

NOTES ON WATTLE BARKS, PART I.

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[With Plates XX, XXI.]

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SINCE the extraction of tannin from wattle bark has given tanners a considerable amount of trouble, it was considered that an examination of the bark structure in conjunction with estimations of the tannin and other contents, should be carried out. A number of samples of barks belonging to the Black or Green Wattle, *Acacia decurrens* Willdenow, group, which forms the principal source of bark in this State, have therefore been examined, and it is proposed in this introductory paper to deal principally with the nature and distribution of the tannins present and the bark anatomy. Although the problems connected with the leather forming properties of tannin and its extraction from wattle bark have received a great deal of attention from the Tanning School attached to the Department of Technical Education, Sydney, this does not apply to the actual distribution of the tannin in the plant tissues. Since the ease of extraction of the tannin depends on the readiness with which it can diffuse out of the tissues it is obvious that the structure of the latter is of importance.

The term "tannin" is used to denote a number of substances possessing somewhat similar properties, and occurring widespread throughout the plant world, being found in the roots, stems, leaves and fruits of the higher plants and even in the filamentous algæ.

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Zimmermann (1896) thus defines tannin: "all those substances which give a blue-black or green-black with iron salts, are commonly designated as tannic acids or tannin. There belong here of the better known compounds, especially pyrocatechin, pyrogallie acid, protocatechuic acid, gallic acid." As defined by Pfeffer, (1903) "tannin is a technical term which has no precise chemical or physiological meaning, for the same microchemical tests with iron salts and potassium bichromate are given by various phenols and phenol compounds, but not by others such as phloroglucin, etc., which have a similar physiological function."

Proctor (1, 1919) on the other hand states that all "tannins give a precipitate or turbidity with gelatine though the sensitiveness of the test is not the same for all tannins. Substances which are like tannins in most other respects but which do not give the gelatine test must be regarded as non-tans."

Tannins may therefore be regarded as products found to occur in plant life, especially in the barks, fruit, wood and leaves. They possess an astringent taste, give greenish or bluish black colourations with iron salts, precipitate gelatine from its solution, give a slightly acid reaction, and combine with the raw hide to produce a leather which has certain chemical and physical properties not common to all kinds of leather.

The tannins are noted for their astringent properties. Villon (1901) describes astringency "as the property of shrivelling the tissues or shutting up the openings of certain organs, as for instance, the papillæ of the tongue, but this is not peculiar to tannins, which however is the astringency par excellence; it is possessed by a large number of styptic salts, such as alum, sulphate of iron, sulphate of zinc, lead acetate and certain acids, such as dilute sulphuric acid, dilute acetic, and gallic acid."

Astringency is a term used to describe the sensation caused by bringing the tongue in contact with certain substances. Although the word is hardly known in the tanning industry, vegetable tannins which cause excessive contraction of the surface of the pelt are sometimes described as being very astringent. In the list of substances mentioned by Villon, *l.c.*, one notes that they possess acid properties and in some cases the power to precipitate gelatine from its solution. Substances which precipitate gelatine probably dehydrate wet animal skin (pelt). Tannins dehydrate pelt with the formation of leather; in this case dehydration of the skin substances is akin to precipitation from solution, and astringency is probably the result of bringing an acid dehydrating substance in contact with cellular or fibrous protein tissue. The dehydration or precipitation of gelatine from its solution is probably caused by the mutual combination of groups in the gelatine and in the tannin molecular aggregates; these groups being the cause of their solubility. That this becomes a complex problem is apparent when we note (Proctor, 2, 1919) that certain substances found as decomposition products of the tannins, catechol and pyrogallol, contain OH groups which have phenolic and alcoholic functions.

The tannins occurring in the wattle bark and dealt with in this paper are only those capable of classification into that group which besides responding to the above definition, are absorbed by hide powder according to the regulations laid down by the Society of Leather Trade Chemists.¹

Perhaps the commonest empirical test for tannins is the use of iron salts, producing certain colour reactions, but this has the disadvantage that the colourations are given by other substances, which do not behave as tannins.

¹ Proctor. Leather Trades Pocket Book.

Potassium bichromate, first recommended by Sanio, was found to be the most satisfactory method of precipitating the tannin in the cells of the tissues in which it occurs. The brownish precipitate is insoluble in water, alcohol, etc. In concentrated aqueous solutions the penetration is quite satisfactory when the bark is treated in small pieces, measuring about 1 cm. square, and sections cut from treated material can be preserved in canada balsam, or glycerine. Zimmerman *l.c.*, states that according to Nickel (1890) various compounds not related to tannins give similar precipitates with potassium bichromate, but it has been found in this research that bichromates give no precipitate with the soluble non-tans left after a hide powder analysis of various samples of wattle bark, *i.e.*, after all the tannins have been absorbed. This is a most important point since it can reasonably be assumed that the precipitate formed in the cells is due to the combination of the tannin and bichromate. As will be shown later potassium bichromate has also the advantage of giving a precipitate in very dilute tannin solutions. Since pyrogallol does not occur in these tannins the insoluble precipitate in this case is not likely to be the oxidation product purpuro-gallol mentioned by Moeller (*c.f.* Zimmerman, *l.c.*). Chromic acid was not found to be so satisfactory as the bichromate.

