THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART III. THE MICROSCOPY OF THE BARK OF *Aspidosperma album* VAHL.

BY J. D. KULKARNI, J. M. Rowson and G. E. TREASE

From the Department of Pharmacy, University of Nottingham, and the Museum of the Pharmaceutical Society of Great Britain

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IN a previous communication[1] the macroscopical characters of the bark of *Aspidosperma album* Vahl. 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 2A, 2B and 2C 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.

Detailed Histology of the Bark of A. album (Figs. 1, 2, 3 and 4)

Cork consisting of some fifteen to thirty layers of rectangular to somewhat tangentially elongated, thin-walled, unlignified or very slightly lignified cells, polygonal in surface view (Fig. 1, D, ck, and Fig. 3, A, ck); R = 8 to **16** to 24 μ, T and H = 12 to **18** to 24 μ. Phellogen (Fig. 1, D, ph, and Fig. 3, A, ph) of one to two layers of thin-walled, rectangular to somewhat tangentially elongated cells; R = 8 to **14** to 20 μ, and T and H = 12 to **18** to 24 μ. Phelloderm (Fig. 1, D, phe, and Fig. 3, A, phe), a well-marked tissue of sclerenchymatous cells, R = 24 to **32** to 40 μ, T = 24 to **35** to 44 μ, H = 16 to **28** to 40 μ; arranged in a more or less continuous band of some four to eight layers of rectangular or slightly isodiametric cells, with small lumen, well-marked simple or branched pits traversing the thick, stratified and lignified walls; together with slightly tangentially-elongated thin-walled parenchymatous cells, R = 27 to **32** to 35 μ, T = 27 to **34** to 40 μ, H = 20 to **28** to 40 μ, some of which contain a single prism of calcium oxalate (Fig. 1, D, pa, and Fig. 3, A, pa). The cortex consists of thin-walled parenchyma together with abundant sclereids, these latter are arranged in a continuous band in the outer cortex (Fig. 1, E, b.sc, and Fig. 3, B, b.sc), and occur as small groups in the inner cortex (Fig. 2, A, g.sc); individual sclereids, R = 40 to **55** to 70 μ, T = 70 to **100** to 145 μ, H = 46 to **66** to 86 μ, isodiametric or tangentially elongated, of various shapes and sizes, with very narrow lumen, or occasionally with somewhat larger lumen, well-marked simple or branched pits traverse the thick, stratified and lignified walls; cortical parenchyma with large intercellular spaces, individual cells R = 32 to **50** to 70 μ, T = 32 to **55** to 70 μ and H = 32 to **44** to 55 μ, thin-walled, very much

