THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA*

PART IV. QUANTITATIVE NUMERICAL STUDIES OF THE LIGNIFIED ELEMENTS IN CASCARA AND IN *Aspidosperma* SPECIES

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BARKS derived from different species of the same genus often resemble one another very closely in the microscopical structure of their elements and cell contents. This has been shown in the previous communications for the barks derived from the genus *Aspidosperma*[1-3], where the detailed histology of *A. ulei*, *A. excelsum* and *A. album* has been described and illustrated. We have examined the detailed histology of the barks of *A. megalocarpon*, *A. oblongum* and *A. quebracho-blanco* and hope to describe them in subsequent communications. In these six co-generic species there are differences in tissue arrangements which disappear on powdering and other means were sought for distinguishing certain of these barks when in the powder form.

In recent years, numerical microscopical studies have been developed to ensure a more complete control of crude drugs, especially when in powder form. Numerical values determined microscopically can be subdivided into the two main classes of ratios, based upon two sets of numbers, and absolute values, based upon counts or upon measurements of area or of length. Compared with the numerous quantitative methods which have been applied to roots, stems, leaves, fruits and seeds in recent years, the number of investigations on barks is small.

Many barks contain sclerenchymatous tissue of stone cells and fibres, usually with heavily thickened walls which are frequently lignified; the striking appearance and the strong reaction for lignin of these cells rendering them easily identifiable. The possibility that these two types of cell-elements might occur in a fixed ratio one to the other in a given species was considered. Should this be proved to be so, it would be useful for identification purposes, and especially so if the ratio values differed between different species. The barks of *Aspidosperma* species contain both sclereids and fibres and were regarded as a suitable group in which to investigate this ratio.

In order to test these premises as stringently as possible within one species, some preliminary work was carried out upon the barks of *Rhammus purshiana*, since a wide range of samples was easily available differing greatly in age and size: also there were no intermediate forms between the sclereids and fibres in this species.

* The subject matter of this communication forms part of a thesis by one of us (J.D.K.) accepted by the University of Nottingham for the degree of Doctor of Philosophy in Pharmacy.

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Cascara Bark

To investigate the ratio between the sclereids and fibres present in cascara bark one may equate the number of each element per mg. of a powder; alternatively, the numbers of each element seen in a given transverse section of the bark may be counted and equated. Since each element has an approximately definite volume, it would also follow that the areas occupied by the sclereids and fibre groups as seen in transverse sections may also be equated. This last method is simple to apply and, if it yields a constant ratio for different samples of cascara bark, it should indicate that the ratio of numbers of sclereids and fibres per mg. is also a constant.

Transverse sections of cascara bark from different samples were cut, cleared by boiling in chloral hydrate solution, stained with phloroglucinol and hydrochloric acid and mounted in glycerine. The lignified areas were then traced on paper by means of an Abbé-type camera lucida. The areas of sclereids and of fibres were determined on the paper by cutting out the areas and weighing them separately. The results of three sets of experiments were tabulated in Tables I, II and III.

It is seen from these results that, although the per cent. area lignified varies between wide limits, the ratio of area of sclereids to area of fibres, as seen in transverse sections, is a constant in samples of cascara bark of different geographical origins, ages or thicknesses and between pieces of bark obtained from different aerial parts of the plant. Hence such a ratio may have diagnostic significance.

Aspidosperma Barks

Attempts were next made to apply this simple method to *Aspidosperma* barks, but the experimental difficulties made this impossible, largely because it was virtually impossible to get entire sections. It was therefore decided to find out if a constant ratio exists between the number of sclereids and the number of fibres present in the powdered barks of *Aspidosperma*.

The barks in No. 90 powder were used for all the quantitative work and microscopical examination showed that they contained sclereids both isolated and in groups. Only the powders of the barks of *A. album* and *A. oblongum* contain groups of fibres as well as the isolated fibres, the other four barks, namely *A. excelsum*, *A. megalocarpon*, *A. ulei* and *A. quebracho-blanco*, contain isolated fibres only. The methods available for counting the numbers of sclereids occurring in these masses were investigated.

