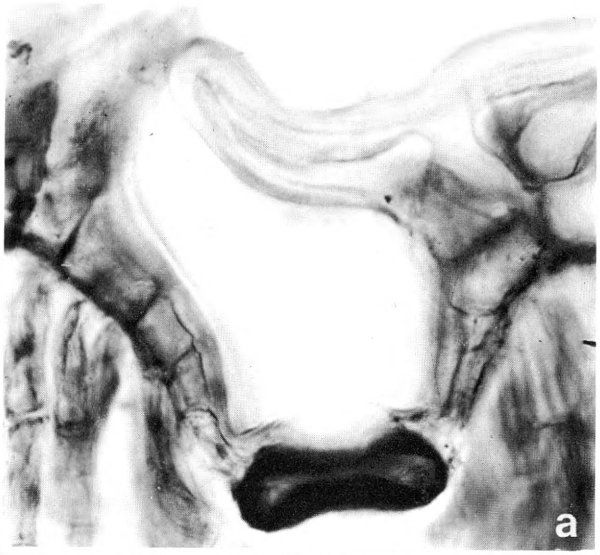
PLATE XI *Bredemeyera colletioides*

Stomatal apparatus and outer part of multiple epiderm.
a Johansen's quadruple staining, b Sudan IV, c–e in polarized light, a ×550, b–c ×800, d–e ×1280.

