

THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART II. THE MICROSCOPY OF THE BARK OF *Aspidosperma excelsum* BENTH.

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IN a previous communication¹ the macroscopical characters of the bark of *Aspidosperma excelsum* Benth. have been described, and illustrated by means of photographs showing the outer and inner surfaces of a typical specimen. The present communication deals with the detailed histology of this bark as seen in sections, macerations and powder. The material used consisted of three samples of bark previously designated 4A, 4B and 4C collected in British Guiana in 1949, 1950 and 1954 respectively. Line drawings to illustrate the diagnostic characters of outer and inner surfaces of this bark and of tissue distribution as seen in smoothed transverse section are given in Figure 1, A, B and C.

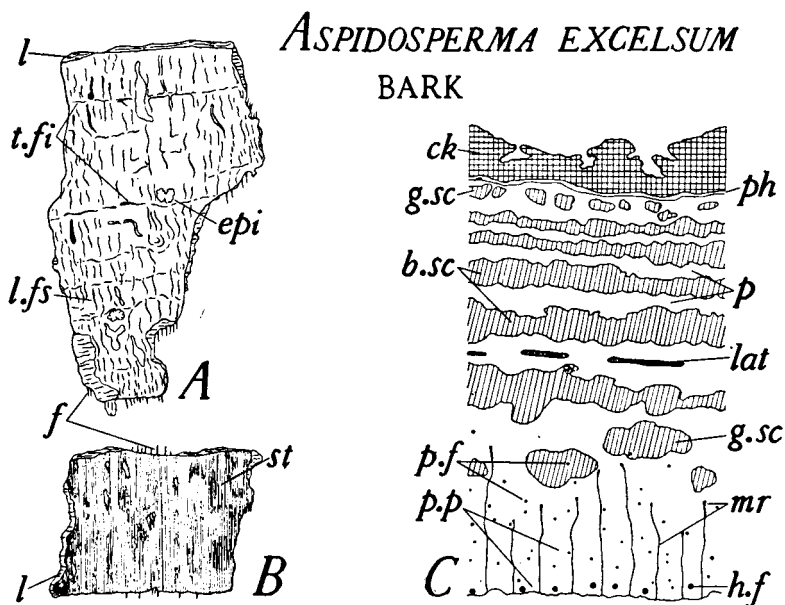


FIG. 1. *Aspidosperma excelsum* bark, macroscopical characters and T.S.:—A, outer surface $\times \frac{1}{2}$; B, inner surface $\times \frac{1}{2}$; C, smoothed T.S. $\times 15$; b.sc, band of sclereids; ck, cork; epi, epiphyte; f, fibre; g.sc, group of sclereids; h.f, phloem fibre with large lumen; l, lamination; lat, latex canal; l.fs, longitudinal furrow; mr, medullary ray; p, cortical parenchyma; p.f, phloem fibre with narrow lumen; ph, phellogen; p.p, phloem parenchyma; st, longitudinal striation; t.fi, transverse fissure.