Calculation of the number of cells in the groups of sclereids in powders

The individual groups of sclereids in No. 90 powder of the barks are usually of two kinds, one consisting of more or less isodiametric stone cells and the other of somewhat elongated stone cells of varying lengths; both types of cells were counted without distinction. By using the method of calculation devised by Wallis and Santra for pimento[4] and for olive stones[5], the number of stone cells in the masses was computed by counting the cells along two directions at right angles to one another and using

TABLE I

S/F RATIO (AREAS) IN CASCARA BARKS OF DIFFERENT THICKNESSES

Pieces	Thickness in mm.	Percentage area lignified	Percentage area of sclereids (S)	Percentage area of fibres (F)	Ratio S/F
1	1·81	0·93	0·52	0·41	1·25
2	1·67	1·33	0·73	0·60	1·21
3	1·95	1·87	1·04	0·84	1·25
4	2·09	1·85	1·04	0·81	1·27
5	2·34	4·78	2·77	2·01	1·31
6	2·27	3·91	2·14	1·76	1·24
7	1·39	7·85	4·35	3·49	1·24
8	1·95	6·82	3·78	3·04	1·24

Mean = 1·25

TABLE II

S/F RATIO (AREAS) IN CASCARA BARKS FROM DIFFERENT SAMPLES (MUSEUM OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN)

Sample	Thickness in mm.	Percentage area lignified	Percentage area of sclereids	Percentage area of fibres	Ratio S/F
1. North America, 1895	2·64 2·43	1·47 1·09	0·80 0·60	0·67 0·49	1·21 1·24
2. Wright, Layman and Umney, 1923	3·89 3·61	4·95 3·42	2·76 1·88	2·19 1·54	1·25 1·25
3. Imported U.S.A., 1930	3·48	1·75	1·02	0·72	1·27
4. H. O. Meek, 1938	1·81 1·95	0·70 1·26	0·40 0·69	0·30 0·57	1·25 1·25
5. H. O. Meek, 1937	5·56	4·03	0·26	1·77	1·29
6. H. O. Meek, 1933	6·39	2·38	1·26	1·13	1·17
7. Imported, 1930	3·41	4·58	2·53	2·04	1·24
8. Mossed, origin unknown	3·89	3·33	1·85	1·48	1·25
9. N. America, no date	2·50	4·06	2·25	1·80	1·25
10. Barts Hospital Museum, 1931 ..	2·92	5·86	3·29	2·57	1·28

Mean = 1·25

TABLE III

S/F RATIO (AREAS) IN CASCARA BARKS. SAMPLES OF DIFFERENT THICKNESSES AND FROM DIFFERENT POSITIONS ON THE SAME PLANT, COLLECTED FROM A CASCARA TREE GROWING AT BIRDSGROVE HOUSE, MAYFIELD, DERBYSHIRE

No.	Height from the ground	Thickness of stem mm.	Thickness of bark mm.	Percentage area lignified	Percentage area of sclereids (S)	Percentage area of fibres (F)	Ratio S/F	Mean S/F
1	7′	5	1·15	Not sufficiently lignified			—	—
2	6′	9	1·25	1·06 1·32	0·65 0·79	0·41 0·53	1·57 1·50	1·53
3	4·5′	16	2·78	1·97 1·98	1·10 1·09	0·87 0·89	1·26 1·22	1·24
4	4·5′	16	2·78	1·52 1·13	0·84 0·63	0·68 0·50	1·24 1·28	1·26
5	6″	31	3·40	3·70 3·93	2·07 2·19	1·63 1·75	1·27 1·25	1·26
6	2·5″	36	4·17	3·57 3·14	1·98 1·75	1·59 1·40	1·25 1·25	1·25

Samples 3 and 4 were taken at the same level on the same branch, No. 3 being from the outer side and No. 4 on the side nearest the trunk.

half their average as the radius of an imaginary equivalent sphere. Twenty-five typical masses were picked up on the point of a needle from a preparation stained with phloroglucinol and hydrochloric acid and each particle was placed on a slide with a drop of glycerine and a coverslip was applied. Counts were then made of cells along the two directions and the number of cells in each particle was calculated from the formula $4/3\pi r^3$. Then each of these particles was disintegrated by adding a drop of nitric acid 50 per cent. and the actual number of cells was counted after a brief maceration. The results are recorded in Table IV.