The gelatine test is regarded by leather trade chemists as the most useful method of detecting the presence of tannin in dilute solutions, but owing to its viscosity it is obviously unsuited for microchemical work. The solution contains 10g gelatine and 100g salt in one litre of water. Proctor (1, 1919) states in reference to gelatine that a precipitate is obtained in extremely dilute solutions of gallo-tannin, or fruit tannin, while the bark tannins give the test if the solutions are not too weak, pine bark and gambier being the least sensitive. Other micro-chemical tests for

tannins are described in the various text books on the subject, such reagents as osmic acid, chromic acid, ammonium molybdate, sodium tungstate, alkaline carbonates, methylene blue and certain alkaloids being found more or less satisfactory.

It was found by experimental work that the gelatine and bichromate tests for tannin do not always give constant results. Apparently with some solutions the sensitiveness of the gelatine reaction with the tannins varies to a considerable extent. We obtained a turbidity with gelatine in a solution of one part of tannin in 100,000 parts of water, but with other solutions from different barks we were unable to get any reaction with a solution of one in 25,000. This apparently is in accordance with results obtained by Thomas and Frieden (1923) who found that the hydrogen ion concentration of the solution is an important factor, controlling to a certain extent the reaction of gelatine with tannin in dilute solutions, and probably our failure to secure recognition of tannin in solutions of one in 25,000 was due to the fact that, with dilution, the hydrogen ion concentration fell below the figure they give as the most suitable for the recognition of tannin in wattle bark. The authors found that the best ratio of tannin to gelatine is two to one, and when the hydron concentration for wattle barks was adjusted to $\text{pH} = 4.5$ to 4, then it was possible to obtain a recognition of one in 200,000. Without adjusting the acidity they only obtained a recognition of one in 20,000 for the same solution. These variations in our results were not so noticeable with the bichromate test, a recognition of one part of tannin in 25,000 to 50,000 being obtained by adding a few drops of a saturated aqueous solution of bichromate of potash to a dilute solution of the soluble matter obtained from various barks. The turbidity was only obtained after standing for some minutes in very weak concentrations.

It is usually accepted that the tannin is more or less in solution in the cell sap and although after the death of the cell, diffusion may occur into the cell wall, it is normally confined within the protoplasmic membranes, and although the tannin undoubtedly possesses the property of precipitating albuminoids it is obvious that it may not necessarily affect protoplasm.

Lloyd (1922) puts forward the theory that two substances are present in the vacuole of the tannin cell, namely, the tannin itself, and another substance with the physical properties of a gel. He also points out that after extraction of oak bark with alcohol the tannin cells are still filled with insoluble material which contains tannin, and he describes this mode of occurrence as the tannin mass, which consists of a complex of substances, tannin being one. Lloyd *l.c.*, quotes Van Wisselingh (1910) as having confirmed the earlier research of Wigand in concluding that the tannin is an essential factor in plant metabolism, being concerned in the building up of cellulose. When the concentration is high, it seems that the view expressed by Lloyd *l.c.*, is correct, namely that the tissues are approaching death and that the tannin does not again enter into the metabolism of the plant. The benefit obtained by a protective device consisting of an outer zone of cells containing a very high tannin concentration cannot be overlooked. (*c.f.* Stahl, 1888) In all the *Acacia* barks so far examined, it has been found that the tannin concentration undoubtedly reaches a maximum towards the outer corky layers. Recent work seems to indicate that tannin is not always a protoplasmic poison, but where it is, it is probably held by a strongly adsorbing body, but where non-toxic, a weaker adsorbing body would allow of its more ready use. This body in certain cases being identified as a carbohydrate. (*c.f.* Lloyd, *l.c.*)

The word "bark" is used here to define the whole of the tissues outside the woody cylinder of the tree, since this portion is always referred to when the term "wattle bark" is used. The definition is more often given as that portion of the outer tissues consisting of the dried up, cortical, and sometimes vascular cells, cut off from the inner living portion by the phellogen or cork cambium, together with the outer corky layers, the whole being principally concerned in the reduction of transpiration, and the furnishing of protection against mechanical injury to the inner conducting tissue.

Dealing first with the anatomical structure it is found that the bark consists primarily of the secondary phloem, a broad zone of conducting tissue extending from the cambium, adjacent to the wood, as far outward as the cortical tissue, the latter being composed of a comparatively narrow area of thin walled cells or parenchyma, outside which is in older bark a narrow band of cork cells, or an epidermal layer in younger barks.