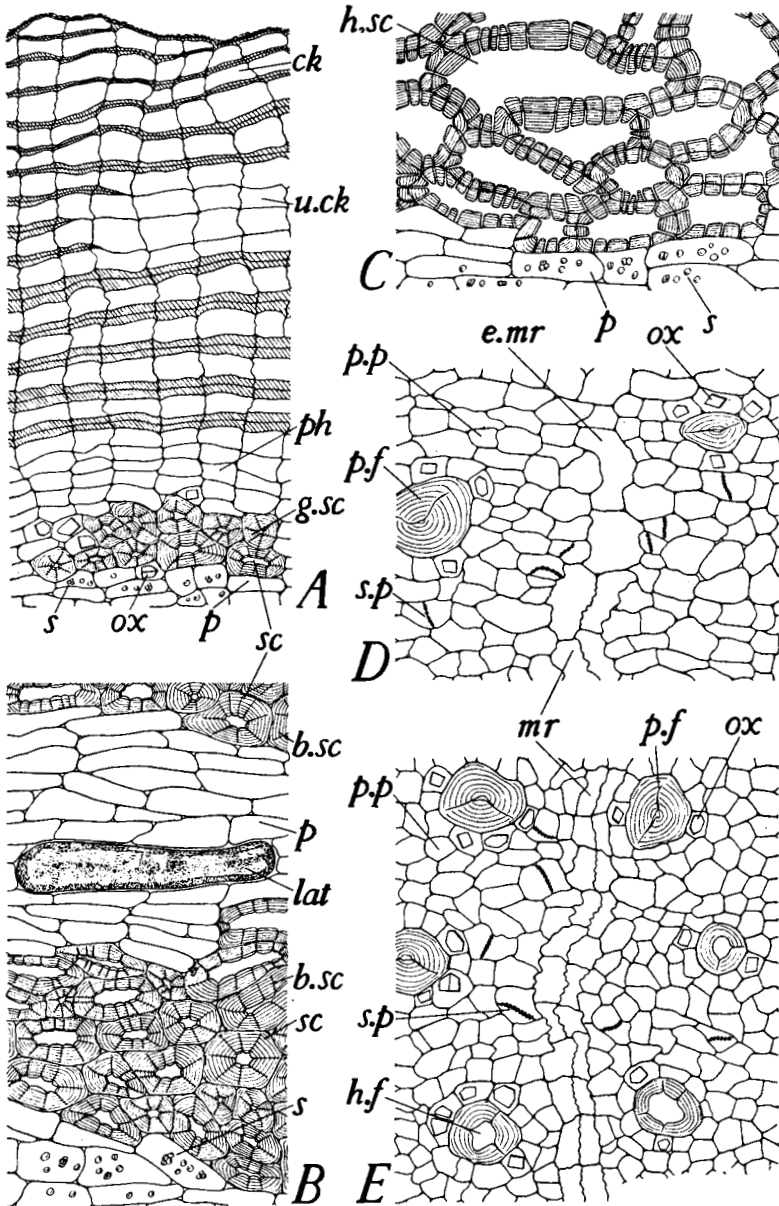


FIG. 2. *Aspidosperma excelsum* bark in T.S.:—A, cork, phellogen and pheloderm; B, cortex; C, inner cortex; D, outer phloem; E, inner phloem; all $\times 200$; *b.sc*, band of sclereids; *ck*, lignified cork with thickened tangential walls; *e.mr*, end of the medullary ray; *g.sc*, group of sclereids; *h.f*, phloem fibre with large lumen; *h.sc*, sclereid with large lumen; *lat*, latex canal; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre with narrow lumen; *p.p*, phloem parenchyma; *s*, starch; *sc*, sclereid with narrow lumen; *s.p*, sieve plate; *u.ck*, unligified or slightly lignified cork.

Detailed Histology of the bark of A. excelsum (Figs. 2 and 3)

