THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART I.

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IN 1949 the Forest Department of British Guiana supplied us with authenticated specimens of the barks of their native species of *Aspidosperma*, some of which, particularly *Aspidosperma excelsum*, were locally credited with medicinal properties[1]. The barks of five species were received and workers in this department have now examined them pharmacologically[2,3,4,5,6,7] and have by means of partition chromatograms obtained information regarding their alkaloidal constituents[8]. This work, which is being continued, is of interest in view of the growing importance of *Rauwolfia* species since the *Rauwolfias* and *Aspidospermas* are closely allied alkaloid-containing plants of the family Apocynaceæ. Woodson[9] recognises 52 species of *Aspidosperma*, many of which are large timber-producing trees. If, therefore, the barks or alkaloids from them prove to be of medicinal value, abundant and cheap raw material is available in many parts of tropical America.

The present paper describes the pharmacognostical macroscopical characters of the five barks used by Lewis and Banerjee[2,3,4,5,6,7] and by Palmer[8], together with the detailed histology of one of them. Further histological and quantitative microscopical work on the other four barks is in progress.

MATERIAL

Barks of five different species were obtained from the Conservator of Forests, British Guiana, and the Conservator of Forests, British Honduras. Different collections of each species are denoted by the letters A, B and C as follows:—

Species	Specimens		
Aspidosperma ulei Mgf., formerly known as *A. vargasii* A.DC, a tree 3 to 20 m. high found in British Guiana, Venezuela and Columbia	1 A	Collected British Guiana	1949
	1 B	,, ,,	1950
	1 C	,, ,,	1954
Aspidosperma album Vahl (R. Benoist), formerly known as *A. woodsonianum* Mgf., a tree 10 to 30 m. high found from north-eastern Columbia to the Amazon Valley	2 A	Collected British Guiana	1949
	2 B	,, ,,	1950
	2 C	,, ,,	1954
Aspidosperma megalocarpon Muell. Arg., formerly known as *A. desmanthum* Benth., a tree 7 to 30 m. high found from south-eastern Mexico to British Guiana	3 A	Collected British Guiana	1949
	3 B	,, British Honduras	1953
	3 C	,, British Guiana	1954
Aspidosperma excelsum Benth., a tree 15 to 35 m. high found in British Guiana and Dutch Guiana	4 A	Collected British Guiana	1949
	4 B	,, ,,	1950
	4 C	,, ,,	1954
Aspidosperma oblongum A.DC., a tree up to 35 m. high found in British, Dutch and French Guianas	5 A	Collected British Guiana	1949
	5 B	,, ,,	1950
	5 C	,, ,,	1954

The above barks have been compared with samples 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 1943[10]. This has been the subject of papers by Schlechtendal[11], Holmes[12], and Short[13].

