THE TANNINS OF THE BLACK CYPRESS PINE (*CALLITRIS CALCARATA* R.Br.) AND THEIR DISTRIBUTION IN THE BARK.

By F. A. Coombs, A.A.I.C., W. McGlynn,
Tanning School, Sydney Technical College.
and M. B. Welch, B.Sc.
Technological Museum.

(With Plates XVII-XVIII.)

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The Black Cypress Pine (*Callitris calcarata,* R.Br.), occurs over a very wide area of the inland portions of the Eastern States of Australia, and in a few localities it even extends as far as the coast. In this paper, it is proposed to deal with the bark, which is an important tannin-bearing material, the work thus being a continuation of that commenced on the Acacias.* The high tannin content of this bark was first pointed out by Baker and Smith,†† in their exhaustive research on the pines of Australia; its anatomical structure was also described. This was followed by two papers from the Technical College Tanning School,† which dealt principally with the leather-forming properties, and gave qualitative tests of the bark and tannins.

A satisfactory extraction of pine bark on a commercial

* Notes on Wattle Barks, Part I., Welch, McGlynn & Coombs. Proceedings, Royal Society, N.S.W., 1923.

†† Pines of Australia.

† Coombs, Comparative Sole-leather Tests with Australian Pine Barks. Collegium (London), September, 1916.

Coombs and Dettman, Notes on Australian Pine Bark. Jour. Soc. Chem. Ind. 1914.

scale was found, however, to be difficult—in this respect differing considerably from the Acacias. It was, therefore, considered necessary to make a more complete examination of the structure of the bark than had hitherto been done, and to compare it with that of Acacia bark, and also to study the actual distribution of the tannin throughout the tissues. Furthermore, it was essential to determine what was the actual cause of the resistance to the penetration of water into the bark, since the ease of extraction depends largely on two factors, namely, the solubility of the tannin, and the ease of water penetration.

Bark Structure.—The mature bark* of *Callitris calcarata* consists entirely of secondary phloem, the outer cortical tissues being lost at a comparatively early stage in the growth of the tree. A section of a twig a few mm. in diameter shows practically the same structure outside the cambium as occurs in the bark of a large tree. Cortical resin passages are replaced by those in the phloem, and the more or less isolated first formed bast fibres are replaced by more regular rows of radially compressed thick walled cells. As pointed out by Baker and Smith, the secondary phloem consists of concentric rows of bast fibres, one cell in thickness, interrupted by uniseriate medullary rays; between the bast fibres are normally three rows of cells, the middle row consisting of phloem parenchyma, and the two outer rows of sieve tubes. (Fig. 3.) The regularity of this arrangement in the Cupressineae is mentioned by De Bary.†

At a distance of about 4 mm. from the cambium in the mature bark there occurs an inner peridermal zone, 3-8 rows of cells in thickness, extending completely round the tree and

* The word "bark" is used to denote all tissues outside the cambium.

† De Bary, Comparative Anatomy of Phanerogams and Ferns. English Translation, Oxford, 1884.

separating the inner living from the outer dead portion. Between this inner periderm and the outside of the bark there are a number of earlier formed peridermal layers at intervals of from 225μ to 1200μ. The cells of the periderm are thin-walled, and show a very marked coloration when treated with a fresh alcoholic solution of chlorophyll, thus indicating the presence of suberisation. The cells are compressed radially, and measure about 45μ x 8μ transversely, and 40μ in length.

The position of the innermost periderm can be easily determined by cutting the fresh bark transversely, when the living portion nearest to the cambium is seen to be white, altering to pink towards the outside. The dead tissues are reddish brown, the line of demarcation from the inner zone being sharply defined, becoming a dirty greyish brown towards the periphery.

The bast fibre cells are important in that their excessive development in a bark must naturally increase the amount of insoluble non-tannins, and correspondingly lower the percentage of tannin. Moreover, they have a direct effect on the difficulty or otherwise of grinding the bark. In the Cypress barks, however, they do not possess the same importance in this direction, as they do in the Wattle barks, where the fibres are grouped into large zones (c.f. Welch, McGlynn and Coombs, l.c.). In *C. calcarata* the cells are flattened in a radial direction (Fig. 3), the average size being 10μ x 40μ x 2000μ in length. The walls are always heavily lignified, the lumen being reduced to a narrow slit. Although near the cambium the radial walls are usually in contact, towards the outer part of the living secondary phloem the cells are often more or less isolated, due to the subsequent increase in circumference of the bark. The degree of separation varies in different barks, but should have the effect of making them easier to grind.