a. Curved stomatal pitcher. Many lamellae in cuticular layer on the right. Plasmatic nodule near the tip of protruding part of cell covering the picher cavity.
b. Section through central pore in stoma covered with diaphragm. The pitcher is curved as in Fig. a and the very delicate channels leading to the nodule are seen in cross-section as dark points (two in each cell). Heavy cutinization of outer walls and cutinized wedges between outer epiderm cells. Rows of cortical palisade cells.
c. Entrance to stomatal pitcher showing birefringency of outer cutinized part and hair-like structures ("wax-hairs"). Near the tips of the protruding parts of the cells forming the entrance are two channels, which appear dark on the left where the wall is shining but show up on the right where the surrounding wall is dark.
d. Guard cells and diaphragm in polarized light.
e. Part of thick outer wall and wedges. In the broad wedge the middle part is birefringent but appears to be transversed by plasmodesmata. Small shining bodies on the surface and two small merging shining areas on the transition between the inner wall rich in cellulose and the outer cutinized part.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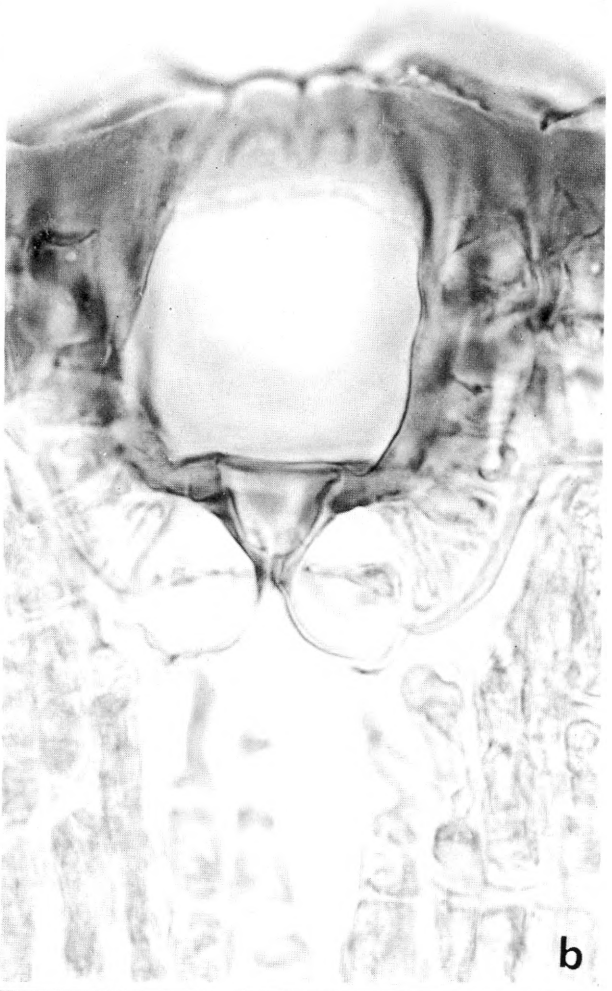
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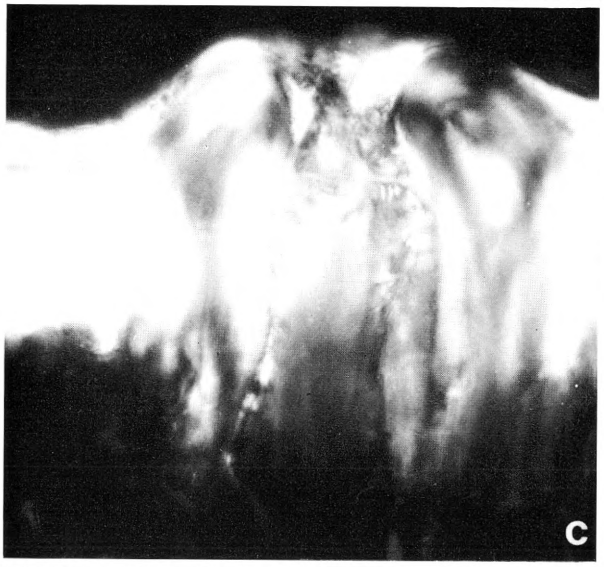
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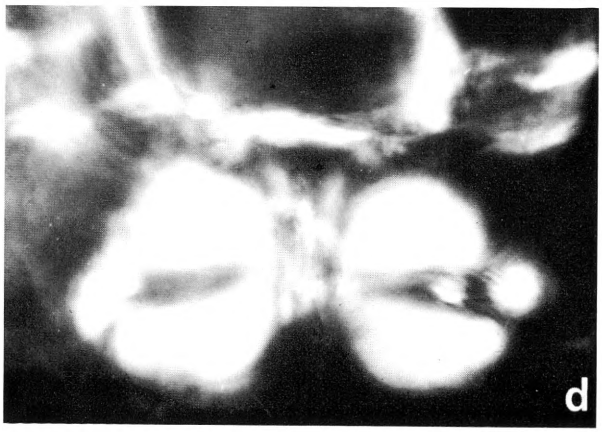
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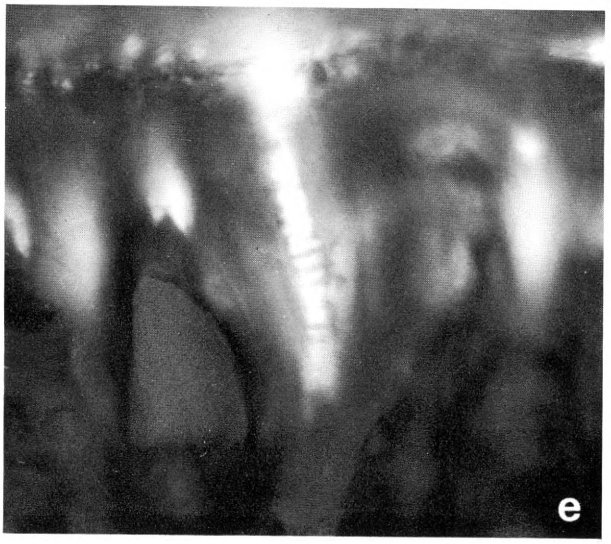
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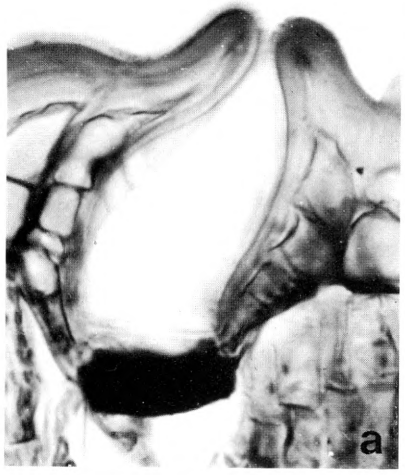
PLATE XII *Bredemeyera colletioides*