TABLE IV

NUMBER OF SCLEREIDS IN PARTICLES SELECTED FROM A NO. 90 POWDER OF THE BARK OF *A. album*

Number of cells along two directions		Total number of cells in each particle	
Longitudinally	Transversely	By calculation	By disintegration
4	3	22·5	24
5	3	33·5	32
5	5	65·5	68
3	3	14·0	16
5	3	33·5	34
6	6	113·0	95
4	4	35·5	30
7	5	113·0	125
4	4	35·5	48
5	4	48·0	31
4	3	22·5	24
6	4	65·5	59
5	4	48·0	44
4	3	22·5	24
5	4	48·0	51
4	4	35·5	33
5	4	48·0	40
5	3	33·5	44
8	3	87·0	78
4	3	22·5	21
4	4	33·5	37
4	3	22·5	19
5	3	33·5	36
5	4	48·0	39
4	4	33·5	33
Total number of cells in 25 particles		1112	1085

Close agreement was noticed in 16 of the experiments between the actual and calculated figures (522 and 526), and this indicated that if a sufficiently large number of particles was examined the errors cancelled out; totals for 25 particles being 1112 and 1085 for the calculation and disintegration methods respectively. These differences are within the error of experiments.

Determination of the number of stone cells and length of fibres per mg. of the powder

A weighed quantity of the powder and of lycopodium was gently triturated for three minutes in a glass mortar with sufficient nitric acid (50 per cent.) to make a thin paste. This trituration was just long enough to disintegrate all the fibres and most of the sclereid groups. Further reaction of the nitric acid was then stopped by the addition of 70 per cent. ethanol. The whole material was then transferred to

a centrifuge tube and the mortar was washed twice with small quantities of 70 per cent. ethanol and the washings transferred to the same centrifuge tube. After centrifuging for ten minutes, the supernatant liquid was carefully drained and 5 ml. of 1 per cent. solution of phloroglucinol in 90 per cent. ethanol was added to the material in the tube, it was shaken well, centrifuged and decanted. A few drops of strong hydrochloric acid were than added whereby the lignified tissues (sclereids and fibres) were stained red. The final volume of the suspension in the tube was made up to 6 ml. by addition of a suspending agent (2 vols. of glycerine, 2 vols. of water and 1 vol. of Mucilage of Tragacanth B.P.). The tube was then oscillated gently to ensure an even distribution. Four slides were prepared. The free sclereids were counted directly and the number of sclereids remaining in groups were calculated as described above. The total sclereids were thus determined in seven strips across the cover-glass as described by Wallis[6]. Further, the lengths of fibres, which are always broken, seen in the same seven strips across the cover-glass were marked on paper with the help of an Abbé-type camera lucida at a magnification of $\times$ 800 and thus the total length of fibres determined.

The methods of experiments and calculations of Wallis[6] were employed. Detailed counts and measurements obtained for three different samples of *A. album* bark are presented in Table V. Similar experiments were then carried on with the powders of various samples of all the other species of *Aspidosperma* and the summarised results are tabulated in Table VI.

The average mean length of fibre in the Aspidosperma *barks*

In order to calculate the number of fibres present in 1 mg. of powdered bark from the length of fibre per mg., which has already been determined, it is necessary to know the average mean length of fibre in each of these six species.

Ten small pieces of bark from each species were macerated in Schultzes maceration fluid for about half an hour. The pieces of bark were then teased out with the help of two dissecting needles to isolate the fibres. At least twenty of these complete fibres were then measured at random with the help of a calibrated eye-piece micrometer. Two samples of each species were used and thus about 400 to 500 microscopical measurements of fibres were obtained for each species. The statistical mean of the fibre lengths was found out by plotting a graph on the "arithmetical probability graph paper", of cumulative frequency as per cent. of total against the mean fibre length of each group. The results of these statistical calculations are tabulated in Table VII.