The sieve tubes are normally very much compressed radially within 0.5 mm. of the cambium. In no case was tannin or starch detected in them, and they are apparently of little direct importance in their influence on the actual amount of tannin yielded on extraction. In Fig. 4 they can be seen as more or less compressed rows of cells, usually empty, on either side of the phloem parenchyma, which possesses dark contents. The sieve tubes measure 6–18μ radially and 20–40μ tangentially, and near the cambium differ little in size or shape from the phloem parenchyma when seen in transverse section. The most important tannin-bearing tissue in the bark is the phloem parenchyma, more especially after enlargement occurs towards the inner peridermal layer (Fig. 2). The section illustrated was cut from a portion of the bark treated with potassium bichromate, which precipitates the tannin in an insoluble form, and has proved the best reagent for the study of the tannin distribution.*

It will be noticed that about half way between the cambium and the innermost periderm, the parenchyma cells undergo a very considerable increase in size: Thus, whereas a typical cell near the cambium measures 40μ x 8μ, near the inner periderm the dimensions are 110μ x 30μ. The increase in the size of these cells can be more clearly seen in Fig. 1, the tannin having been dissolved out, leaving the cell walls. Outside the periderm (Fig. 2), there is an apparent decrease in the tannin contents of the cells, but this is evidently due to the breaking away of portions of the cell contents during sectioning. The most striking feature of this section is the almost complete absence of tannin near the cambium, and the gradual increase in the tannin (seen as black masses in the illustration), till a maximum is

* Lloyd. The Occurrence and Function of Tannin in the Living Cell. Trans. Roy. Soc., Canada, 1922.

reached near the innermost peridermal zone. A more highly magnified transverse section, about midway between the cambium and the inner periderm, which has been treated with potassium bichromate (Fig. 4), clearly shows the regularly arranged phloem parenchyma cells, with their dark contents.

This gradual tannin increase outwards is in accord with what was found to occur in the Acacia barks, where practically the whole bark is analogous to the innermost living part of the Cypress barks. There is nothing in the bark of *Acacia mollissima* to correspond to the outer dead secondary phloem tissues which form the greater portion of the bark of *Callitris calcarata*. Thus in the Acacia bark, a considerable amount of tannin is present in the primary and secondary cortical tissues, and in the broad fan-shaped medullary rays, whereas in the Cypress barks, the cortical tissues are lacking, and the medullary rays are narrow and uniseriate. Although tannin is present in many of the medullary ray cells, it is not always so (c.f. Figs. 2-4), and they cannot be regarded as an important tannin-bearing tissue.

One of the most characteristic features of the Cypress barks is the very large development of resin passages in the phloem. They occur in more or less concentric rings (Fig. 2), and measure about 120μ in diameter, minimum = 60μ, maximum = 250μ, anastomosing freely in a tangential plane, but rarely radially. The contents of the innermost ducts are fluid in fresh bark, clear viscous drops being exuded on a cut surface; those of the outer ducts are amorphous, non-fluid and resinous, appearing microscopically as irregular, angular, cracked fragments. The alteration in the physical character is due possibly either to the loss of the more volatile constituents of the oleo-resin or to oxidation or both. The ducts are readily observed

macroscopically in a transverse cut of the outer bark as white concentric rings. A well-defined epithelial layer surrounds each duct, and it is interesting to note that microchemical reactions show undoubtedly the presence of a high tannin concentration in these cells (Fig. 4), although no evidence of it has been found in the ducts themselves. Haas and Hill * state that it has been found that the amount of tannin varies inversely in *Pinus* as the resin content. Although no determinations have been made in the Callitris barks to ascertain whether a similar variation occurs, one would hardly expect to find much seasonal alteration in the whole bark, since the portion which preponderates and which contains a considerable amount of resin is non-living, and cannot alter except by decomposition. Nevertheless, it seems possible that there is a close relationship between tannin and resins since tannin is so prominent in the epithelial cells. The solubility of the contents of the ducts in alcohol is unchanged by the loss of the more fluid constituents; in all cases the resins readily dissolve in that solvent.