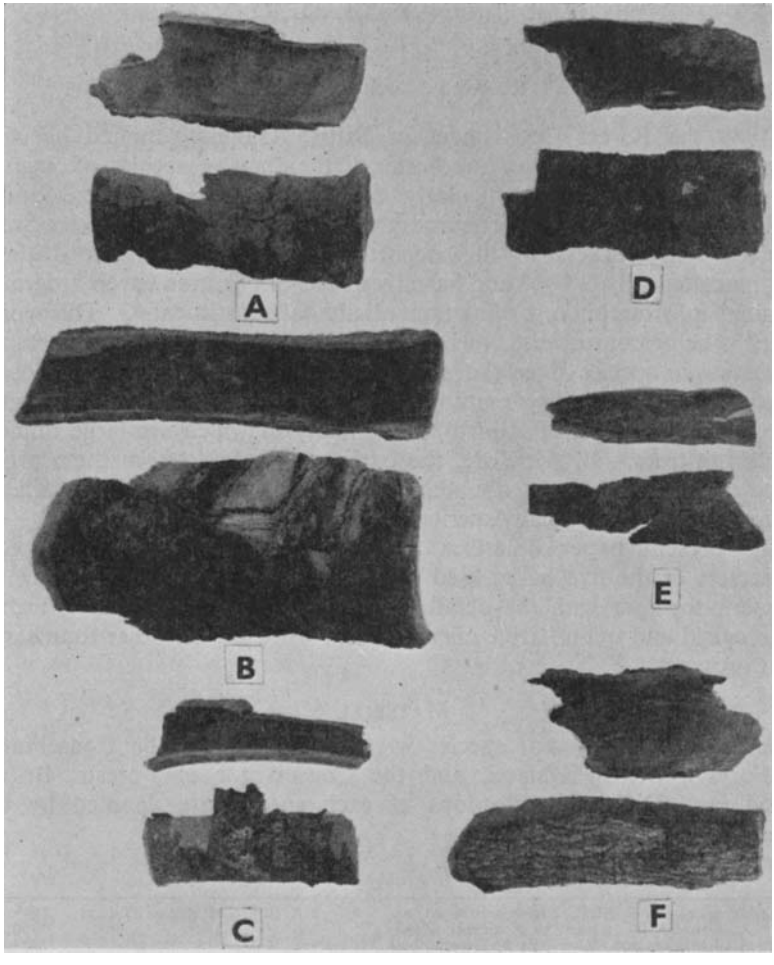

FIG. 1. Photographs of outer and inner surfaces of Aspidosperma barks × $\frac{1}{8}$. A, *A. quebracho-blanco*; B, *A. album*; C, *A. ulei*; D, *A. oblongum*; E, *A. excelsum*; F, *A. megalocarpon*.

The specimen A in Figure 1 was collected by Dr. Martin Cardenas in 1953 at Cochabamba in Bolivia.

EXTERNAL CHARACTERS

Aspidosperma ulei (Fig. 1C). Curved or channelled pieces up to 16 cm. long, 7 cm. wide and 10 mm. thick. Outer surface consisting of scaly cork, which readily exfoliates exposing a nut-hard yellow cortex. The

cork is light brown in colour and shows large transverse lenticels, transverse cracks and patches of greyish-green lichen. The lenticels are well-marked in the rossed bark and are seen to occur in rings at intervals varying from about 5 to 25 mm. Inner surface pale yellow to yellowish-brown, longitudinally ridged and striated. Difficult to fracture; transverse fracture yellowish, except in the cork, and consisting of an outer granular zone and an inner fibrous zone. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma album (Fig. 1B). Specimen 2 B occurs in curved pieces up to 26 cm. long, 11 cm. wide and 10 mm. thick. Outer surface warty, with patches of thin, dark grey cork partly covered with lichen. The surface warts are up to 5 mm. in diameter, corky and easily powdered and from cinnamon-brown to dull red in colour. Transverse rings about 3 to 7 mm. apart of conspicuous lenticels and occasional deep transverse cracks several cm. in length. Inner surface yellowish to grey, uniformly coloured or patchy, surface usually striated longitudinally but in the larger pieces patches of the innermost zone of fibres tend to exfoliate. Fracture, short in the outer part which appears granular, fibrous in the inner part. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma megalocarpon (Fig. 1F). Almost flat pieces having a tendency to curve lengthwise in the direction of the cork; up to 18 cm. long, 8 cm. wide and 8 mm. thick. Outer surface consisting of a grey to reddish-brown outer cork with a patchy or almost complete coat of whitish lichen. Outer cork with longitudinal fissures and transverse cracks, easily rubbed off exposing a thin, brick-red inner cork. Inner surface cinnamon-brown to grey, finely striated longitudinally. Fracture short in the outer part, fibrous in the inner part. Odour, indistinct; taste, bitter. The specimen from British Honduras (3 B) was indistinguishable from those from British Guiana.