The conclusion arrived at by Coester, (1894) namely, that the outer limit of the bast formed by a composite ring of sclerenchyma, (*i.e.*, thick walled cells) is a characteristic feature of the Mimoseæ, in which group the Acacias are included, is in the majority of cases correct for the barks of the *Acacia decurrens* group. In certain cases, however, the ring has been found to be broken, and therefore cannot be regarded as a specific character. Similarly it has been found that although the cork cells are usually developed superficially as pointed out by Coester, *l.c.*, the phellogen not arising much below the epidermis yet in certain bark specimens examined, undoubted evidence was found that the cork cells may develop well within the sclerenchymatous ring, proving that a phellogen may subsequently arise in a deep seated position. The cork cells are flattened

radially with moderately thick walls, the actual thickness of the periderm being small, as a rule not more than 0.2 mm. The outer bark surface is often scaly due to the separation of the masses of cork cells, caused by the increase in circumference of the growing tree.

Both the sieve tubes and companion cells which occur in tangential bands between rows of phloem parenchyma cells, collapse within a short distance of the cambium. These areas of collapsed cells are persistent throughout the secondary phloem, (Plate XXI, fig. 4) and possess a strong affinity for stains, but the parenchyma cells remain unchanged except for an increase in size. The amount of space occupied by these collapsed tissues in a mature bark is unimportant and they can therefore have little effect upon the tannin content of the whole.

The phloem parenchyma cells which contain a large percentage of the total tannin measure about 0.037 mm. in length, being directed longitudinally, by about 0.01 mm. in diameter, and are almost circular in cross section. As they become further removed from the cambium, due to the growth of newer cells the increase in size becomes more marked, until finally they may measure 0.04 mm. in diameter often becoming flattened, with the longer axis directed tangentially. There is no proportional increase in vertical length.

Of even greater importance in their influence on the tannin yield are the medullary rays, which are either uniseriate or multiseriate, broadening considerably in the outer portion of the bark, with a considerable increase in the cell size to about 0.05 mms. in maximum diameter. Near the cambium the longest axis is directed radially; the older cells are flattened tangentially.

The degree of development of the bast fibres is however of considerable importance in its influence on the tannin

content, for several reasons. In the first place the cell walls are extremely thick, the lumen in the older cells almost disappearing, and since the specific gravity of this lignified tissue is about 1.6, the insoluble matter in a comparatively small amount of fibre is equivalent in weight to that present in a much larger amount of thin walled tannin bearing cells. Thus a bark containing even a moderate development of fibre has an increased percentage of insolubles, and a lower percentage of total solubles, including tannin, on analysis. Secondly, since no tannin is shown by any microchemical test to be present in the cell wall or in the lumen of these fibre cells, they can yield practically nothing on extraction. In certain barks examined in which the tannin content was low the development of bast fibre was exceptionally high, and *vice versa*, in a bark showing a tannin content of 46.93%¹ the amount of fibre was extremely low. In the inner portion of the secondary phloem the area of the fibre groups in cross section may amount to as much as 50% in some barks. The diameter of the bast fibres is small, averaging about 0.009 mms., the maximum size of the groups being in the vicinity of 0.6 mm. in a tangential direction by about 0.1 mm. in width. More or less surrounding the fibre group is a single row of short thick walled crystal bearing cells the contents being apparently calcium oxalate.

Surrounding the vascular tissue is usually a more or less complete chain of stone cells, (sclereides), irregular in shape with heavily lignified walls and containing no tannin. The cortical tissues outside this zone are tannin bearing, and usually chlorophyll is present. The outer corky cells of the periderm are small, with thick suberised walls, the

¹ As far as we are aware this is the highest recorded analysis of any bark of the *A. decurrens* group. The complete analysis was, tannin = 46.93%; soluble non tannins = 9.85%; insolubles = 34.45; moisture = 8.77%.

brown contents evidently consisting principally of phlobaphenes. The phelloderm is scarcely developed. It has been pointed out that in certain cases the phellogen may develop in a deep seated position, but this is rare. Even in barks of considerable thickness the epidermis may still be persistent in some cases. Occasionally a row of pockets containing what is evidently gum occur concentrically in the secondary phloem. The contents give no reaction with potassium bichromate when the tissue is treated in bulk; they are then insoluble in water. The cavity is apparently formed by the disintegration of the phloem tissues.

A whitish glaucous deposit of wax is occasionally found in the barks of some trees. The powdery substance is partly soluble in 100 per cent. alcohol, and is soluble in chloroform.