A microscopic examination of the bark also shows the presence of a large amount of starch in the phloem parenchyma, the medullary rays and the epithelial cells of the inner living zone of bark (Fig. 4). Peacock† has pointed out that in *Heuchera americana* tannin is most abundant in October, and least in May, whereas starch reaches a maximum in March. In the Callitris barks, no seasonal determination of starch has been made, but it is significant that starch occurs only in those cells in which tannin is found. The grains can be seen in Fig. 3 as small rounded masses (measuring about 5μ in diameter); in the

* Hass & Hill. Chemistry of Plant Products, Longmans Green, 1921.

† Peacock. American Jour. Pharmacy, 1891, p. 172.

cells in which tannin is most evident the grains are often closely packed. In no case were starch grains found outside the innermost periderm, and it is evident that it is converted to some other carbohydrate, or broken down either just prior to the formation of the corky layers, or utilised by the dying cells after their isolation by the periderm. Since starch is a carbohydrate food storage body, one would expect to find seasonal variation, probably reaching a maximum in the winter. The specimens collected at Dubbo in June certainly showed abundant starch when examined microscopically.

If thin sections of the fresh inner bark be transferred to water, solution of the tannin is practically instantaneous, and subsequent treatment with potassium bichromate or ferric chloride shows no indication of tannin in the cells. A few scattered parenchymatous cells near the periderm, however, possess yellow contents which are insoluble in cold water, but which darken when treated with solutions of iron salts. The contents of the cells of the outer bark are in general, progressively less soluble in cold water towards the outside, with a change in colour to a deep brown. The contents of the outermost cells are completely insoluble even in boiling water or alcohol, and this also applies to scattered cells, even near the innermost periderm. The contents of these cells are clear and somewhat granular, amorphous, reddish brown, and often with irregular cracks or markings. In a typical bark in which the inner living portion is 4.5 mm. in thickness, the insoluble masses are comparatively rare in the 3 mm. of dead tissue adjacent to the innermost periderm; then in the outer portion which varies considerably in thickness from 3 mm. to 30 mm. on account of the furrowed nature of the bark, there is a gradual increase in the insolubility of the cell contents. It seems, therefore, that there is a gradual

alteration of the cold-water-soluble colourless tannins to a cold-water-soluble red tannin, then to a hot-water-soluble red tannin, and finally to a red insoluble phlobaphene. Practically all the insoluble cell contents are darkened with ferric chloride, but isolated cells show little or no alteration. When a bark section is treated with ferric chloride, there is an indication of tannin in the cell walls, but this is evidently only due to adsorption of the tannin brought into solution during the examination, since no tannin was shown in the walls when the sections were treated directly with potassium bichromate.

Extraction.—It is not yet suggested that the difference in the ease of extraction of pine and wattle barks is due to the variation of the solubility of the tannin. All barks used for the extraction of tannin are usually broken up into small particles before the introduction into the extraction vats. The smaller the particles, the easier the extraction, provided that the water can pass freely through the mass and wet each particle. It should be noted that although a plant is obtainable for dealing with powdered bark, without exception all Australian tanneries have plants for the extraction of bark in coarse lumps, and these plants, whilst quite satisfactory for the treatment of wattle bark, are apparently unsuitable for pine.

The factor of water-penetration was dealt with by a series of experiments with wattle and pine barks. The first experiment was the extraction of soluble matter from the bark with water at ordinary summer temperature, and also at 50° C. Working under these conditions, one portion of the bark was ground to powder, and another portion was crushed to pieces, as used in the tannery. This gave two comparative tests with fine and coarse bark. If the extraction results were equal, it would be considered as evidence that the forces opposing water penetration were

negligible, but if a considerable difference were shown between the two results, it would indicate that the water penetration and the subsequent diffusion of the tannin from the bark were retarded by unknown factors.

Apparently water passes freely into the bast cells, and it has been noted that the tannin diffuses just as freely from these cells. The size of the cells is approximately 1/600in. x 1/3000in., and 1,500,000 would be required to cover one square inch, so that the grinding of the bark could not be expected to rupture all the cells. Ruptures may occur as a result of contraction of the cell walls after the bark has been dried, but there is no evidence to show that this does take place. Therefore, the theory at present must be one that assumes that the tannin is removed by diffusion through the undamaged walls of the cells. After that the tannin could be expected to choose the path of least resistance and diffuse outwards to places of lower concentration.

The results obtained by extracting coarse and fine wattle bark showed that the fine bark at ordinary temperatures gave up 7.3%, and at 50° C. 2.4% more soluble matter than the coarse. These figures are taken as an indication that the forces opposing water penetration are small so far as wattle bark is concerned.