Aspidosperma excelsum (Fig. 1E). Curved or channelled pieces up to about 16 cm. long, 4 to 6 cm. wide and 3 to 8 mm. thick. Outer surface finely furrowed, greyish or brownish-black, patches of epiphytes. Inner surface pale yellowish-brown or reddish-brown, finely striated longitudinally. Fracture short. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma oblongum (Fig. 1D). In flat or slightly curved pieces up to 15 cm. long, 7 cm. wide and 3 to 4 mm. thick. Outer surface rough from numerous transverse and longitudinal cracks, greyish or brownish-black, with whitish or greenish patches of lichen. Cork not easily separated. Inner surface pale yellow to almost black, not furrowed but finely striated longitudinally. Fracture short and laminating, the surface showing, even with the naked eye, bands of sclerenchyma and projecting phloem fibres. Odour, indistinct; taste, bitter and aromatic.

Microscopy

Microscopical examination of sections and powders of the British Guiana barks shows that they, together with the bark of *Aspidosperma quebracho-blanco*, possess a number of characters of cell-structures and cell-contents in common, whilst differing in other histological details.

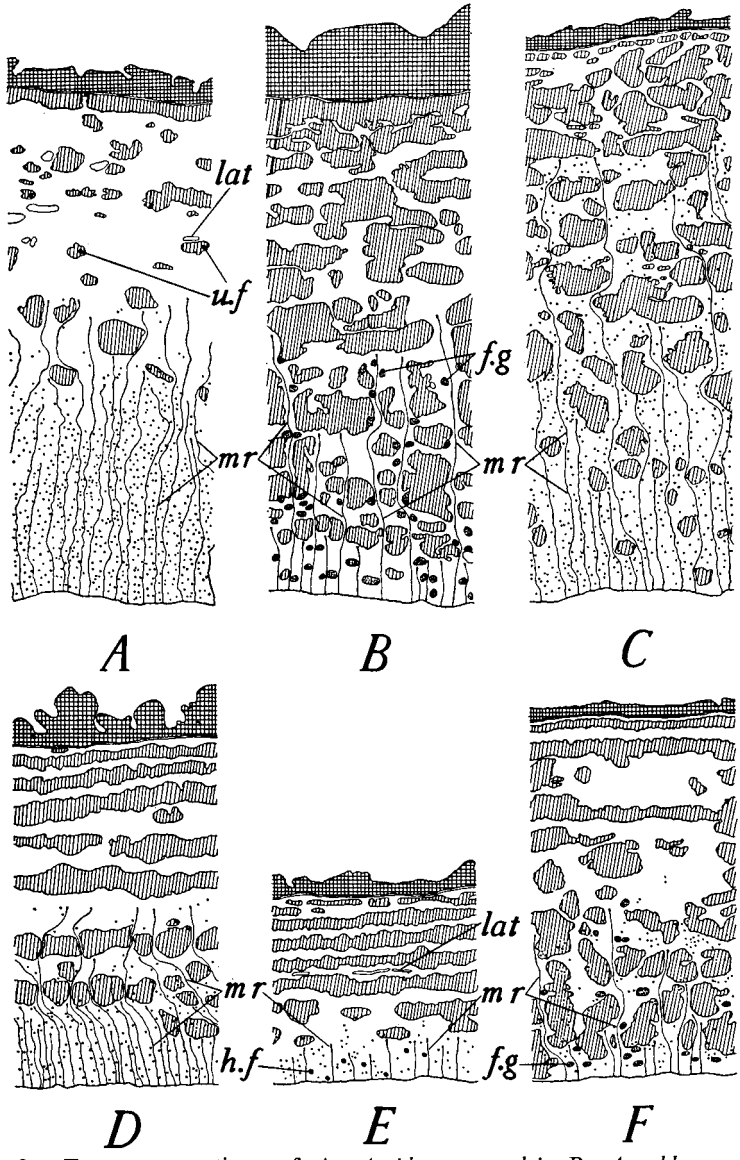

FIG. 2. Transverse sections of A, *Aspidosperma ulei*; B, *A. oblongum*; C, *A. quebracho-blanco*; D, *A. megalocarpon*; E, *A. excelsum*; F, *A. album*. All at × 8, cross-hatched area, cork; diagonally-hatched area, sclereids; *f.g*, fibres in groups; *h.f*, fibre with large cell-cavity; *lat*, latex canal; *mr*, medullary ray; *u.f*, unlignified fibre.