Cork consisting of some eight to sixteen layers of tangentially elongated brick-shaped cells of two types, arranged in groups to form a discontinuously stratified tissue, the greater number of cells with inner and outer tangential walls heavily thickened and lignified (Fig. 2, A, *ck*, and Fig. 3, A, *ck*); other cells with thin walls which are unlignified or only slightly lignified (Fig. 2, A, *u.ck*, and Fig. 3, A, *u.ck*); cells polygonal in surface view; R = 12 to 22 to 30 μ , T = 15 to 35 to 55 μ , H = 18 to 24 to 30 μ . Phellogen (Fig. 2, A, *ph*, and Fig. 3, A, *ph*) of one to three layers of thin-walled, tangentially-elongated cells; R = 6 to 10 to 15 μ , T = 22 to 28 to 36 μ , H = 14 to 22 to 28 μ . Phelloderm (Fig. 2, A, and Fig. 3, A), a well-marked tissue of sclerenchymatous cells arranged in small groups (Fig. 2, A, *g.sc*, and Fig. 3, A, *g.sc*), together with slightly tangentially-elongated parenchymatous cells, some of which contain a single prism of calcium oxalate; the sclerenchyma consisting of large isodiametric cells 22 to 60 μ in diameter, with small lumen, well-marked simple or branched pits traversing the thick, stratified and lignified walls. The cortex consists of groups of sclereids arranged in five to seven tangential, more or less continuous bands (Fig. 2, A, *b.sc*, and Fig. 3, A, *b.sc*) and separated by bands of cortical parenchyma; sclereids of the outer bands and parenchymatous cells are similar to those present in the phelloderm. Much tangentially-elongated latex canals, R and H = 36 to 54 to 86 μ , T = 560 μ to 2150 μ (Fig. 2, B, *lat*, and Fig. 3, B, *lat*), occurring in one or more of the bands of cortical parenchyma, which lie between the sclereid bands; latex, which is granular in appearance, is stained yellow with iodine solution. Towards the inner region of the cortex, the bands of sclerenchyma consist of thick-walled sclereids as described above, together with sclereids possessing a very large lumen, very well defined simple pits and thick, stratified and lignified walls, R = 25 to 36 to 54 μ , T = 54 to 85 to 144 μ , H = 18 to 36 to 90 μ (Fig. 2, C, *h.sc*, and Fig. 3, C, *h.sc*). No defined endodermis or pericycle are distinguishable but in this region discontinuous groups of thick-walled sclereids occur, resembling those of the phelloderm but also containing, at times, a small number of axially elongated, thick-walled fibres which are identical with those occurring throughout the phloem and to be described below.

Phloem, which is up to 40 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, phloem fibres and medullary rays. Sieve tubes may be distinguished with oblique, compound sieve plates on the end walls (Fig. 2, D and E, *s.p*, and Fig. 3, D, *s.p*). Phloem parenchyma with few intercellular spaces (Fig. 2, D and E, *p.p*, and Fig. 3, D, *p.p*), of thin-walled cells R = 18 to 22 to 36 μ , T = 25 to 34 to 40 μ , H = 44 to 85 to 134 μ ; having compound pits on the vertical walls and containing starch granules; prisms of calcium oxalate occur singly in a few of these cells (Fig. 3, D, *c.ox*). Phloem fibres, always isolated and surrounded by thin-walled parenchymatous cells each containing a prismatic crystal of calcium oxalate (Fig. 2, D and E, *ox*, and Fig. 3, D, *ox*), of two types; the greater number are scattered throughout the phloem