Different results were obtained with the pine bark, and the experiment was carried out in the following manner: Equal quantities of fine and coarse bark were treated with water which was removed as tan-liquor. This was repeated until five liquors were drawn off the bark. Each liquor was analysed and the sum of the results is shown below. For further details regarding this experiment see "Extraction of Tannin from Wattle Bark."*

* Extraction of Tannin from Wattle Bark. New South Wales Tanning Committee, Technical Gazette, Sydney, Vol. 12, pt. 2, 1922.

Extraction Results.—Pine Bark.

	Ordinary temperature.		Temperature 50°C.	
	Coarse.	Fine.	Coarse.	Fine.
Tannin	19.18 grms.	29.42 grms.	25.34 grms.	33.89 grms.
Non-tannin ..	12.88 „	13.41 „	13.58 „	14.40 „
Total Solubles ..	32.06 „	42.83 „	38.92 „	48.29 „

The maximum amounts of the total tannins and non-tannins extracted were 70 and 83 per cent. respectively.

These returns show that the fine bark at ordinary temperature gave up 33.5%, and at a temperature of 50°, 24% more soluble matter than the coarse bark. This certainly indicates that water penetration into pine bark and the subsequent diffusion of the soluble substance is retarded to a considerable extent, especially when compared with wattle bark.

The total solubles are made up of tannins and non-tannins, and it is important to note that the difference in the above figures is due more to the variation in the amounts of the tannins than to the non-tannins. The latter only show a difference in favour of fine bark of 4.1% at ordinary temperature and 6% at 50° C., while the corresponding figures for the tannin are 53.4 and 33.7%. If the smallest particles of the bark used are constant as regards structure and cell contents, then these figures for tannins and soluble non-tannins would indicate that the reason for a greater and more regular extraction of the total non-tannins in comparison with the total tannin must be due to the tannin being more difficult to bring into solution, or that for certain reasons its diffusion from the bark is retarded to a considerable extent. Further work has shown that the smallest particles are not constant as regards cell contents, and, moreover, the structure of the inner living bark differs from the outer portion, due to the absence of the suberised peridermal layers. These occur

at frequent intervals of from 0.25 mm. to 1 mm.; whereas the pieces of coarsely ground bark often measure up to 10 mm. x 5 mm. x 5 mm.; therefore the opportunities for water penetration in these large pieces are considerably reduced, and can only occur tangentially between the impervious cell layers. It is important to note that in the outer portion, the soluble non-tannins are reduced to a minimum, as will be shown later by analyses, whereas in the inner bark zone which is devoid of impervious layers, and into which water can readily penetrate, the soluble non-tannins reach a maximum. It is easy to understand, therefore, that an extraction of coarse or fine bark removes the soluble non-tannins practically with the same ease; whilst the tannin, which is distributed throughout the whole bark, is obviously less easily removed from the outer portion, apart from any variation in its actual solubility.

The complete removal of the tannin from the bark by the usual methods can only be accomplished with the aid of hot water, but no substance is returned as tannin unless it be soluble in water at 15° to 20°. Catechol tannins which have changed to the insoluble state are classed as phlobaphenes. These phlobaphenes are rarely found in the living secondary phloem, but they undoubtedly exist in the ross, or outer layers, especially in the pine bark.

If ordinary ground bark be covered with water, that portion which is not absorbed soon shows signs that some of the tannin has passed into solution and is diffusing from the bark. If the temperature be kept constant at about 20°, the amount of tannin diffusing from the bark after 24 hours' exposure reaches a very low figure, which can only be increased again at the same temperature by lowering the tannin concentration of the surrounding liquor. This change in concentration is brought about by removing the old and replacing it with a weaker liquor or water.

When the diffusion of tannin from the bark practically ceases it could be assumed that the tannin concentration in the bark structure and surrounding liquor is the same. At this stage if the tannin be all alike as regards its solubility, and omitting adsorption, it should be possible to calculate the total tannin originally in the bark from the concentration and volume of the surrounding liquor, when the volume of liquor retained by the bark is also known. This means that under these conditions the total tannin in the bark could be estimated from the tannin found in a known proportion of the total volume of liquor. This known proportion can only come from the liquor surrounding the bark, and a few experiments have shown that its tannin values are always too low even if the total tannin be calculated as that amount extracted at 20°. When the total tannin is considered as that amount soluble at 20°, but extracted at the usual high temperatures, then this figure is very much too low.