The present paper describes the detailed investigation of one of them, *Aspidosperma ulei.*

Sclerenchyma Distribution in Transverse Sections

All the Aspidosperma barks examined contain sclereids and phloem fibres, but, as will be seen from Figure 2, the sclerenchyma arrangement

varies considerably in different species. In *A. ulei* (Fig. 2A), *A. album* (Fig. 2F), *A. quebracho-blanco* (Fig. 2C) and *A. oblongum* (Fig. 2B) the sclereids are mainly or entirely in isolated groups, whereas in *A. megalocarpon* (Fig. 2D) and *A. excelsum* (Fig. 2E) the sclereids are mainly arranged in bands. Phloem fibres, as shown by black dots in Figure 2, are present as isolated fibres in *A. ulei, A. quebracho-blanco, A. megalocarpon* and *A. excelsum* (Fig. 2, A, C, D and E), whereas in *A. oblongum* and *A. album* (Fig. 2, B and F) the fibres are arranged in groups containing two to several components. These isolated fibres and fibre-groups are sometimes enclosed within the sclereid groups.

Detailed Histology of A. ulei (Figs. 3 and 4)

Cork consisting of numerous layers of very much collapsed reddish-brown and tangentially-elongated cells, which, when expanded by treatment with 80 per cent. sulphuric acid (Fig. 3, A and B, *ck*, and Fig. 4, A, *ck*), are seen to be brick-shaped, walls suberised, non-lignified or only slightly lignified; R = 4 to **7** to 11 μ, T = 54 to **84** to 115 μ, H = 4 to **9** to 18 μ*; polygonal in surface view. Phellogen (Fig. 3, A, *ph*, and B, and Fig. 4, A, *ph*), 1 or 2 layers of thin-walled tangentially-elongated cells; R = 10 to **15** to 20 μ, T = 60 to **85** to 110 μ and H = 7 to **10** to 15 μ. Phelloderm a well-marked tissue within this phellogen (Fig. 3, A and B), of sclerenchymatous cells, together with some parenchyma; the greater amount of sclerenchyma consisting of large isodiametric cells 20 to 60 μ diameter, with small lumen, well-marked simple or branched pits, thick, stratified and lignified walls, arranged as a compact tissue, some 15 layers broad, interspersed with few isodiametric parenchyma cells, some of which contain prismatic calcium oxalate crystals, and extending to the cortex. In places, several layers of tangentially-elongated parenchymatous cells lie between the phellogen and the broad band of sclereids; also in this outer zone of phelloderm there may be one or several layers of tangentially-elongated sclereids (Fig. 3, A and B, and Fig. 4, A, *e.sc*), R = 15 to **20** to 25 μ, T = 62 to **78** to 108 μ, H = 11 to **15** to 18 μ, with small lumen, thick, lignified and stratified walls, traversed by well-marked pits; each of these elongated sclereids is surrounded by thin-walled, small parenchymatous cells, containing solitary prismatic calcium oxalate crystals (Fig. 3, B, and Fig. 4, A, *ox*). Cortex of large, thin-walled, tangentially-elongated parenchymatous cells containing starch (Fig. 3, C, and Fig. 4, B, C and D, *p*); together with large isodiametric or somewhat tangentially-elongated latex canals, the contents of which appear to be granular and are stained yellow with iodine solution. R = 85 to **125** to 180 μ, T = 240 to **500** to 1680 μ and H = 100 to **145** to 210 μ (Fig. 3, C, and Fig. 4, C, *lat*). Scattered throughout the cortex are large groups of isodiametric sclereids similar in structure to those found in the phelloderm, surrounded by

* R, T and H indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their *Botanical Pen Portraits* (1923). The symbol H indicates height, the measurement in the longitudinal direction, which has been used instead of L, as used by these workers.