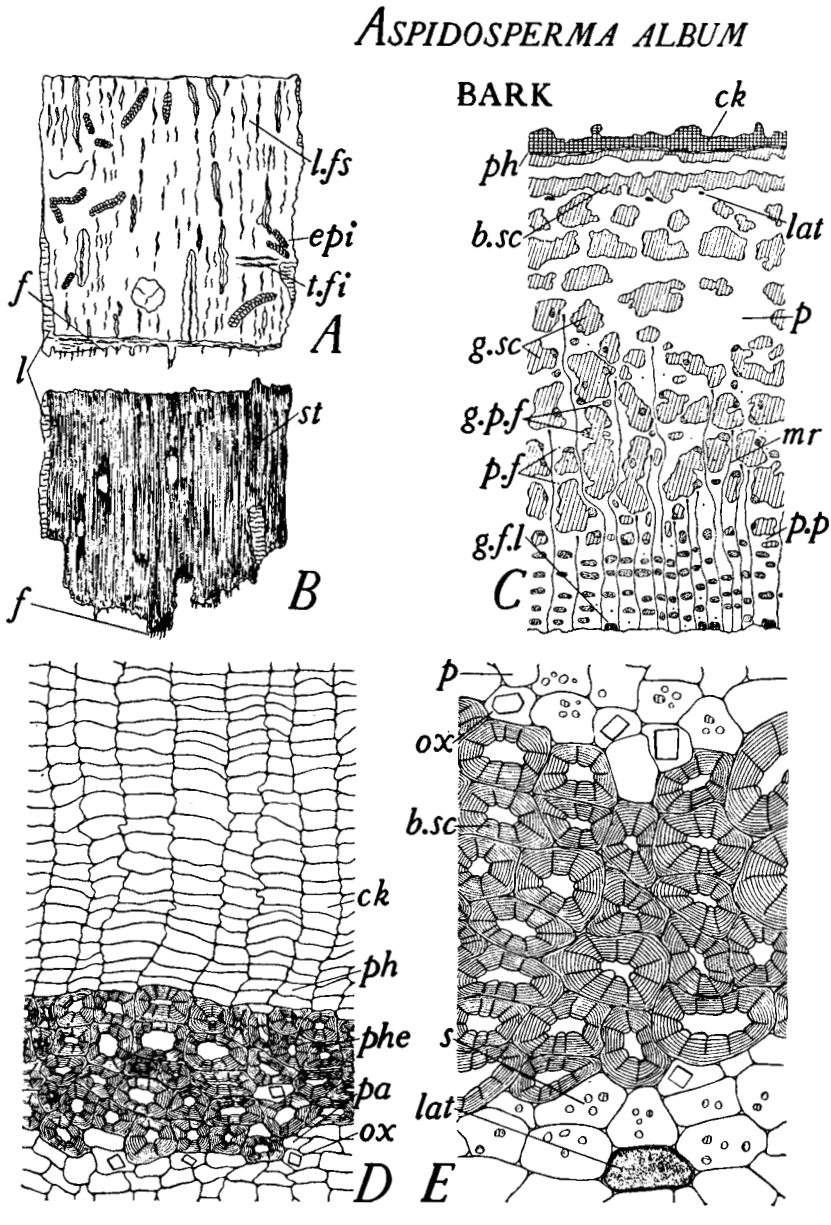

Fig. 1. *Aspidosperma album* bark, macroscopical characters and T.S.:—A, outer surface x ¼; B, inner surface x ¼; C, smoothed T.S. x 10; D, cork, phellogen and phelloderm; E, cortex; D and E, x 200; b.sc, band of sclereids; ck, cork; epi, epiphyte; f, fibre; g.f.l, group of phloem fibres with large lumen; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; l, lamination; lat, latex canal; l.fs, longitudinal furrow; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; pa, cortical parenchyma found associated with phellodermic sclereids; ph, phellogen; phe, phellodermic sclereids; p.f., isolated phloem fibre with narrow lumen; p.p, phloem parenchyma; s, starch; st, longitudinal striation; t.fi, transverse fissure.