Entrance of stomatal pitchers.
a Johansen's quadruple staining, b–e no staining, glycerol mounted slides made from herbarium material, e in polarized light. a ×433, b–d ×1950, e ×1280.

a. Stomatal pitcher (longitudinal section) which is slightly curved.
b. Tip of protruding part of subsidiary cell forming entrance to pitcher. On the left dense covering of "wax hairs". In the wall the plasmatic nodule and several very delicate strands issuing from it.
c. The entrance and outer part of stomatal pitcher showing wax hairs and "wax bridge" on the transition to the pitcher cavity. In the protruding cell wall several very fine strands (ectodesms). Fan-shaped arrangement of the strands above dark area in anticlinal wall.
d. Entrance of stomatal pitcher in which all "wax hairs" are concentrated at the entrance and stop at the "wax bridge" which in this case appears to be particularly dense.
e. Entrance and outermost part of stomatal pitcher. At arrows birefringence of two very delicate channels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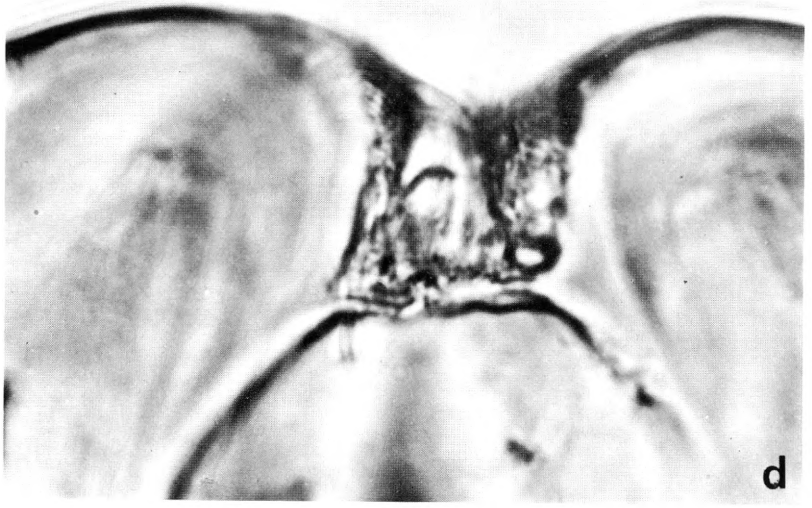
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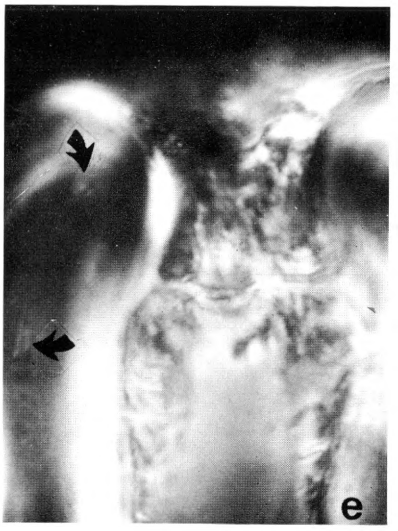
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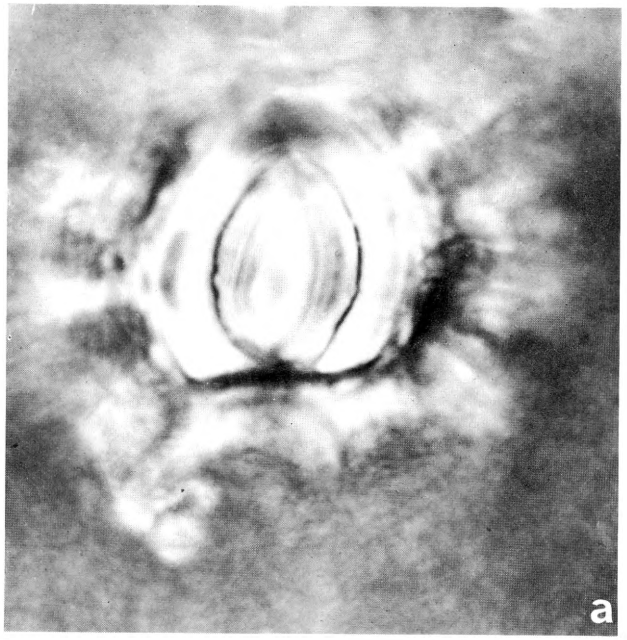
PLATE XIII *Bredemeyera colletioides*