thin-walled isodiametric parenchymatous cells each containing a prismatic calcium oxalate crystal. Towards the inner region of the cortex some of the groups of sclereids may be associated with single unlignified or only slightly lignified large fibres (Fig. 3, C, *uf*), with very small lumen, thick striated walls, traversed by a few simple pits along which splitting

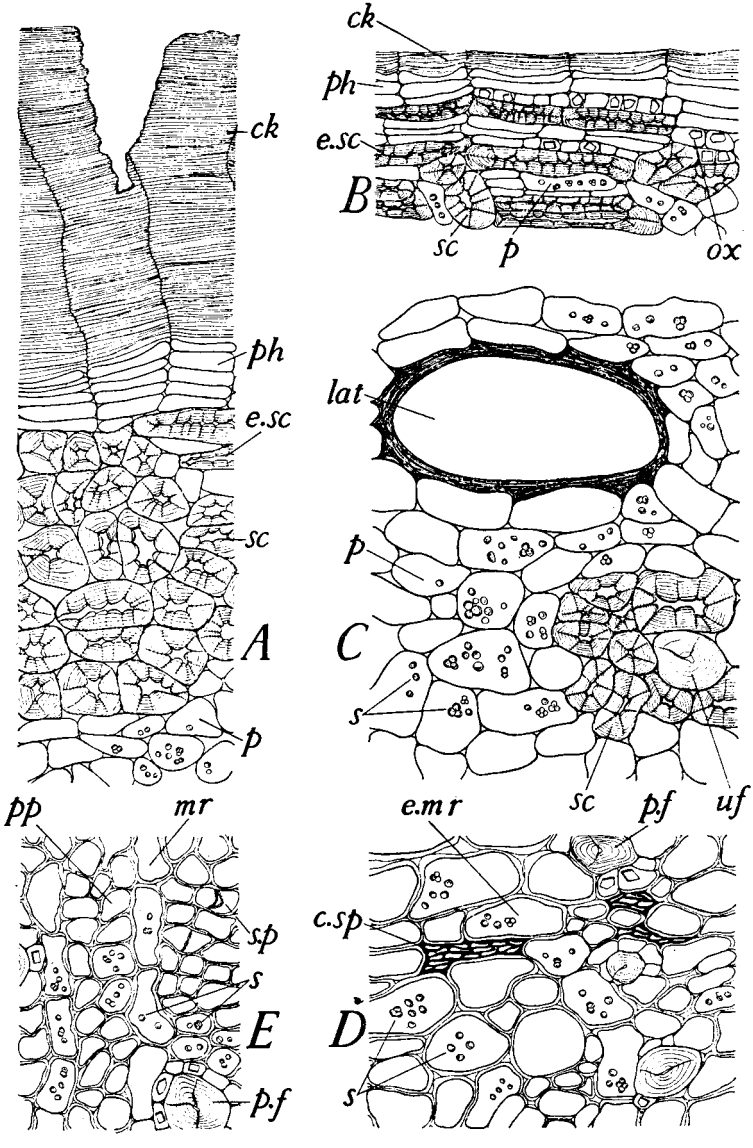

FIG. 3. T.S. *Aspidosperma ulei* × 200. *ck*, cork; *c.sp*, collapsed sieve tubes; *e.mr*, end of the medullary ray; *e.sc*, elongated sclereid; *lat*, latex canal; *mr*, medullary ray; *ox*, calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre lignified; *pp*, phloem parenchyma; *s*, starch; *sc*, sclereid; *s.p*, sieve plate; *uf*, unlignified fibre.