The distribution of the tannin is principally in the outer parenchymatous cells of the medullary rays, in the primary and secondary cortex, and also in the phloem parenchyma. Plate XX, fig. 1 shows a transverse section of a portion of the bark of *Acacia decurrens* measuring about 4 mms. in thickness. The bark was given the preliminary treatment with potassium bichromate before sectioning. It is seen that the concentration of tannin bearing cells with dark contents, reaches a maximum both inside and outside the narrow clear band of stone cells occurring towards the outer edge.

Plate XX, fig. 2, shows an enlargement of portion of the same section within the lower rectangle near the cambium. The tannin bearing cells of the phloem parenchyma are usually arranged in short chains at right angles to the medullary rays, the cells of the latter being somewhat elongated and also showing a reaction for tannin. These cells are in each case thin walled; the thicker walled bast fibre groups are already numerous and contain no tannin.

The concentration of these darker cells increases with the distance from the cambium at the right hand edge of the figure. Fig. 3 (Plate XXI) also shows an enlargement of portion of the same section as Fig. 1 (Plate XX), but nearer the outer edge of the bark, as shown in the upper rectangle. The enormous increase in the tannin bearing cells is at once apparent, which, together with a considerable enlargement in the size of the cells indicates a greatly increased tannin content. The particular portion shows by no means a maximum of tannin cells, as can be seen by an examination of fig. 1. Running across the section is one of the considerably broadened multiseriate medullary rays, consisting of comparatively large cells; on either side of the ray are the non-tan bearing cells of the bast fibre groups.

A strip of bark was removed from the butt to the top of a tree about 25' in height, and a series of six sections cut at regular intervals. The bark samples were given the preliminary treatment with a concentrated aqueous solution of potassium bichromate. An examination of the sections shows clearly that the amount of tannin is highest in the bark near the base of the trunk. Commencing at the bottom of the tree we have:—

Section 1. Thickness of bark 5 mms. Inner portion of 0·9 mm. in width containing comparatively little tannin; outer portion contains a large number of tannin bearing cells.

Section 2. Thickness of bark 4 mms. Inner portion 0·6 mm. in thickness containing little tannin.

Section 3. Thickness of bark 4 mms. Tannin more evenly distributed through secondary phloem.

Section 4. Thickness of bark 2·40 mms. Very little tannin in inner portion of 0·9 mm.

Section 5. Thickness of bark 2·40 mms. Tannin comparatively evenly distributed in secondary phloem, but number of tan bearing cells fewer than in 1 and 2.

Section 6. Thickness of bark 1.9 mms. Comparatively little tannin in inner 0.6 mm. Much greater concentration outside this limit.

In all the above sections the greater concentration is found in the cells of the medullary rays where they broaden out, especially in the cells just within the sclerenchymatous sheath corresponding to the pericycle, and in the cells of the primary and secondary cortex, all these being filled with a dark brown precipitate. In many of the cells the contents appear vacuolated; in many cases starch grains are present. The cell walls are scarcely stained, a decided difference from what obtains in the spent bark.

The Melbourne Board of Enquiry (1892) states that the best months for stripping the bark are September, October, November, and December, and also that while the bark strips easily after rain the quality is inferior. Ewart (1912) states that the best time for stripping is the spring or early summer when the sap is rising, since at that time not only does the bark come off more easily, but it is in a better condition for tanning. We are in accord with the statement that spring is undoubtedly the best time for stripping, more particularly on account of the fact that the removal of bark is much easier at that time. This is evidently due to the increased growth of new cells from the cambial layer, which being thin walled are therefore more readily separated. A similar explanation can apparently be given to the fact that the bark is more easily stripped after rain. As far as our investigation has gone there is no evidence to show that the quality of the tannins varies during the different seasons of the year, or after a wet period, except where large increase in new growth would augment the non-tans and insolubles, and thereby increase the risk of fermentation in the extraction vats and tan liquors. This increase in the other constituents might therefore lower the percentage of tannin in the bark

although actually a slight increase in the total quantity of tannin may occur. So far we have not obtained any direct evidence that the percentage of tannin varies at different times of the year.

Under normal conditions the amount of tannin in the bark of the tree is proportional to its age. This does not mean that old trees necessarily contain more, or a greater percentage of tannin than young trees, since the amount of fibre present is a factor, and thickness is usually more important than age in its influence on the tannin content. The lack of tannin in the freshly formed cells of the inner bark has been pointed out. (Plate XX, fig. 1). It is evident that the amount of tannin present bears a direct relationship to the age of the cells, since those nearest the cambium contain comparatively little tannin when compared with those nearer the outer cortex. If then it is correct that the percentage of tannin in the bark increases as the percentage of young cells in the bark decreases, then one would expect to find the greatest percentage of tannin at the end of a period of minimum growth, which would normally correspond to the end of the winter months, and therefore this should be the time to strip the bark to obtain the highest yield of tannin. Probably the maximum increase in thickness occurs in the spring and summer whilst during the autumn and winter there is a greater increase in the tannin production. The evidence obtained microscopically as to the distribution was confirmed by the analysis of two samples of bark which were taken and the outer rind removed. Each was then split parallel to the surface into two equal halves, and analysed with the following results:

	Young Tissue.		Old Tissue.	
No. of bark	1	2	3	4
Tannin	17.92	17.02	27.11	29.24
Non-tannin	10.06	12.43	7.89	10.53
Insoluble	63.87	64.15	55.8	53.51
Water	8.15	6.4	9.2	6.72
	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>

It will be noted from the above figures that the older tissues contain the greater amount of tannin whereas the younger cells contain a higher percentage of soluble non-tans. This to a certain extent is similar to results obtained when analysing samples of bark taken from different parts of the tree; the bark at the butt with a maximum of old cells containing more tannin than the bark at the top of the tree, which contains a larger percentage of younger cells. These results seem to point to the fact that the new tannin-bearing cells contain certain substances that are not tannins but apparently change to tannins as the age of the cell increases.

Williams (1915) states that "the percentage of tannins in the thicker portions of the bark felled in winter is appreciably higher in most cases than in the case of bark of corresponding thickness felled in summer. It appears as if there is a greater proportion of tannin in the lower portion of the tree during the winter months and this is what one might expect seeing that the sap is usually concentrated more or less towards the base of the tree in the autumn and winter months." If this is correct one would expect to find the tannin in a plastic condition near the cambium whereas the concentration evidently reaches a maximum furthest from the actively conductive tissues of the secondary phloem.

It seems probable that there is little alteration in its position once the tannin is elaborated. An attempt made to prove that the parent bodies of the tannins are found in the soluble non-tans was not successful. Wilson (1916) states that the soluble non-tans after heating changed colour, with the production of tannin. The wattle bark extracts we have examined also changed to a red colour upon heating, but no indication of tannin was found in this solution with the gelatine test.

In order to determine the amount of moisture in the bark as it occurs on the tree, samples were weighed immediately after stripping. When dry, after a long exposure to air, they were again weighed, and the moisture and tannin contents determined by the usual method. The results were as follows:—

	1	2	3	4	5
Tannin	17.08	17.35	16.15	9.43	14.37%
Non-tannin	4.29	4.53	4.62	4.51	5.96%
Insolubles	22.34	24.55	24.78	28.09	27.05%
Water	56.29	53.57	54.45	57.97	52.62%

From the above figures it is possible to obtain some idea of the concentration of the solution of the tannins and total solubles in the cell, *e.g.*, if all the water present acts as a solvent for this material we find that:—

	(1)	(2)	(3)	(4)	(5)
Concentration of total solubles	=27.5	29.0	27.6	19.4	27.8%
„ tannin	=22.0	23.0	21.4	13.1	19.7%

This assumption however is obviously incorrect. If we assume that the percentage of moisture in the cell walls is 50,¹ the concentration of the solubles is largely increased. Allowing then 50% of water in the insolubles, we find that the concentration of the total solubles and tannins are as follows:—

	(1)	(2)	(3)	(4)	(5)
Concentration of total solubles	=38.6	43.0	41.1	31.8	44.3%
„ tannin	=30.9	34.1	32.0	21.5	31.3%

These figures are very probably by no means the highest that can be obtained, but they are sufficient to show that a very high concentration is reached, if the tannin is wholly

¹ This is a very conservative figure. Pfeffer *l.c.*, estimates that the percentage of moisture in the cellulose cell walls is 70–90; in the lignified walls, 50%. In the Acacia bark only a proportion of the cell walls are lignified, and moreover the amount of water in the other insolubles present, *e.g.* starch, albuminoids, etc., would be considerably in excess of this amount.

in solution in the cell sap. Under ordinary conditions such a solution would be extremely viscous.

After stripping, the bark is air dried, the moisture content being reduced from about 55 per cent. on the wet weight to normally about 10 per cent., by exposure to ordinary atmospheric conditions. The moisture is probably present in the bark in three ways, (1) as free water in the cells, (2) as water present in combination with the cell contents, and (3) as moisture absorbed by the cell walls. In the air dried bark it is probable that the moisture is distributed between the cell walls and the cell contents, which in this case are principally tannin. The theory has been advanced that the contraction which occurs in drying, and the subsequent crushing of the bark, results in small cracks. No evidence has, however, so far been found that the rupture of the cell walls actually takes place, but it is a fact that the bark must be crushed before the majority of the tannin can be extracted. This crushing must however rupture certain of the cells, and by reducing the size of the particles of bark, would allow the readier penetration of water and hence give rise to a more efficient extraction.