Transverse sections of stomatal pitchers and surface view of guard cells. ×1280.

a–b. The same stoma.
a. Plane of diaphragm sharp. Diaphragm with elliptic opening.
b. Central pore between guard cells sharp.
c and d. Transverse sections of outer part of two different pitchers. In pitcher c the stomatal aperture is straight under the opening (dark area in the middle, cp. Plate XIV a–b for continuation at lower focussing). Pitcher d is curved and the stomatal aperture displaced in relation to the outer part of the pitcher (dark round area below, for continuation, see Plate XIV c–d).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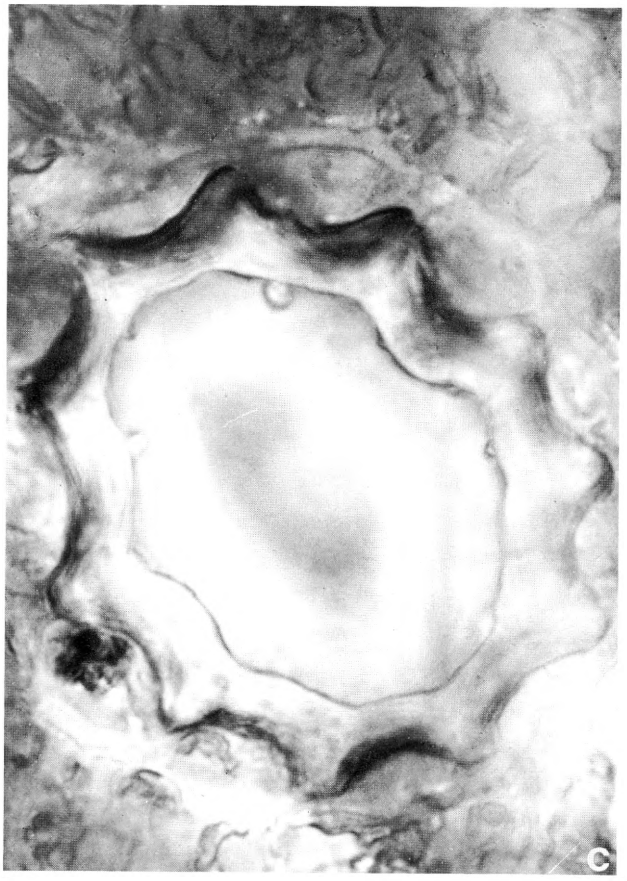
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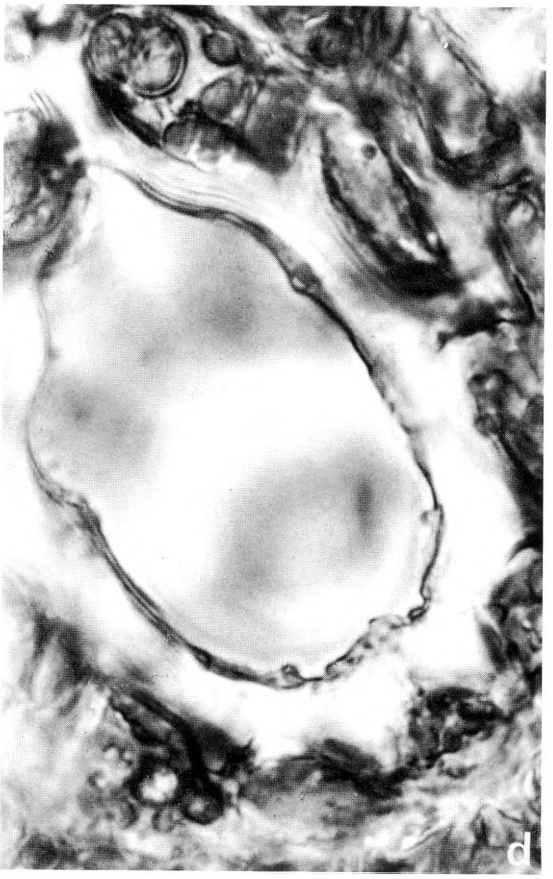
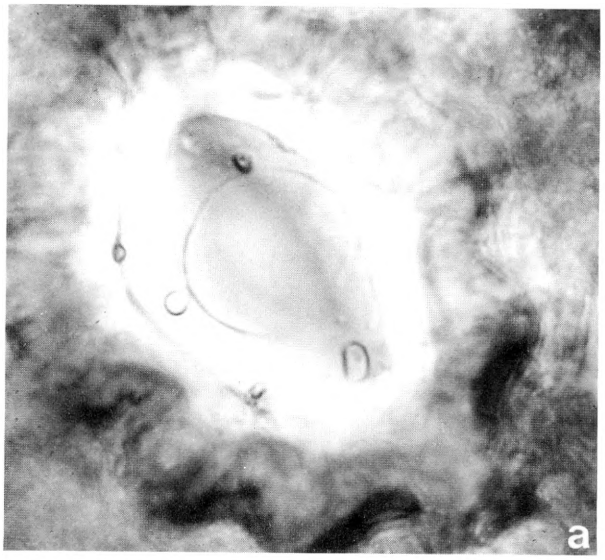
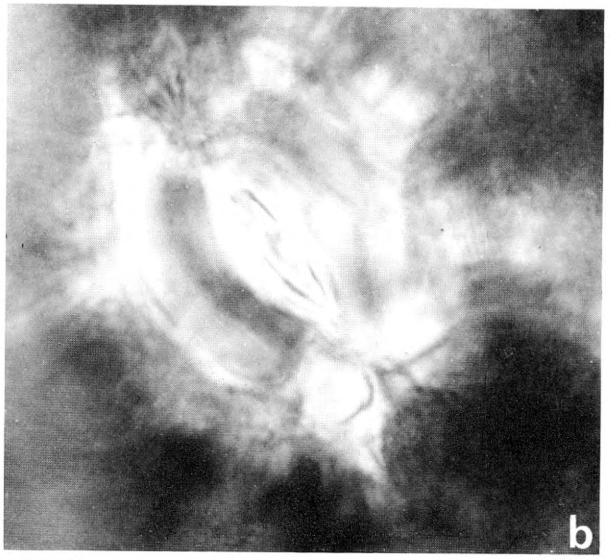