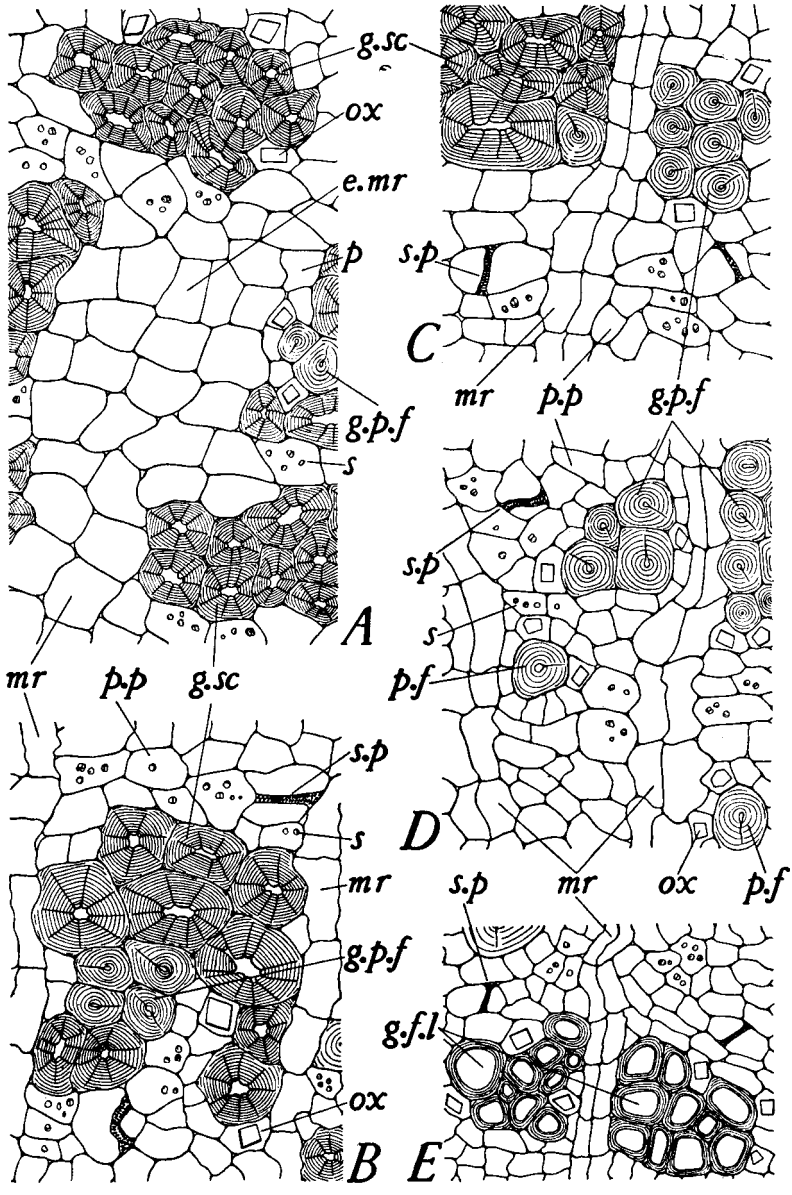

Fig. 2. *Aspidosperma album* bark in T.S.:—A, inner cortex; B, outer phloem; C, phloem; D, inner phloem; E, innermost phloem; all x 200; e.mr, end of medullary ray; g.f.l, group of phloem fibres with large lumen; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; p.f, isolated phloem fibre with narrow lumen; p.p, phloem parenchyma; s, starch; s.p, sieve plate.

tangentially elongated and containing starch granules (Fig. 1, E, p, Fig. 2, A, p, and Fig. 3, A, B and C, p). Much longitudinally-elongated latex canals, R and T = 32 to **44** to 55 μ, H = 98 to 300 μ, lying parallel to the vertical axis or very slightly obliquely thereto, are found associated with the inner boundary of the cortical band of sclereids and also in the cortical parenchyma (Fig. 1, E, lat, and Fig. 3, C, lat); the latex, which is very finely granular in appearance, is stained yellow with iodine solution. No defined endodermis or pericycle are distinguishable, but in this region discontinuous groups of thick-walled sclereids occur, resembling those of the cortex, but also containing, at times, axially elongated, thick-walled fibres, either singly or, more frequently, in groups of two to six fibres, which are identical with those occurring throughout the phloem and to be described below.

Phloem, which is up to 70 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, phloem fibres, medullary rays and sclereids, and can be subdivided into four regions. In the outermost region groups of stone cells are present, some being associated with groups of fibres; in the second region, the groups of fibres are more or less surrounded by the sclereid groups; the third region is characterised by the presence of fibres either isolated or in groups and by the absence of sclereids; in the innermost region of phloem, fibres, both isolated and in groups, are found together with a few groups of a second type of fibre with very large lumen. Sieve tubes may be distinguished with oblique, compound sieve plates on the end walls (Fig. 2, B, C, D and E, s.p, and Fig. 4, A, s.p). Phloem parenchyma with large intercellular spaces, of thin-walled cells, having compound pits on the vertical walls and containing starch granules, R = 16 to **24** to 32 μ, T = 32 to **42** to 55 μ, and H = 48 to **70** to 90 μ (Figs. 2, 3 and 4, p.p). Phloem fibres, R and T = 25 to **32** to 40 μ, H = 800 to **1750** to 2600 μ, of two types; the greater number, either isolated (Fig. 2, p.f) or in groups (Figs. 2, 3 and 4, g.p.f) scattered throughout the phloem, are spindle shaped, with bluntly pointed ends, walls thick, lignified, stratified, traversed by a few simple pits, along which splitting may have occurred, lumen very narrow; crystal sheath surrounding the isolated phloem fibres and groups of fibres, except when these are embedded within groups of sclereids (Figs. 2, 3 and 4). A smaller number of fibres, occurring in groups towards the innermost region of the phloem, differ from those described above in the presence of walls which are somewhat thinner, the large lumen being 8 to **20** to 32 μ in diameter, crystal sheath surrounding some groups of these fibres but not always present. Medullary rays (Fig. 2, A, B, C, D and E, mr, and Fig. 3, E, mr) very wavy, due to displacement by groups of sclereids and fibres, one to two cells in width but becoming up to five cells wide (Fig. 2, A, e.mr) towards the periphery of the phloem, 15 to 20 cells in height, the cells, R = 40 to **62** to 86 μ, T = 20 to **25** to 32 μ, and H = 16 to **28** to 35 μ, straight to somewhat wavy in outline and containing starch granules.

Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4- compound; individual granules with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter (Figs. 1, 2, 3 and 4, s).

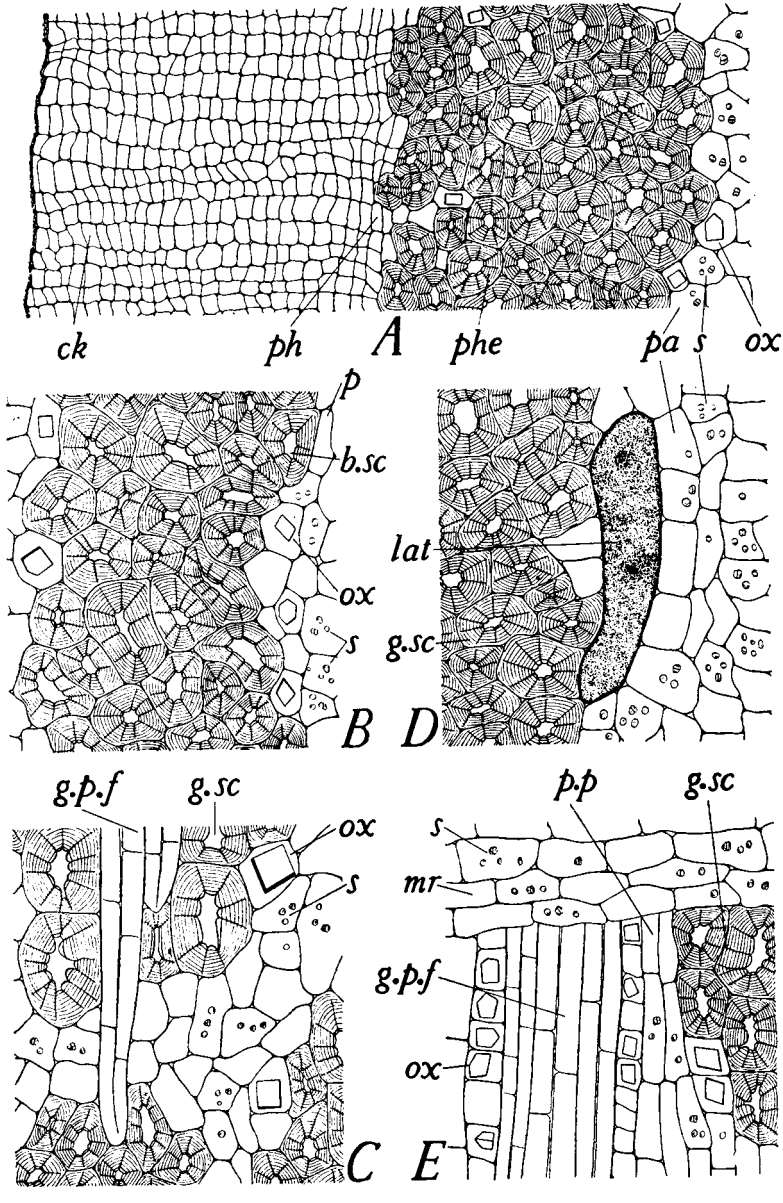

Fig. 3. *Aspidosperma album* bark in L.S.:—A, cork, phellogen, phelloderm and outer cortex; B, outer cortex; C, outer phloem; D, cortex; E, inner phloem; all x 200; b.sc, band of sclereids; ck, cork; g.p.f, group of phloem fibres with narrow lumen; g.sc, group of sclereids; lat, latex canal; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; pa, cortical parenchyma found associated with phellodermic sclereids; ph, phellogen; phe, phellodermic sclereids; p.p, phloem parenchyma; s, starch.