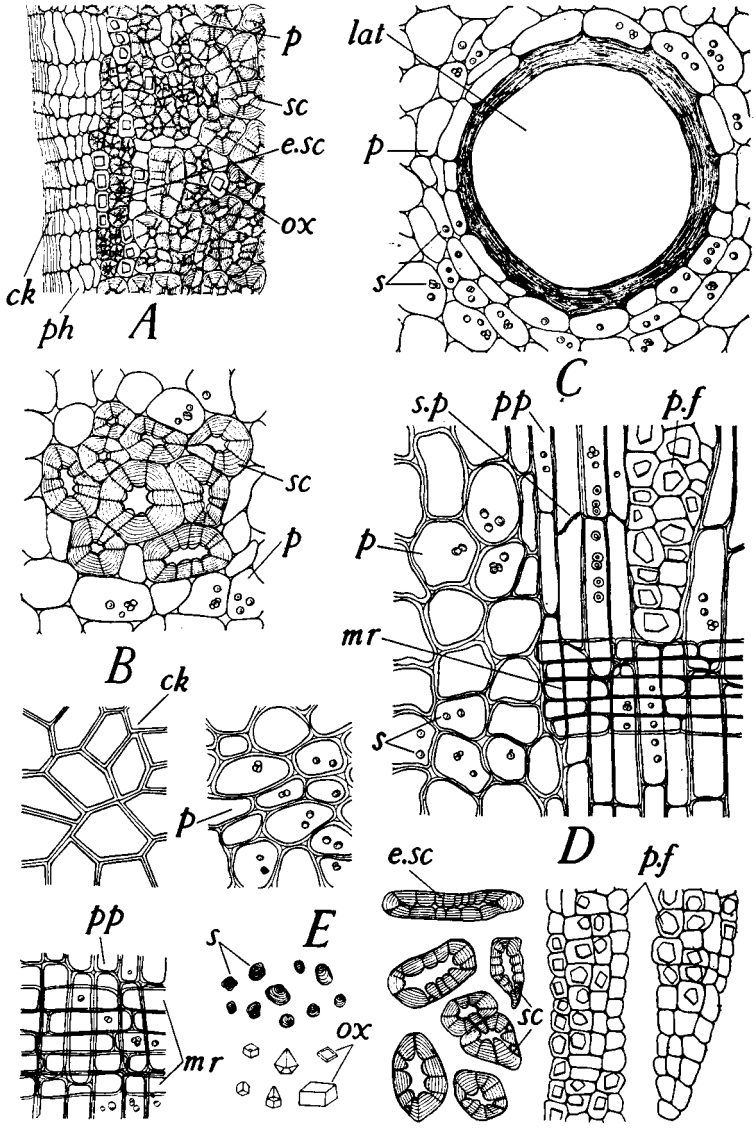

FIG. 4. L.S. and powder of *Aspidosperma ulei* × 200. *ck*, cork; *e.sc*, elongated sclereid; *lat*, latex canal; *mr*, medullary ray; *ox*, calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre; *pp*, phloem parenchyma; *s*, starch; *sc*, sclereid; *s.p*, sieve plate.

may have occurred, 35 to **56** to 70 μ diameter, and up to 1000 μ long, with bluntly pointed ends; calcium oxalate prism sheath absent. Phloem, which is up to 60 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, sclereid groups, phloem fibres and medullary rays. Sieve tissue is very much collapsed (Fig. 3, D, *c.sp*), except in

the innermost region of phloem, where sieve tubes may be distinguished with oblique, compound sieve plates (Fig. 3, E, *s.p*, and Fig. 4, D, *s.p*). Phloem parenchyma (Fig. 3, E, and Fig. 4, D, *pp*) is thin-walled, with compound pits on the vertical walls, its cells contain starch; sclereid groups large, chiefly in the outer part of the phloem, of isodiametric cells similar to those found in the cortex, and surrounded by thin-walled isodiametric parenchymatous cells containing single prismatic calcium oxalate crystals; phloem fibres (Fig. 3, D and E, and Fig. 4, D, *p.f*) numerous, mostly isolated or very rarely in groups of 2, lignified, with very small lumen, thick striated walls, traversed by a few simple pits along which splitting may have occurred, 55 to **65** to 80 μ diameter, 2500 to **3650** to 5370 μ long, with bluntly pointed ends, similar to the lignified fibres found in the cortex, except that the phloem fibres are surrounded by thin-walled parenchymatous cells, each containing a prismatic calcium oxalate crystal (Fig. 3, D and E, *ox*, and Fig. 4, D, *ox*). Medullary rays (Fig. 3, E, and Fig. 4, D, *mr*), 1 to 2 cells wide and 5 to 7 cells in depth, very wavy and much displaced by the groups of sclereids through which the rays do not pass. The cells are R = 30 to **48** to 65 μ, T = 15 to **22** to 32 μ, H = 36 to **52** to 65 μ, and contain starch grains. Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4-compound; individual grains, with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ. Calcium oxalate as square, rectangular or obliquely rectangular prisms or as small cubes of various sizes, measuring up to 35 μ, are found associated with sclereid groups and around fibres in different zones of the bark; there is no relationship between crystal shapes and the region of bark in which they occur (Fig. 3, B, D and E; Fig. 4, A, *ox*).