The specific surface of the bark must reach a fairly high figure, as noted by the size of the cells, and therefore adsorption is a factor to be considered when extracting tannin from barks, etc. That the amount of tannin found in the liquor which can be drained away from the bark is always too low to permit a correct volumetric calculation may be largely due to adsorption, and the fact that complete extraction requires water at a high temperature suggests that the theory that a rise in temperature reduces the adsorption power of charcoal may be applied in this case.

The solubility of the tannins must be considered here, and it should be noted that the high temperatures 80° to 100° required for the extraction of tannin soluble in water at 20° have always been considered necessary for a complete extraction. Proctor and Parker* carried out certain

* Proctor and Parker. Jour. Soc. Chem. Ind., 1895, p. 635.

experiments which gave results obtained from various tanning materials when extraction was carried out at various temperatures ranging from 15° to 100°.

They showed an optimum temperature for extraction for each material at which more tannin could be extracted than at any other, though a small additional amount could be obtained by boiling the extracted residue.

If we examine their optimum figure for wattle bark we find that the maximum percentage of tannin extracted was at 70° to 80°, with only a 6% loss for extraction at a higher temperature ranging between 90° to 100° C. This result and others that are more prominent, show that tannin is certainly destroyed at the higher temperatures, and one might say that a satisfactory and workable extraction can only be obtained by using a high temperature, but there will always be a loss of tannin which will reach a maximum for any material when the tannin that can be extracted at the lower temperatures is exposed to the highest temperature, and a minimum when these tannins which are extracted at the lower temperatures are removed and not exposed to the highest temperature.

This optimum temperature, then, is a point where the extra amount of tannin extracted by an increase in temperature just exceeds the amount destroyed when the temperature remains constant for each extraction.

The destruction of catechol tannins by heat is generally recognised, but apparently the pine tannins as they exist in the bark are more sensitive to this factor than the wattle tannins. Extraction experiments with pine bark on a practical scale have shown that the tannin cannot be satisfactorily removed from the usual coarse pieces at a low temperature.

When the higher temperatures were reached for the same work, the extraction results were still too low when compared with results obtained from wattle bark under similar conditions. Analyses of the spent bark and liquors containing extracted tannin show that tannin was destroyed in the pine bark at the higher temperatures.

This result might be described as a want of stability of the pine tannins at a high temperature—tannins changing to the insoluble phlobaphenes—but further experiments have shown that there are other factors.

Proctor and Parker's research shows that tannin is destroyed over a period of a few hours by using a high temperature, and analytical figures are now given for a comparative experiment with four solutions of pine tannin made up from one pine bark liquor. The tannin concentrations were the same for these solutions, but two were exposed on a water bath for four hours at a temperature of 98°. This experiment was expected to show if these pine tannins were affected by heat under conditions of temperature comparable with those prevailing when extracting for analysis. The tannins used for this experiment were extracted at temperatures not exceeding 50°.

	Normal.			4 hrs. at 98° C.	
	1	2		3	4
Tannin	4.40	4.42	. .	4.34	4.36
Non-Tannin.	3.80	3.77	. .	3.87	3.83
Total Solubles. . . .	8.20	8.19	. .	8.21	8.19

These results show that pine tannin extracted at a low temperature and then exposed to a temperature of 98° for three hours, only lost 0.93 per cent. of the total tannin. This seems to show that these tannins are not sensitive to high temperatures, and such a result is not in agreement

with the theory that the large amount of tannin destroyed during the leaching of this bark is due to high temperatures.

One factor in the destruction of tannins during an extraction process might be starch. Microscopical examination has shown that there is a considerable amount of starch in the inner portion of the bark. An unfiltered starch solution, 1 grm. per litre, when added to a solution of pine tannins, gives a cloudy liquor which soon deposits insolubles, and the filtered starch solution when added to a filtered solution of pine tannins, analytical strength, gives a cloudy liquor which deposits overnight.

When a solution of pine tannins is mixed with a starch solution the resulting cloudy solution responds to heating, and the iodine test in a manner similar to that described below.