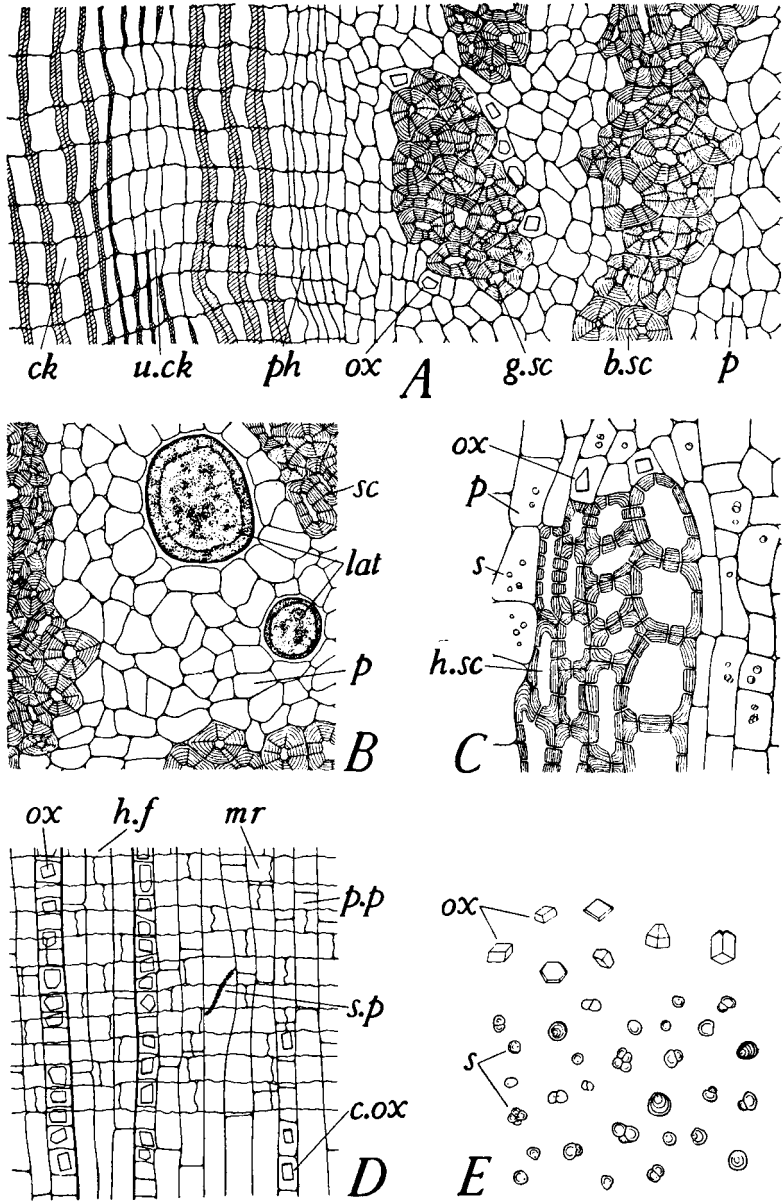


FIG. 3. *Aspidosperma excelsum* bark in L.S. and powder:—A, cork, phelloderm and outer cortex; B, cortex; C, inner cortex; D, phloem; E, calcium oxalate crystals and starch granules, as seen in powder; all $\times 200$; *b.sc*, band of sclereids; *ck*, lignified cork with thickened tangential walls; *c.ox*, phloem parenchyma cells containing prisms of calcium oxalate; *g.sc*, group of sclereids; *h.f*, phloem fibre with large lumen; *h.sc*, sclereid with large lumen; *lat*, latex canal; *mr*, medullary ray; *ox*, crystal of calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.p*, phloem parenchyma; *s*, starch; *sc*, sclereid with narrow lumen; *s.p*, sieve plate; *u.ck*, unligified or slightly lignified cork.

(Fig. 2, D and E, *p.f.*) and are spindle-shaped with bluntly pointed ends, R and T = 36 to 50 to 62 μ , H to 1550 to 2210 to 2800 μ , walls thick, lignified, stratified, traversed by a few simple pits along which splitting may have occurred, lumen very small; a smaller number of fibres occurring towards the innermost region of the phloem are somewhat thicker, R and T = 40 to 60 to 80 μ , the walls are somewhat thinner and the lumen is large, 18 to 30 to 45 μ in diameter; these fibres resemble those described above in all other characters (Fig. 2, E, *h.f.*, and Fig. 3, D, *h.f.*). Medullary ray (Fig. 2, D and E, *mr.*, and Fig. 3, D, *mr.*), straight, two cells wide but becoming one cell wide towards the periphery of the phloem (Fig. 2, D, *e.mr.*), 15 to 25 cells in height; the cells R = 25 to 40 to 55 μ , T = 15 to 25 to 36 μ , H = 12 to 18 to 22 μ , very wavy in outline and containing starch granules.

Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4-compound; individual granules with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter (Figs. 2 and 3, *s.*). Calcium oxalate, as square, rectangular or obliquely rectangular prisms or small cubes of various sizes, measuring up to 25 μ , associated with the groups of sclereids and as a sheath around both types of fibres; there is no relation between the shapes of the crystals and the region of bark in which they occur (Figs. 2 and 3, *ox.*).