An interesting feature connected with this work is found when extraction results with Adelaide and South African barks are compared. The former has always the appearance of a bark which has been stored, and thoroughly dried, since it is distinctly red—a sure sign of exposure. The South African bark is pale in comparison with that of Adelaide, and certainly does not appear to have been cut for any length of time. The Adelaide bark appears to give up its tannin more readily than the South African bark. Some Australian tanners believe that the longer the period that is allowed to elapse after the time of stripping, the easier is the extraction of the tannin from the bark. [Coombs, 1919.]

Small pieces of the fresh bark were placed in potassium bichromate solution. Sections were also cut from fresh untreated bark, transferred to water, and then into a saturated aqueous solution of bichromate. It was found that the longer the preliminary treatment with water, the less pronounced was the reaction given by the potassium bichromate. The rapidity of the diffusion of the tannin from the cells is indicated by the fact that even after as short a period as five seconds in water, an appreciable difference could be found when compared with sections from material which had first been treated with the bichromate solution. All sections were of uniform thickness. After five seconds the brown precipitate was most pronounced in the medullary rays, and also in the tannin bearing phloem parenchyma cells. After ten seconds a still further reduction in the intensity of the colouration was observed, similarly after fifteen seconds. After six minutes the contents of the medullary rays, and also of isolated cells in the cortex, were stained. After thirty-six minutes the medullary rays were still distinct and also certain cells of the secondary cortex and phloem. After five hours a few cells of the the medullary rays were affected but there was comparatively little difference between this and the thirty-six minutes exposure. A section which after four hours in cold water was boiled and then transferred to bichromate showed no reaction in the medullary ray cells, but certain of the cells in the secondary phloem became somewhat yellowish, and in places the cell walls were stained a light brown. Similar results were obtained by boiling sections cut from fresh bark.

From these results it seems evident that the greater percentage of the tannin in the fresh bark is readily soluble in cold water, the rapidity of solution depending on the amount of surface exposed. There does not appear to be

any clear evidence to support the prevalent idea that the solubility of the tannin increases with the length of time bark is kept after stripping, but further work remains to be done on this subject.

A portion of the spent bark after extraction of the tannin under ordinary commercial conditions was examined, after a further treatment with alcohol and chloroform, by means of paraffin sections. Many of the cells of the outer expanded portion of the medullary rays were found to be wholly or partially filled with almost clear, colourless, amorphous, slightly granular contents, sometimes showing signs of striation. Numerous starch grains were present both in these cells and also in those apparently without other contents. In the tissue of the secondary cortex many of the parenchyma cells also possessed contents. The irregular branching groups of collapsed sieve tubes were decidedly coloured, being especially prominent in contrast with the lighter coloured surrounding cells. The most prominent portion of the medullary rays was nearest the cambium, the contents being light brown in colour; further out the cells of the rays were often devoid of contents, at other times containing an amorphous mass. Here also starch grains were numerous.

Similar sections treated with potassium bichromate gave the most pronounced colouration in the collapsed groups of sieve tubes and companion cells, these becoming brown. The more or less striated cell contents of the broad medullary rays, parenchyma, etc., were only slightly stained, and the cell walls were considerably more prominent. The innermost cells of the medullary rays showed a very pronounced colouration, as also obtained in the adjacent parenchyma.

With ferric chloride, the same changes were observed, the collapsed sieve tube areas, especially those near the

cambium being stained to a bluish grey, though they show no tannin reaction in fresh bark. It seems evident, therefore, that the tannin is absorbed by the cell walls during the diffusion of the contents in water. The walls of the heavily lignified bast fibre cells showed very little alteration. With iodine solution, the lignified tissues were stained yellow. With chlor-zinc-iodine, the contents of the parenchymatous cells became somewhat violet, the collapsed sieve tube areas, however, were scarcely affected. The probable presence of a cellulose-like body in the parenchymatous cells seems to confirm the conclusion arrived at by Lloyd, l.c., as to the occurrence of an adsorption equilibrium between the tannin and a second body, in certain cases. As already pointed out no evidence was found of any rupturing of the walls of the cells in which the tannin occurs.

It is proposed to make a thorough investigation both chemically and anatomically of the barks of the *Acacia decurrens* group in particular. In the very complete investigation made on the wattle barks (Maiden 1891), over thirty years ago, the Lowenthal method of analysis was used, and the results may differ from those obtained by the modern hide powder method. A close study of the seasonal variation, if any, in the tannin content, by obtaining a series of bark specimens from the same trees will also be made.

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Explanation of Plates.

PLATE XX.

Fig. 1. Transverse section of the bark of *Acacia decurrens* showing the distribution of the tannin after the precipitation with potassium bichromate. The tannin-bearing cells increase in number and size from the cambial zone at the bottom of the figure, towards the outer corky tissues seen at the top. $\times 14$.