Powder. Yellowish in colour; cork cells in surface view polygonal and reddish-brown in colour; sclereids in more or less intact groups with crystal sheath, or separated (Fig. 4, E, *e.sc* and *sc*); individual sclereids, which are isodiametric or elongated, have heavily lignified walls with simple or branching pits; portions of phloem fibres (Fig. 4, E, *p.f*) and occasional portion of unlignified fibre from the cortex as described previously; phloem parenchyma (Fig. 4, E, *pp*), with thin pitted walls may be associated with cells of medullary rays (Fig. 4, E, *mr*); cortical parenchyma of thin-walled cells which are tangentially-elongated (Fig. 4, E, *p*); starch abundant, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ (Fig. 4, E, *s*); calcium oxalate prisms of various shapes and sizes up to 32 μ in maximum length (Fig. 4, E, *ox*), as described previously.

Microscopical Measurements of Five Aspidosperma Barks

A quantitative microscopical method for distinguishing between powdered barks of closely allied species such as these Aspidospermas, which is based on the ratio between their stone cells and fibres, will be the subject of a further communication. The following figures, each based on about 250 measurements, made from macerations of the bark and from powders, are recorded as an indication that such measurements

alone are insufficient to distinguish between the many different barks from this genus.

MEASUREMENTS IN μ

	Sample	Fibres		Sclereids		Cork cells	Starch
		Length	Width	Length	Width	Tangential	Grains
A. ulei	1 A	2530–5370	65–80	40–140	25–60	12–25	4–7–15
	1 C	2500–5340	55–75	35–130	30–60	12–25	4–7–15
A. album	2 A	835–2585	25–40	35–125	25–75	25–50	4–9–18
	2 C	795–2500	25–45	30–125	25–65	25–45	4–9–20
A. megalocarpon ..	3 A	695–1615	30–40	25–100	20–60	25–45	4–11–18
	3 C	630–1600	25–40	25–115	15–60	20–40	4–11–18
A. excelsum ..	4 A	1530–2780	35–75	20–70	15–35	15–35	4–7–15
	4 C	1500–2725	35–75	25–80	15–40	20–40	4–7–15
A. oblongum ..	5 A	1295–2640	35–60	40–110	20–55	25–60	4–10–15
	5 C	1335–2670	30–55	30–115	25–55	25–55	4–10–15

DISCUSSION

The 52 species of Aspidosperma have been classified by Woodson[9] and by Pichon[14]. Although we and our colleagues at Nottingham have examined only a few of these species, our findings, based on entirely different characters, support the accepted botanical groupings.

In *A. ulei* (Woodson's Series III):

1. The stone cells are in groups.
2. The alkaloids antagonise the pressor response to adrenaline in spinal cats[5].
3. The bark contains at least 9 alkaloids, no one of which predominates in amount[8]. This is also true of 2 other species belonging to this series, *A. tomentosum*[15] and *A. australe*[16].