Isolation of individual cells by maceration (Fig. 4, A and B)

In order to isolate the latex canal cells, transverse sections of about 100 μ in thickness were cut and the exact position of the latex canal located under a high power of magnification: the two bands of sclereids, in between which the latex canal lies, were then separated with the help of two fine dissection needles, leaving the whole latex canal together with the surrounding band of cortical parenchyma. The parenchyma was removed as far as possible with the help of two fine needles and the remainder dissolved out by the action of 80 per cent. sulphuric acid. The latex canal (Fig. 4, A and B, *lat*) is a long tube without any indication of transverse walls; its contents appear granular and are stained yellow with iodine solution. The phloem fibres, which have been described above (Fig. 4, A and B, *p.f.* and *h.f.*) were isolated by maceration with Schultze's maceration fluid.

Powder. Greyish dark-brown in colour: cork cells polygonal in surface view and reddish-brown in colour, the greater number with thick lignified inner and outer tangential walls and some cells with thin unlignified or slightly lignified walls (Fig. 4, C, *u.ck* and *ck*): sclereids of various shapes and sizes, with thick, stratified, lignified walls; of two types, the greater number with very narrow lumen, having simple or branched pits, others with large lumen and well-defined simple pits (Fig. 4, D, *sc* and *h.sc*): phloem fibres usually somewhat broken, of two types, the greater number with very narrow lumen, fewer cells with large lumen; both types of fibres with thick, stratified lignified walls, traversed by a few simple pits, and surrounded by a sheath of thin-walled parenchymatous cells, each containing a single prismatic crystal of calcium