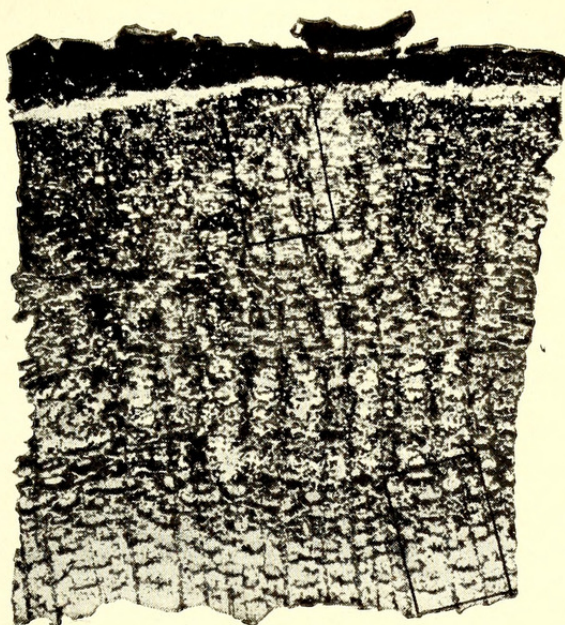


Fig. 1.

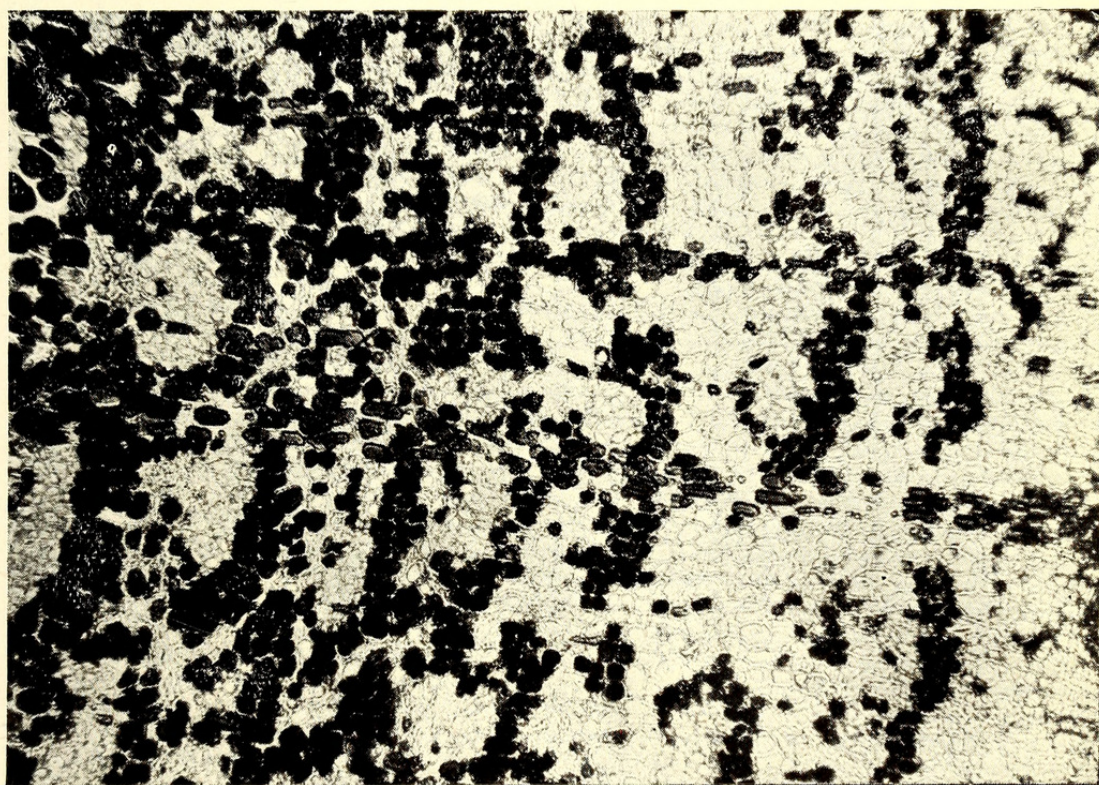


Fig. 2.