d

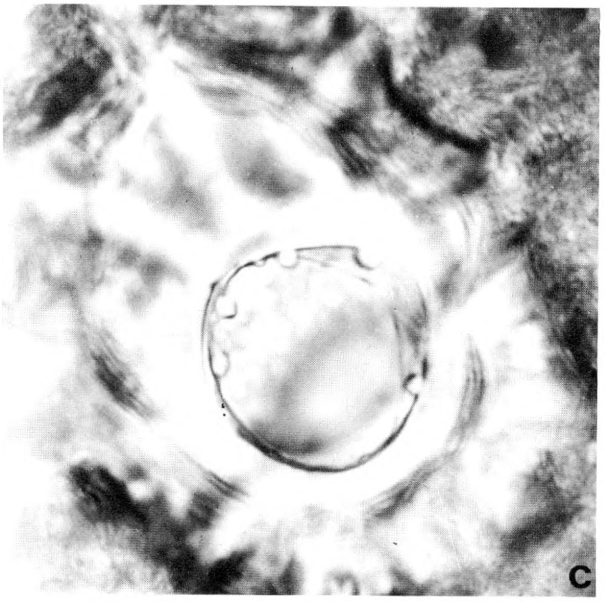
PLATE XIV *Bredemeyera colletioides*

a–d surface views of diaphragm and guard cells. e longitudinal section through mouth of pitcher, f transverse section of mouth of pitcher.
a–d, f ×1280, e ×1950.