To determine the number of fibres per mg. of the powdered barks of Aspidosperma

By dividing the mean values of the lengths of fibres per mg. (Table VI) by the mean lengths of the fibres (Table VII), the mean number of fibres per mg. in the powdered barks have been derived. The results are tabulated in Table VIII.

TABLE V

COUNTS OF THE NUMBERS OF SCLEREIDS AND MEASUREMENTS OF TOTAL LENGTHS OF FIBRES (BROKEN PIECES) PER mg. POWDERED BARK OF *A. album*

Sample	Suspension	Number of sclereids per mg.	Fibre length per mg. in mm.
1949	I	8058, 8778, 8292 Average: 8376	118·25, 131·18, 125·16 Average: 124·86
	II	9148, 9109, 9175 Average: 9144	123·72, 125·20, 117·87 Average: 122·26
	III	8574, 8902, 9306 Average: 8927	120·46, 127·13, 124·53 Average: 124·04
1953	I	9246, 8492, 8921 Average: 8886	115·78, 121·44, 125·94 Average: 121·05
	II	8713, 8345, 9146 Average: 8734	116·48, 123·45, 128·16 Average: 122·36
1954	I	9140, 8986, 9456 Average: 9194	132·45, 129·28, 114·99 Average: 125·57
	II	8188, 8491, 9255 Average: 8645	130·04, 118·60, 114·78 Average: 121·14
Grand average		8844	123·04 mm.

TABLE VI

AVERAGE NUMBERS OF SCLEREIDS AND AVERAGE TOTAL LENGTHS OF FIBRES PER MG. IN THE POWDERS OF *Aspidosperma* BARKS

Species	Average number of sclereids per mg.	Average total length of fibres per mg. in mm.
A. album	8844	123·04
A. excelsum	7059	16·08
A. megalocarpon	11705	47·19
A. oblongum	11228	26·50
A. ulei	5749	65·59
A. quebracho-blanco	5052	40·80

TABLE VII

AVERAGE MEAN LENGTHS OF FIBRES IN THE *Aspidosperma* BARKS

Species	Sample	Mean lengths of the fibres in μ	Mean lengths in μ
A. album	1949 1954	1752 1798	1775
A. excelsum	1949 1954	2196 2208	2202
A. megalocarpon	1949 1954	1140 1222	1181
A. oblongum	1949 1954	1878 1930	1904
A. ulei	1949 1954	3684 3844	3764
A. quebracho-blanco	6B 6C	862 838	850

To determine the ratio of number of sclereids to number of fibres in the powdered barks of Aspidosperma

Finally, having determined for each species the number of sclereids per mg. and having calculated the number of fibres per mg., these two values were equated. The ratio was determined by dividing the values of number of sclereids per mg. (Table VI) by the number of fibres per mg. (Table VIII), and the results are tabulated in Table IX.

TABLE VIII

NUMBER OF FIBRES PER mg. OF THE POWDERED BARKS OF *Aspidosperma*

Species	Lengths of the fibres per mg. in mm.	Mean lengths of the fibres in mm.	Calculated number of fibres per mg.
A. album	123·04	1·7750	69
A. excelsum	16·08	2·2020	7
A. megalocarpon	47·19	1·1810	40
A. oblongum	26·50	1·9040	14
A. ulei	65·59	3·7640	18
A. quebracho-blanco	40·80	0·85	48

TABLE IX

S/F RATIO (NUMBER OF SCLEREIDS TO NUMBER OF FIBRES) IN THE POWDERS OF THE *Aspidosperma* BARKS

Species	Number of sclereids per mg. (S)	Number of fibres per mg. (F)	Ratio S/F
A. album	8844	69	128
A. excelsum	7059	7	1008
A. megalocarpon	11705	40	293
A. oblongum	11228	14	802
A. ulei	5749	18	319
A. quebracho-blanco	5052	48	105

DISCUSSION

Preliminary work on the bark of cascara sagrada has shown conclusively that in transverse sections the ratio of area of sclereids to area of fibres is a constant in samples of different geographical origins, ages or thicknesses and between pieces of bark obtained from different aerial parts of the plant. These results are tabulated in Tables I, II and III.