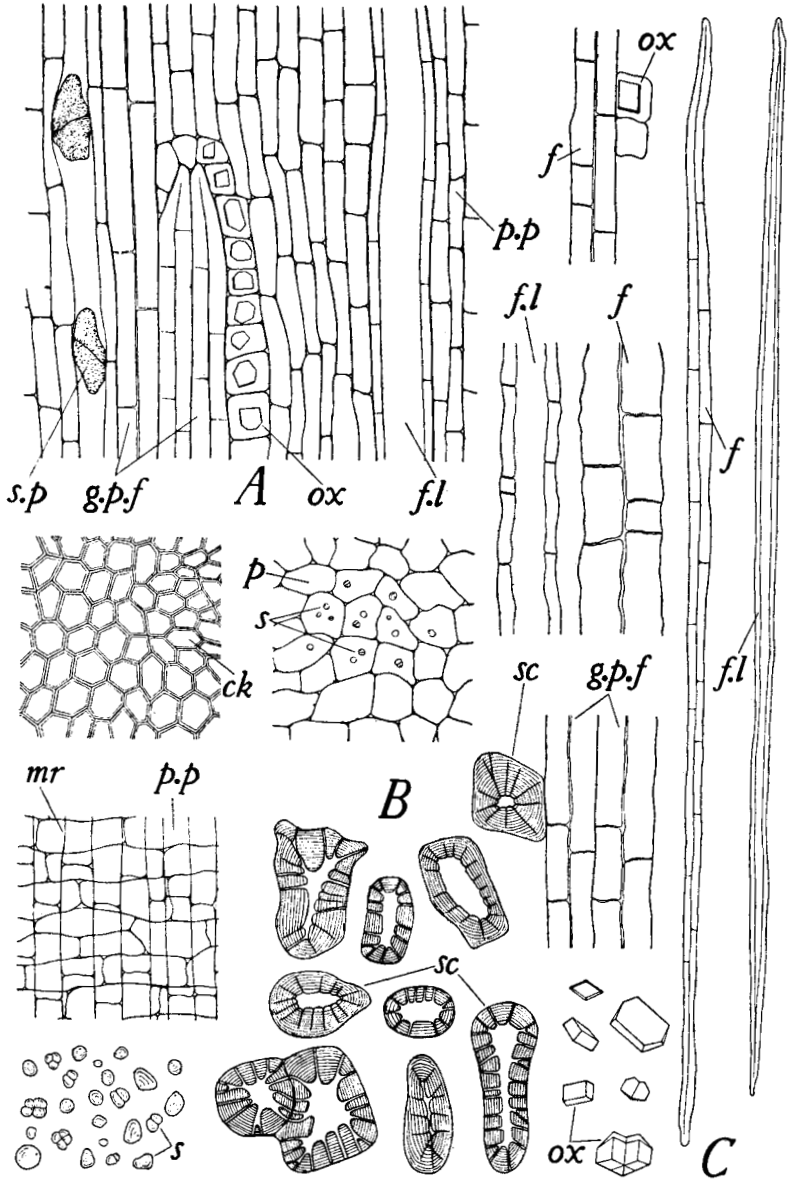

Fig. 4. *Aspidosperma album* bark in L.S., powder and macerate:—A, innermost phloem x 200; B, various components as seen in powder x 200; C, fibres, isolated by maceration x 50; ck, cork; f, phloem fibres with narrow lumen; f.l, phloem fibre with large lumen; g.p.f, group of phloem fibres with narrow lumen; mr, medullary ray; ox, crystal of calcium oxalate; p, cortical parenchyma; p.p, phloem parenchyma; s, starch; sc, sclereid; s.p, sieve plate.