a–b. The same stoma. – a. Plane of diaphragm sharp with circular opening – b. Plane of central pore between guard cells sharp.
c–d. The same stoma. — c. Excentric circular opening in diaphragm. Five small rounded teeth and some delicate threads (probably wax) at the margin of the opening. — d. Central pore seen obliquely below opening in diaphragm.
e. Upper parts of four uppermost subsidiary cells cut, showing plasmatic nodules, channels and delicate strands (ectodesms?) radiating towards the periphery.
f. Mouth of pitcher seen from inside. Five connections to cell lumens cut. On the left the connecting channels merge and form nodules.


a


b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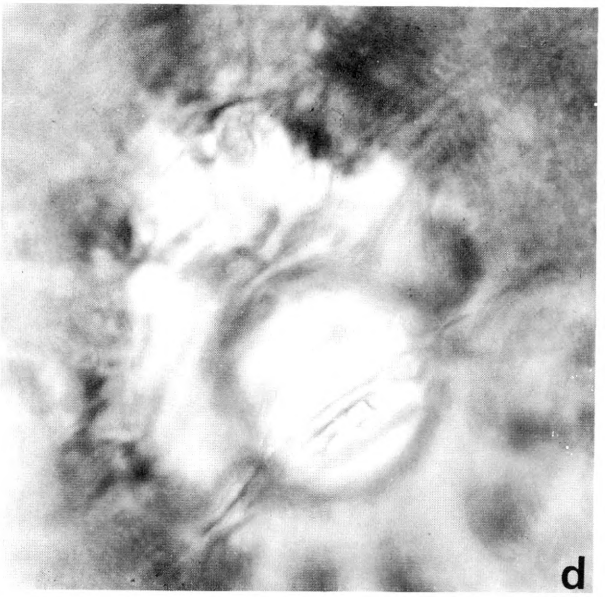
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e

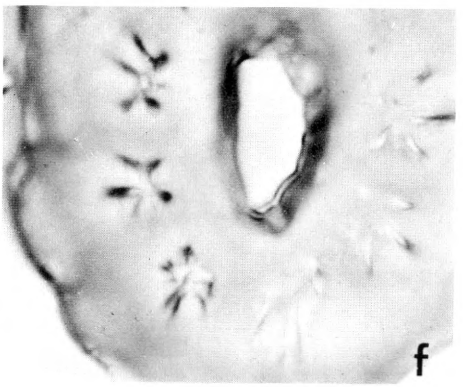

f

PLATE XV *Bredemeyera colletioides*

Sections through multiple epiderm. a–b outermost cells with thick cuticular layer, c area with three stomatal pitchers, d–e longitudinal sections of guard cells. — All stained with Johansen's quadruple stain. — a ×1950, b ×1342, c ×320, d–e ×537.

a. Clear layering in cuticular layer, in the lefthand cell one delicate channel connecting cell-lumen with area which is filled with radiating dark structures (ectodesms?).
b. Cell showing two plasmatic platelets from which very delicate strands radiate towards the periphery.
c. The two stomatal pitchers on the left are cut through the guard cells, in the second from the left the diaphragm formed by the outer ledges is seen. On the right protruding mouth of a pitcher (with five plasmatic nodules).
d–e. d. Section through central opening in diaphragm. e. Section through the diaphragm.