Similar ratios of areas could not be investigated because of section cutting difficulties in the barks of *Aspidosperma*, also a similar ratio in the entire bark would not have solved the problem of identification of these barks when in powdered form. In consequence, direct counts of the numbers of sclereids and a measure of the numbers of fibres present in each mg. of powdered barks were made.

A partial maceration of powdered *Aspidosperma* barks completely disintegrated the fibre masses but only incompletely disintegrated the

sclereid groups. Using the lycopodium method of Wallis[6], counting isolated sclereids and calculating the numbers in sclereidal masses by the method of Wallis and Santra[4,5], concordantly reproducible results were obtained for the number of sclereids per mg. in each species. The results in Table IV confirm the findings of Wallis and Santra[4,5] that the method of calculation gives satisfactory results for sclereid masses. It will be noted from Tables V and VI that the numbers of sclereids per mg. of powdered barks were constant, within experimental error, for each of the six species. Individual species could not be distinguished by this number alone, although three separate groups may be observed.

In order to determine the number of fibres present in 1 mg. of powdered bark an indirect method had to be employed since the fibres present in these fine powders were much broken. Hence total length of fibre per mg. was readily obtained by direct microscopical measurements; an independent measure of average fibre length in each species was made and, from these pairs of values, the numbers of fibre per mg. of powdered barks were calculated for each species.

From Tables V and VI, it will be seen that mean fibre length per mg. of powder is a constant, within experimental error, for barks of the six species examined. Moreover, four of the six species may be distinguished with some certainty, by this value alone, but values for *A. megalocarpon* and *A. quebracho-blanco* are similar as shown in Table VIII. In the same Table it will be seen that the mean fibre length, uniform within a species, differs considerably between species and ranging from 0·85 mm. in *A. quebracho-blanco* to 3·76 mm. in *A. ulei.* These values possess some diagnostic usefulness when the entire or broken barks are examined.

The number of fibres per mg. of barks as calculated and shown in Table VIII, is also a diagnostic character for each species but a clear distinction between the six species under examination is not possible on this character alone.

When the S/F ratio is calculated from these numbers of elements present per mg. of powdered barks, the values shown in Table IX were obtained. It will be seen that a clear distinction between each of the six barks can be made by means of this value alone. This separation is reinforced when the value of S (number of sclereids per mg.) and of F (number of fibres per mg.) are also considered.

Summary and Conclusions

1. The ratio of the areas of sclereids to fibres as seen in transverse sections of barks of *Rhamnus purshiana* has been shown to be a constant for samples differing considerably in sizes, ages or geographical origins.

2. The average mean fibre lengths in barks of six species of *Aspidosperma* have been determined.

3. The numbers of sclereids and numbers of fibres per mg. of powders of these six barks have been counted.

4. The ratio S/F (number of sclereids: number of fibres) is a constant for barks of each species and it serves to distinguish between them.

REFERENCES

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DISCUSSION

The paper was presented by DR. J. D. KULKARNI.

DR. T. E. WALLIS (London) said that the authors had noted that the mean length of fibres per mg. offered a useful criterion for distinguishing barks, and suggested that they might have referred to the work of Dequeker, who was the first worker to suggest that that character could be used. It was a remarkable fact that the ratio of sclereids to fibres should be constant for a particular bark. Both types of sclerenchyma were separate in function. Sclereids hardened the tissue and fibres gave resilience and tensile strength. It would be of interest to know whether the authors could give any reason why the plant should produce those two tissues in constant proportions.

DR. J. D. KULKARNI, in reply, said that the authors had found the original method of Wallis and Santra for estimating the number of fibres and sclereids very useful.

DR. J. M. ROWSON, in reply, said that the authors could not say why the plant produced fibres and stone cells in constant proportions.