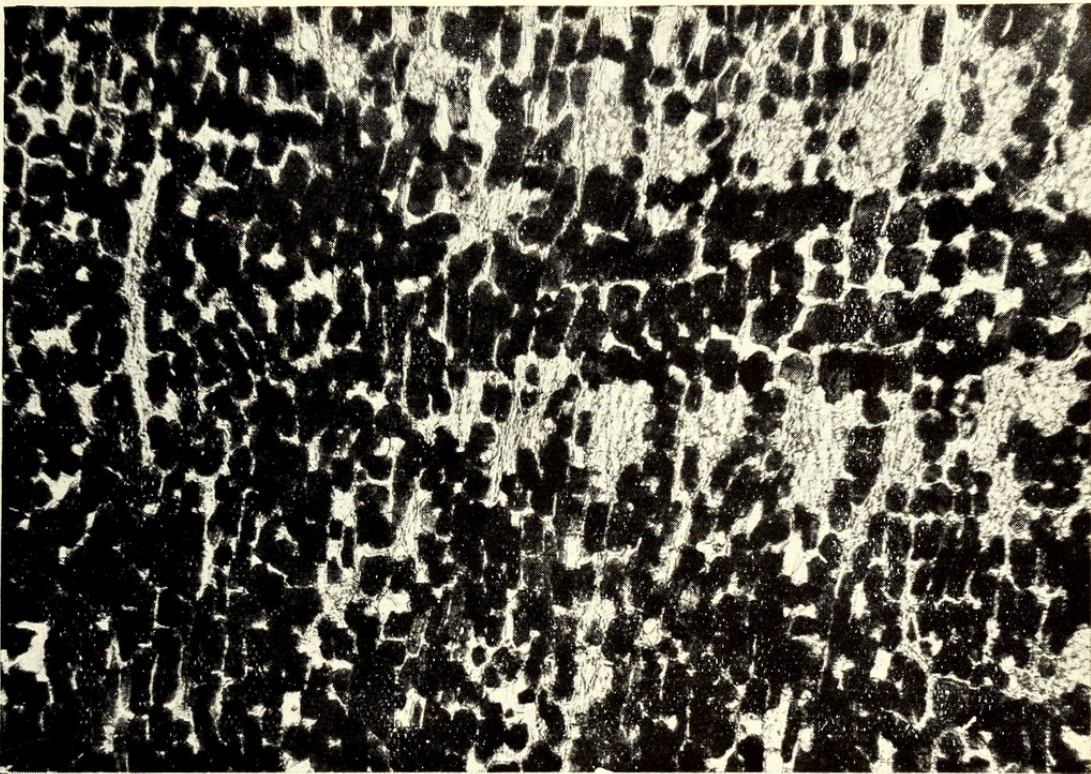


Fig. 3.

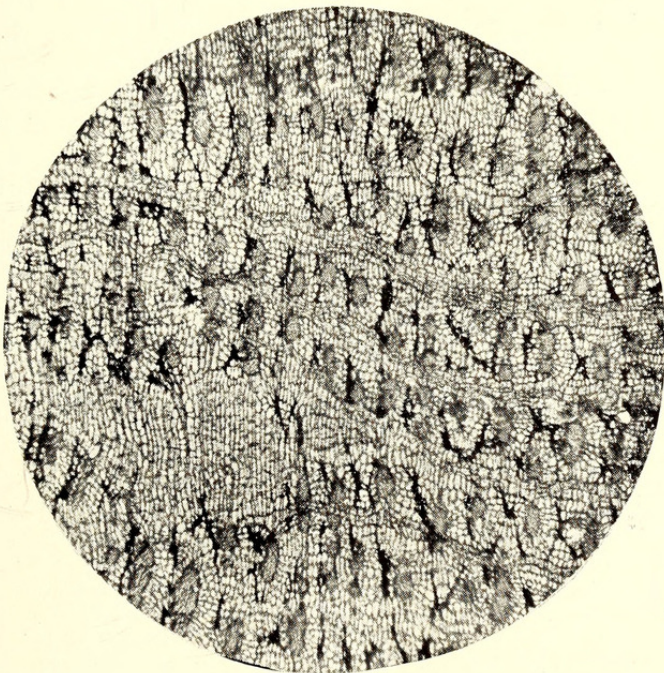


Fig. 4.

Fig. 2. Transverse section of that portion of bark seen in the lower rectangle in Fig. 1. Towards the right hand edge are the newest cells of the secondary phloem, which are practically devoid of tannin, the latter becoming more pronounced in the older cells of the phloem parenchyma. Tannin is also present in the cells of the medullary rays, (running horizontally in the figure) at a very early stage, but the companion cells, sieve tubes and bast fibres show no evidence of it. $\times 95$.

PLATE XXI.

Fig. 3. Transverse section of that portion of bark seen in the upper rectangle in Fig. 1. This section shows the enormous development of tannin-bearing parenchymatous cells in the broader fan-shaped medullary rays, and in the phloem parenchyma. There is no evidence of tannin in the bast fibre zones (seen as groups of small, clear thick walled cells), or in the collapsed sieve tubes and companion cells. $\times 95$.

Fig. 4. Transverse section of the bark of *Acacia decurrens* after removal of the tannin, showing portion of the secondary phloem. The narrow, dark coloured areas represent the collapsed groups of sieve tubes and companion cells. The isolated bast fibre zones are separated by the medullary rays, which are seen as more or less regular bands of cells, and also by the phloem parenchyma cells. $\times 24$.



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