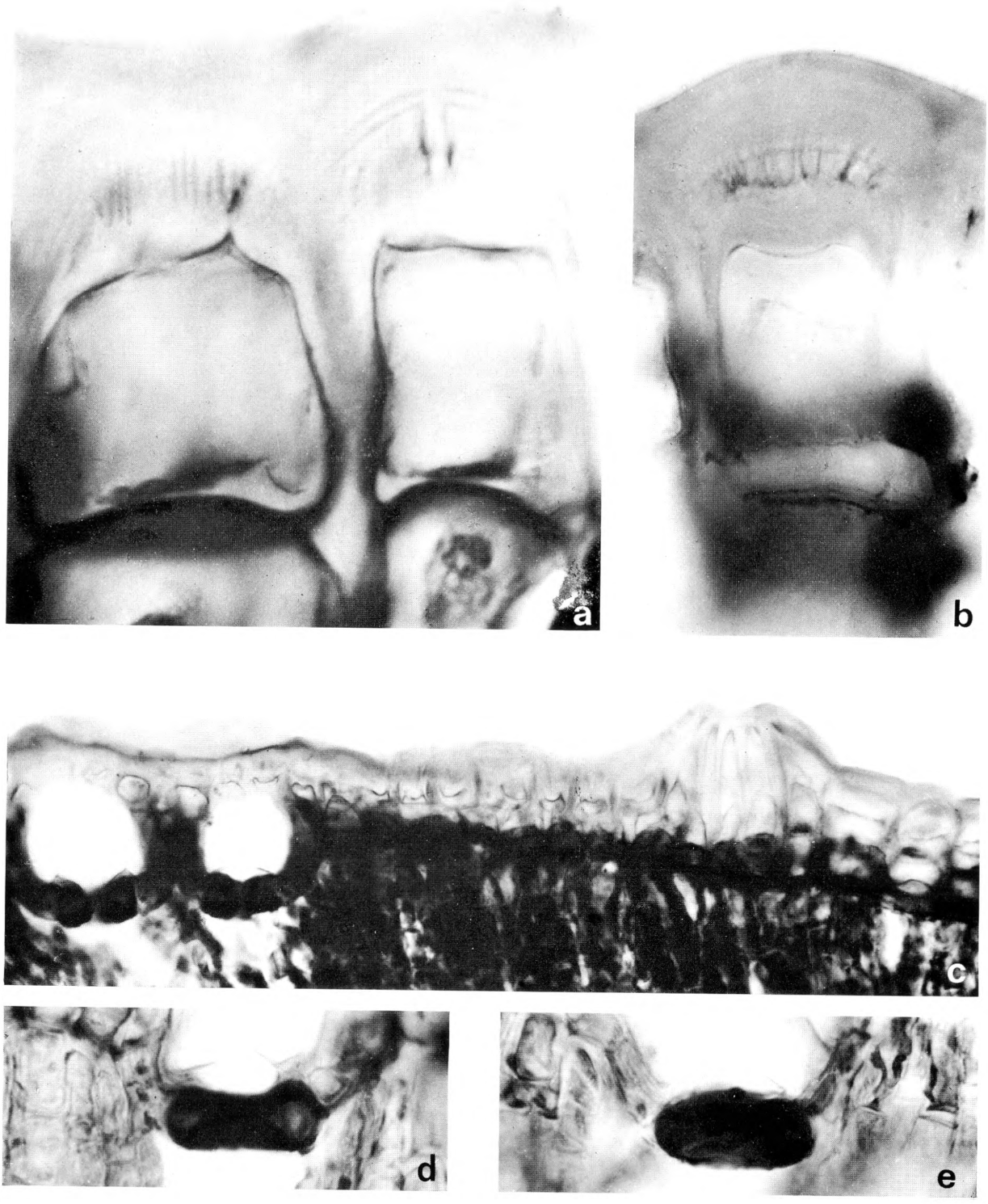
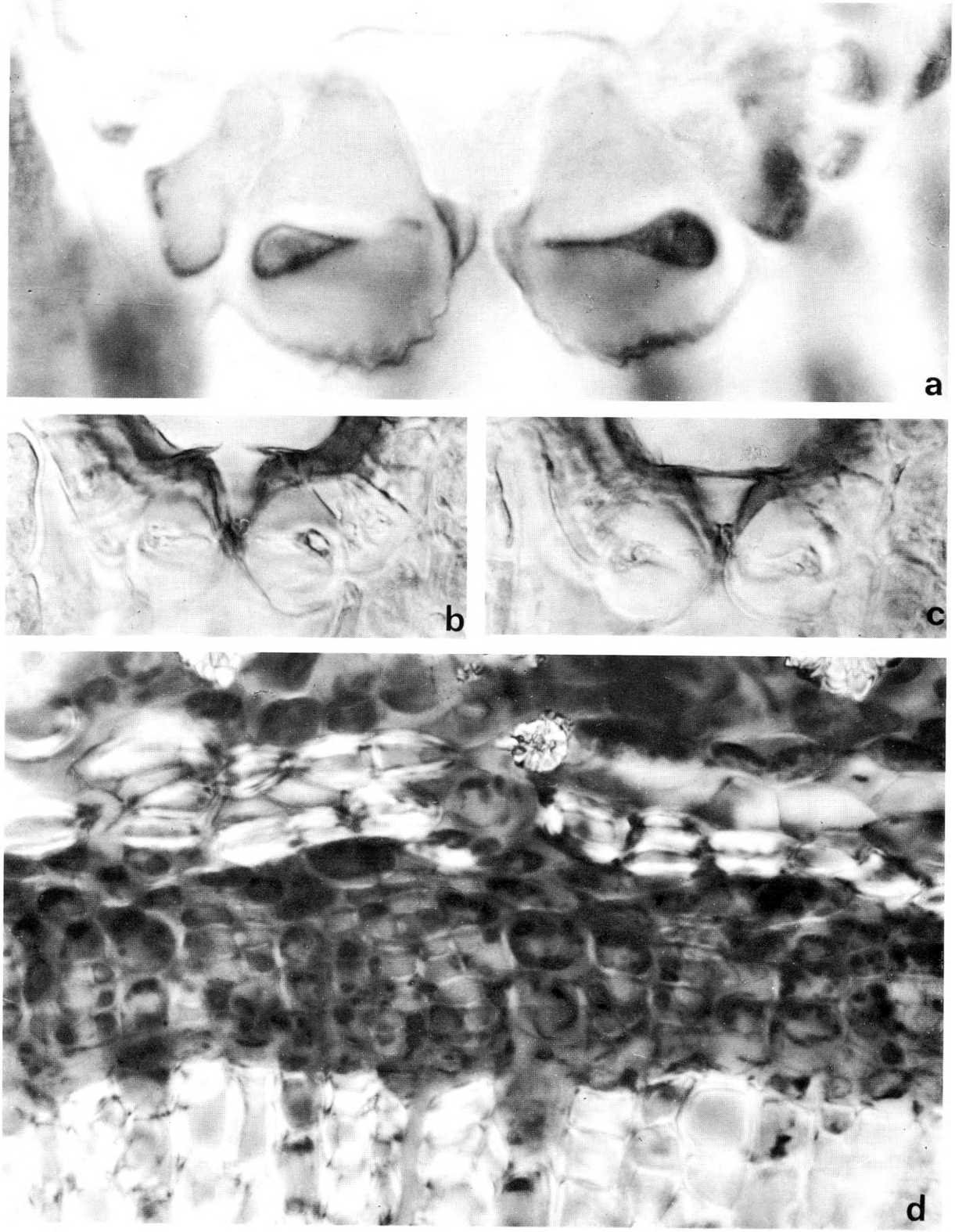

a
b
c
d
e

PLATE XVI *Bredemeyera colletioides*

a–c transverse sections of guard cells. — d. transverse section of extraxylary fibre band, phloem, cambium and xylem. a–c stained with Sudan IV, d as seen in polarized light. a ×1950, b–d ×800.

a. Diaphragm covering large part of front cavity. Walls of central pore heavily cutinized, inside the central pore the surface is folded and covered with a cuticle.
b–c. Stoma at two different focussings showing opening in the diaphragm (b) and heavy cutinization of front cavity.
d. Crystal druses in inner part of cortex, extraxylary fibre band (pericyclic, with passage of living parenchymatous cells in the middle), phloem and poorly developed cambium, xylem with many rays.


a
b
c
d

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