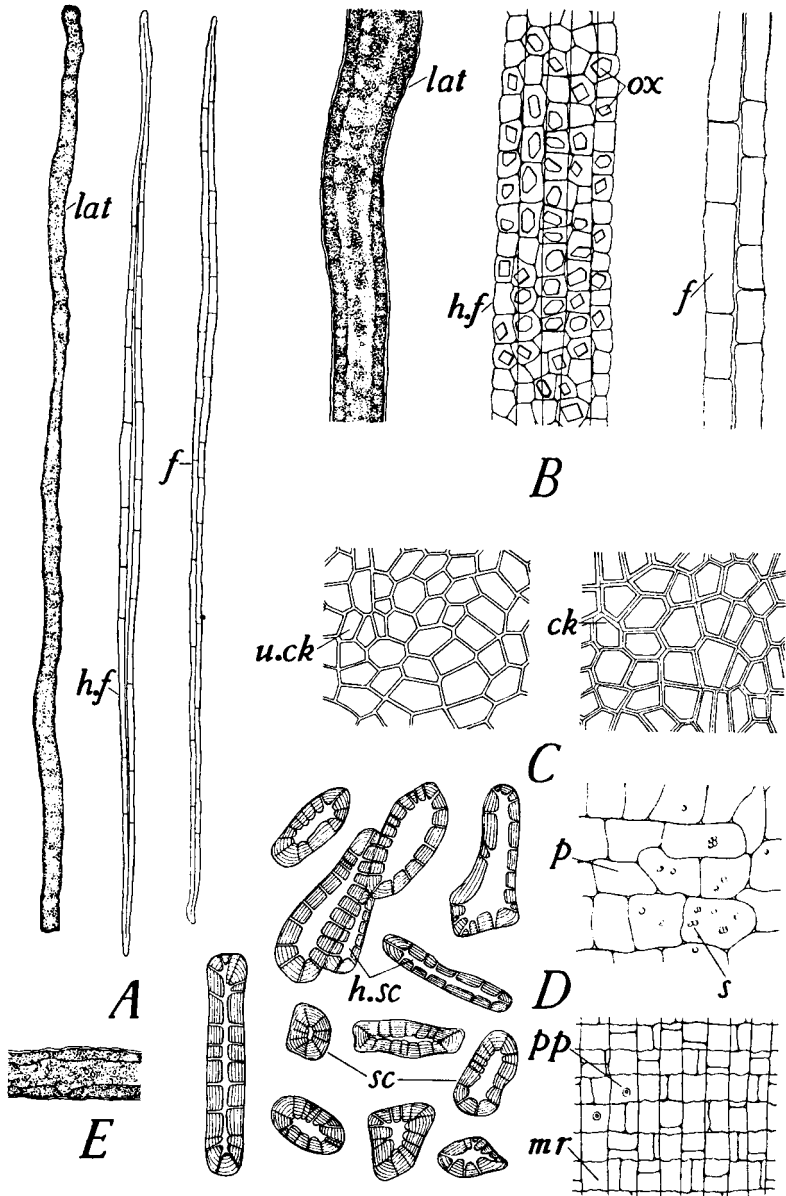


FIG. 4. *Aspidosperma excelsum* bark, macerate and powder:—A, latex canal and fibres, isolated by maceration; B, C, D and E, various components as seen in powder; A $\times 50$, B to E $\times 200$; ck, lignified cork; f, phloem fibre with narrow lumen; h.f, phloem fibre with large lumen; h.sc, sclereid with large lumen; lat and E, latex canal; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; p.p, phloem parenchyma; s, starch; sc, sclereid with narrow lumen; u.ck, unligified or slightly lignified cork.

oxalate (Fig. 4, B, *p.f.* and *h.f.*): phloem parenchyma (Fig. 4, D, *p.p.*), with thin pitted walls, may be associated with cells of the medullary rays (Fig. 4, D, *mr*), the cells of which are very wavy in outline and contain starch granules: cortical parenchyma of thin-walled cells which are tangentially elongated (Fig. 4, D, *p*): starch abundant, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter (Fig. 3, E, *s*): calcium oxalate prisms of various shapes and sizes and up to 25 μ in maximum length (Fig. 3, E, *ox*), as described previously.

DISCUSSION

The diagnostic characters of the bark of *Aspidosperma excelsum* are summarised.

1. Cork cells, reddish-brown, tangentially-elongated, of two types; the greater number of cells with thick, lignified outer and inner tangential walls; the smaller number of cells with thin unlignified or very slightly lignified walls, arranged in small groups amongst the thick-walled cells to form an interruptedly stratified tissue.

2. Phelloderm, mainly of thick-walled sclereids arranged in groups.

3. Latex canals of the cortex, very much tangentially elongated.

4. Sclereids arranged in from 5 to 7 more or less continuous tangential bands in the wide cortex; cells isodiametric or slightly tangentially-elongated, of various shapes and sizes, having thick stratified lignified walls; of two types; the greater number with very narrow lumen and with simple or branched pits, others with large lumen and well defined simple pits.

5. Phloem fibres, with thick stratified lignified walls, traversed by a few simple pits and surrounded by a calcium oxalate crystal sheath; of two types, the greater number, which are scattered throughout the phloem, with very small lumen, others, which are present toward the innermost region of phloem, with very large lumen.

6. Phloem parenchyma, with thin walls and compound pits.

7. Narrow medullary rays, the cells with thin, very wavy walls and containing starch granules.

8. Starch in all parenchymatous tissue, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ in diameter.

9. Calcium oxalate of square, rectangular and obliquely rectangular prisms or as small cubes of various sizes, measuring up to 25 μ in maximum length, associated with fibres, sclereid groups or bands, and in the phloem parenchyma.

The macroscopical and microscopical characters of the bark of *Aspidosperma quebracho-blanco* Schlecht., a drug formerly included in the U.S.P. and B.P.C., and described in the U.S. Dispensatory 1943², have been the subject of papers by Schlechtendal³, Holmes⁴, and Short⁵. In a previous communication, by two of the present authors, on "The Pharmacognosy of the Aspidosperms Barks of British Guiana"¹, the macroscopical and microscopical characters of *Aspidosperma ulei* Mgf.