In *A. excelsum* and *A. oblongum* (Woodson's Series VI):

1. Stone cells mainly in bands.
2. The barks resemble one another pharmacologically but differ from the barks of the other species examined. For example, the alkaloids have marked hypotensive and sympathetic properties. Some of them are indole derivatives[2–5].
3. *A. excelsum* bark has not yet been fully investigated but its alkaloids resemble those of *A. oblongum*[6]. Chromatograms from *A. oblongum* show 3 alkaloids; the major one being yohimbine[8].

In *A. album* and *A. megalocarpon* (Woodson's Series IX):

1. Stone cells mainly in bands, and in groups in the inner region of cortex and phloem; dimensions of cells and starch grains similar.
2. The barks resemble one another pharmacologically but differ from the barks of the other species examined[2–5].
3. The barks of both species contain 2 major alkaloids and other minor ones. The total alkaloids found are similar in amount, being 0·71 and 0·74 per cent. respectively[8].

The diagnostic characters of *A. ulei* are as follows:—

1. Presence of very much collapsed unlignified cork, consisting of reddish-brown and tangentially-elongated cells.

2. Phelloderm mainly consisting of sclerotic tissue.
3. Presence, in the outer zone of phelloderm, of one or several layers of tangentially-elongated sclereids.
4. Large isodiametric or somewhat tangentially-elongated latex canals.
5. Large groups of sclereids of isodiametric cells are present in cortex and phloem which are surrounded by thin-walled parenchymatous cells, some of which contain a single prism of calcium oxalate.
6. Unlignified or slightly lignified large single fibres associated with the groups of sclereids in the inner region of cortex.
7. Lignified large fibres, usually single, sometimes in groups of 2, may be associated with the groups of sclereids, but mainly without, surrounded by calcium oxalate crystal sheath, are present throughout the phloem region.
8. Starch is present in cortical parenchyma, phloem parenchyma and also in the cells of medullary ray.

Summary

1. The macroscopical characters of 5 *Aspidosperma* barks from British Guiana are described.
2. The diagnostic histological characters of the bark of *Aspidosperma ulei* have been illustrated and described.
3. The dimensions of cork cells, stone cells and fibres of 5 *Aspidosperma* barks are recorded.
4. Differences in sclerenchyma arrangement are recorded and illustrated. These appear to be correlated with the botanical classification of the *Aspidospermas* and with certain observations made on the pharmacology and alkaloidal constituents of these barks.

We desire to thank Dr. J. M. Rowson and Dr. T. E. Wallis for their interest and assistance in this work; the Conservators of Forests in British Guiana and British Honduras for the supply of material, and our Nottingham colleagues for keeping us informed on the progress of their pharmacological and chemical work on these barks.

References

1. *Br. Guiana Forest Bull.*, No. 2 (n.s.), 1948, 39.
2. Banerjee and Lewis, *Nature, Lond.*, 1953, **171**, 802.
3. Banerjee and Lewis, *J. Pharm. Pharmacol.*, 1954, **6**, 246.
4. Banerjee and Lewis, *ibid.*, 1954, **6**, 466.
5. Banerjee, Lewis and Sharp, *ibid.*, 1954, **6**, 660.
6. Banerjee, Ph.D. Thesis, Nottingham, 1954.
7. Banerjee and Lewis, *J. Pharm. Pharmacol.*, 1955, **7**, 42.
8. Palmer, M.Pharm. Thesis, Nottingham, 1954.
9. Woodson, Jr., *Ann. Missouri Bot. Gard.*, 1951, **38**, 119.
10. *The Dispensatory of the U.S.A.*, 1943, p. 1490.
11. Schlechtendal, *Bot. Zeit.*, 1861, **XIX**, 137.
12. Holmes, *Pharmaceutical Journal and Pharmacist*, 1926, **62**, 4.
13. Short, *ibid.*, 1926, **62**, 103.
14. Pichon, *Bull. Mus. Nat. d'Hist. Nat.*, 1947, ser. 2, **19**, 362.
15. Floriani, *Rev. centro. estud. farm. bioquim*, 1935, **25**.
16. Orazi, *Ann. assc. quim. argentina*, 1946, **34**, 158.