Calcium oxalate, as square, rectangular or obliquely rectangular prisms or small cubes of various sizes, measuring up to 32 μ, associated with the groups of sclereids and as a sheath around both types of fibres; there is no relationship between the shapes of the crystals and the region of the bark in which they occur (Figs. 1, 2, 3 and 4, ox).

Powder. Fawn in colour: cork cells polygonal in surface view and slightly reddish-brown in colour (Fig. 4, B, ck): sclereids of various shapes and sizes, with thick, stratified walls, either with narrow lumen or with a large lumen, having simple or branched pits (Fig. 4, B, sc): phloem fibres usually somewhat broken, isolated or in groups of two to three fibres, of two types, the greater number with very narrow lumen and frequently surrounded by a sheath of thin-walled parenchymatous cells, each containing a single prismatic crystal of calcium oxalate, a number of fibres are associated with sclereids, fewer fibres with large lumen either with or without prism-crystal sheath; both types of fibres with thick, stratified lignified walls, traversed by a few simple pits (Fig. 4, B, f, f.l and g.p.f): phloem parenchyma (Fig. 4, B, p.p) with thin walls, may be associated with cells of the medullary rays (Fig. 4, B, mr), the cells of which are somewhat wavy in outline and contain starch granules: cortical parenchyma of thin-walled, tangentially elongated cells containing starch granules (Fig. 4, B, p): starch abundant, simple or 2- to 4- compound, individual grains with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter (Fig. 4, B, s): calcium oxalate prisms of various shapes and sizes and up to 32 μ in maximum length (Fig. 4, B, ox), as described previously.

Discussion

The diagnostic characters of the bark of *Aspidosperma album* are:—

1. Cork cells, pale reddish-brown and rectangular to somewhat tangentially elongated, thin-walled, unlignified or very slightly lignified.

2. Phelloderm mainly sclerotic, of an irregular band of sclereids three to eight layers in radial thickness.

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

4. Sclereids arranged in one, more or less continuous tangential band in the outer cortex, and in groups in the inner cortex and outer phloem, having thick, stratified walls, with simple or branched pits and with narrow or large lumen.

5. Phloem fibres, isolated or in groups of two to fifteen fibres, with thick stratified lignified walls traversed by a few simple pits and with or without surrounding crystal sheath; of two types, the greater number, which are scattered throughout the phloem, with very small lumen, others, which are present towards the innermost region of phloem, always in groups, with very large lumen.

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

7. Medullary rays, narrow, wavy, the cells with thin, somewhat wavy walls and containing starch granules.

8. Starch in all parenchymatous tissue, simple or 2- to 4- compound,

individual grains with eccentric hilum, spherical, ovoid or plano-convex and up to 28 μ in diameter.

9. Calcium oxalate as square, rectangular and obliquely rectangular prisms or as small cubes of various sizes, measuring up to 32 μ in maximum length, associated with fibres, sclereid groups or bands, and cortical and phloem parenchyma.

In previous communications[1,2], the macroscopical and microscopical characters of the barks of *Aspidosperma ulei* Mgf. and *Aspidosperma excelsum* Benth. have been described and compared with those of the bark of *Aspidosperma quebracho-blanco* Schlecht. The bark of *Aspidosperma album* Vahl. possesses many characters in common with these three barks. Thus it occurs in thick, curved or channelled pieces, the abundant cork being furrowed and fissured externally and bearing epiphytic lichens or liverworts, the inner surface is longitudinally striated; the odour is indistinct but the taste is bitter and aromatic. Histologically, each of the four 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 or 2- to 4- compound and up to 28 μ in diameter, are found in each of the four barks.

A character common to the three barks *A. album*, *A. excelsum* and *A. ulei* is the presence of latex-containing canals in the cortex; these are much longitudinally elongated, running slightly obliquely in *A. album*, more tangentially elongated in *A. excelsum*, and slightly tangentially elongated or somewhat isodiametric in *A. ulei*, these latex canals can only be seen in the powders of the bark of *A. excelsum*. No latex canals occur in the bark of *A. quebracho-blanco*.