have been described. These two barks, along with that of *Aspidosperma excelsum* Benth., possess many characters in common. Thus they all occur in thick, curved or channelled pieces, the abundant cork being furrowed and fissured externally and bearing epiphytic lichens, the inner surfaces are longitudinally striated; the odour is indistinct but the taste of each is bitter and aromatic. Histologically, each of the three barks possesses a sclerotic phelloderm and abundant isodiametric sclereids, 20 to 60 μ in diameter with thick walls and small lumen, arranged in masses in the cortex. The phloem contains sieve tubes with compound sieve plates upon the oblique end walls; the medullary rays are narrow; the scattered phloem fibres are mainly of large spindle-shaped cells with thick walls, traversed by simple or compound pits and with small lumen; each fibre is surrounded by a parenchymatous sheath of cells containing prismatic crystals of calcium oxalate. Similar calcium oxalate crystals are associated with the sclereid groups. Starch granules, simple and 4 to 7 to 15 μ in diameter or 2- to 4-compound are found in each of the three barks.

Additional characters common to the barks of both *A. excelsum* and *A. quebracho-blanco* are, the presence of one type of isodiametric sclereid in the phelloderm, arranged in groups (the second type, of tangentially elongated, large sclereids found in *A. ulei*, being absent), and the absence of fibres from the cortex and the presence in the phloem of two types of fibres possessing either narrow or wide lumen. (*A. ulei* bark has two types of fibre, each with narrow lumen and thick walls, the first type, which are few in number, unligified or only slightly lignified and up to 1000 μ in length, are associated with the sclereidal groups of the inner cortex, those of the second type, lignified and up to 5370 μ in length, are scattered throughout the phloem either isolated or in groups of 2).

A character common to the barks of both *A. excelsum* and *A. ulei* is the presence of latex-containing canals in the cortex; these are the more tangentially elongated but of smaller diameter in vertical section in *A. excelsum* and for this reason are readily found in powders of that bark, whilst in the powdered bark of *A. ulei* the latex canals are much broken and are difficult to distinguish. No latex canals occur in the bark of *A. quebracho-blanco*.

The characters, described and illustrated above, which distinguish the bark of *A. excelsum* from those of *A. ulei* and *A. quebracho-blanco* are the reddish-brown colour of the outer surface; the fracture, which is short and laminated in the cortical region due to the arrangement of the cortical sclereids in tangential bands; cork cells not collapsed, of two types, the larger number with thick lignified tangential walls; presence of a second type of somewhat tangentially elongated cortical sclereids with large lumen; also a narrow phloem 40 per cent. of the bark in thickness free from stone cells and with straight medullary rays one to two cells in width; calcium oxalate crystals are the smallest, being up to 25 μ in length. In contrast the bark of *A. ulei* has a dark reddish-brown outer surface, is hard to break; cork cells are much collapsed, of one type only with thin walls; cortical sclereids with small lumen are arranged in

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small groups which are relatively few in number; the phloem is 60 per cent. of the bark in thickness with a few small groups of sclereids in the outer region and with wavy medullary rays one to three cells in width; calcium oxalate up to $35\ \mu$ in length. Also in contrast the bark of *A. quebracho-blanco* is very dark brown in colour with shortly fibrous fracture; cork cells not collapsed, thin-walled; cortical sclereids with small lumen, arranged in large groups which are very numerous; phloem wide, 80 per cent. of the bark in thickness, with abundant groups of sclereids throughout, often traversing the very wavy medullary rays which are up to four cells in width, calcium oxalate up to $30\ \mu$ in length.

SUMMARY

1. The histology of the bark of *Aspidosperma excelsum* has been described and illustrated.
2. The diagnostic characters by which this bark may be identified and distinguished from those of *A. ulei* and *A. quebracho-blanco* are tabulated and discussed.
3. The dimensions of cork, phellogen, sclereids, cortical parenchyma, latex canals, phloem parenchyma, fibres, starch and calcium oxalate crystals are recorded.

REFERENCES

1. Trease and Kulkarni, *J. Pharm. Pharmacol.*, 1955, 7, 463.
2. The Dispensatory of the U.S.A., 1943, 1490.
3. Schlechtendal, *Bot. Zeit.*, 1861, XIX, 137.
4. Holmes, *Pharm. J.*, 1926, 116, 4.
5. Short, *ibid.*, 1926, 116, 103.