The barks of *A. album* and *A. quebracho-blanco* agree in the presence of one type of cork cells which are non-lignified, thin walled and are not collapsed; also in the presence of abundant groups of sclereids in all parts of the phloem except the innermost region. These two barks and that of *A. excelsum* are free from fibres in the cortex but possess phloem fibres of two distinct types; *A. album* differs however in that the narrow-cavitied fibres are usually arranged in groups whilst those with wide cavities always occur in groups. The bark of *A. album* may also be distinguished by the isodiametric sclereids of the phelloderm arranged in a continuous band and by the intermingling of two types of cortical sclereids in one continuous band and in scattered groups.

Summary

1. The histology of the bark of *Aspidosperma album* has been described and illustrated.

2. The diagnostic characters by which this bark may be identified and distinguished from those of *A. ulei*, *A. excelsum* 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. Kulkarni, Rowson and Trease, *ibid.*, 1955, 7, . 905

DISCUSSION

The papers were presented together by J. D. KULKARNI.

MR. G. R. A. SHORT (London) congratulated Mr. Kulkarni on his excellent drawings. He heard today that these barks had active principles with a rauwolfia action. Dr. Rowson, commenting recently on a paper that he (Mr. Short) had written some years ago on aspidosperma, had told him that he and the other authors today agreed with what he had then said. The paper was written in 1926, and was the outcome of a little controversy with the late Mr. E. M. Holmes on the nomenclature of *Aspidosperma quebracho-blanco*. Mr. Holmes had a sample sent to him from Central America which had a rose-pink inner bark. He considered that this was not *Aspidosperma quebracho-blanco*, because it was not white, but *A. quebracho-colorado*; but this was not correct, because that name was given to a bark in another family. He had endeavoured to explain this in his paper, but felt sure that he had not convinced Mr. Holmes. He wondered whether any further species were to be examined. He understood that there were 65 species of aspidosperma. Could the authors give any explanation of the curious colours of the inner bark of *A. quebracho-blanco*? Those he had seen varied from cream through rose and red, and some samples were clove brown. Was this clove brown due to tannin?

DR. F. FISH (Glasgow) dealing with the first paper commented on the variation in size of the latex canals illustrated. He thought it rare for a drug to contain isolated fibres with a prismatic crystal sheath and if this was unique, as he thought, it was worth special mention as a diagnostic character. The phloem and medullary ray parenchyma contained starch granules, although this was not shown in the paper. Nor was there any indication of the striations on the starch granules. The maximum size of starch granules from the three species thus far examined was quoted as 15 mμ. In the second paper, with four species, the whole lot were grouped with a maximum of 28 mμ. If there was such a difference between the *A. album* and the other three barks, it might have been used as a distinguishing feature. He also asked whether it was necessary to submit the two papers separately. It might have been better, as had been done in the delivery that morning, to put them together, because there was some repetition in the summary of the second.

MR. KULKARNI, in reply, said that the colours of the barks varied considerably. They had examined Mr. Short's sample of 1926 and found it to be exactly the same as those which they had received in 1949, 1950 and 1954. There were 52 species in this genus, and they had worked on only six of them. *A. quebracho-blanco* was well known; *A. ulei* had been described in Part I; *A. excelsum* and *A. album* had been described in the present papers, and *A. megalocarpon* and *A. oblongum* were being investigated. The sample collected by Mr. Holmes was in the Society's museum, and he had examined it. After cutting a few transverse sections, he found that it was a different bark, because it had striated cork and did not compare at all with either Mr. Short's sample or their own recent samples collected from British Guiana.

DR. ROWSON, also replying to the discussion, said that at present they could not offer any explanation of the wide variation in colour of the inner bark of *A. quebracho-blanco*. He agreed with Dr. Fish that they should have described the starch striations and he accepted his criticism of the references to the size of the granules. He would add one comment on the drawings. It would be realised that the latex canals did not run perfectly vertically in one specimen or perfectly horizontally in the other. Some had been isolated and true transverse sections shown.