This starch factor was tested by making up a solution containing a certain amount of tannin and 0.5 grm. of starch, previously treated with water and brought to 100°, then cooled down to 18° and added to the tannin. The solution was then made up to a litre and analysed. For comparative purposes another solution containing the same amount of tannin per litre and no starch was also analysed. The tannins used were obtained from 250 c.c. of a pine liquor extracted at a temperature ranging from 20° to 50°. This pine liquor was a bright, clear solution, and the same volume was used for each test. The following are the results from the duplicate tests:—

	No. 1. With Starch.	No. 2. With Starch.	No. 3. Without Starch.	No. 4 Without Starch.
Tannin	3.166 grm.	3.186 grm.	3.607 grm.	3.589 grm.
Non-tannin ..	2.544 „	2.520 „	2.481 „	2.515 „
Total Soluble ..	5.710 „	5.706 „	6.088 „	6.104 „

The solutions receiving starch show a loss of 11.7% tannin and a non-tannin increase of 1.4%. These starch and tannin mixtures responded to the iodine test, but not after filtering, which indicates that all the starch was precipitated with that portion of the lost tannins shown above. The tannin and starch combination behaved like tannins that remain as suspended matter in a cold solution, but dissolve in sufficient quantity to give a much brighter solution at the higher temperatures.

From the work done on these barks we find that a considerable amount of suspended matter remains in the solution extracted at 100°. This suspended matter was first considered to consist of substances like tannin that are only soluble in hot water, but after our tests with starch we consider that it is possible that a large portion of it is the insoluble tannin and starch combination.

If other tanning materials contain starch in like quantities, then it is possible that a certain proportion of the tannin destroyed, as shown by Procter and Parker's figures with optimum temperatures, could be attributed to starch. Starch granules would not be expected to destroy tannin when extraction proceeds at a low temperature, but this would be reversed at high temperatures suitable for dissolving the starch.

The most important factor noted as far as water penetration is concerned, is that the greater portion of the tannin is in the outer bark, where we find barriers set up by corky layers, which prevent water penetration in certain directions.

Whilst dealing with the question of resistance to water penetration, another factor to be considered is the resinous condition of these barks. These resins occur in passages, as already pointed out, all through the structure, and while the

crushed bark may be smeared with them to a certain extent, there is nothing to show that they are the cause of any great opposition to the penetration of water, although as mentioned in the description of the anatomical structure of the bark, the resinous contents of the ducts are in close proximity to the tannin bearing epithelial cells.

As only a portion of the tannin is extracted at a low temperature, it might be considered that this result is related to a difference in the cell walls, whereby tannin is extracted from some cells with cold water and other cells only give up their tannin when hot water is used. Such an assumption is, however, scarcely warranted, since the tannin is only contained in thin walled cells, which would offer a minimum of resistance to the penetration of water, but which would scarcely show a variable penetration factor at any one temperature.

Bark Layers.—It has been shown by a microscopical examination of the bark that the phloem cells cut off by the cambium, and their contents, are subjected to certain changes as they become older. These changes, as far as the tannin content of the cells is concerned, have been followed by making analyses of certain layers of the bark, it being split longitudinally into three divisions. Thus the innermost living portion was stripped off, and the remainder was again divided into two by the removal of the outermost ross. These layers were numbered in turn, 1, 2, and 3, No. 1 being the innermost, i.e., nearest the cambium. The ross which was removed corresponded to that usually removed by bark strippers when working on a commercial scale, and consists of cells more or less disintegrated on account of exposure to weather.

Samples taken at the end of December, at Dunedoo, N.S.W., three feet from ground.

No. 1 *Tree.* 50 ft. high, 12 inches diameter.

Layer.	Weight.	Tannin.	Non-tannin.	Insoluble.	Water.	Total.
No. 1	107 grm. ..	19.64	19.47	49.39	11.5	100.0
No. 2	1013.7 „ ..	21.07	4.8	62.63	11.5	100.0
No. 3	125.4 „ ..	3.98	3.74	80.78	11.5	100.0
No. 2 *Tree.* 36 ft. high, 7 inches diameter.						
No. 1	106.5 „ ..	20.28	17.68	50.54	11.5	100.0
No. 2	616.5 „ ..	20.48	4.43	63.59	11.5	100.0
No. 3	93.0 „ ..	2.71	3.57	82.22	11.5	100.0
No. 3 *Tree.* 40 ft. high, 12 inches diameter.						
No. 1	153.5 „ ..	28.79	15.56	44.15	11.5	100.0
No. 2	368.1 „ ..	24.42	4.36	59.72	11.5	100.0
No. 3	333.9 „ ..	6.86	2.57	79.07	11.5	100.0

From an examination of these figures it will be seen that the outer portion of the bark has lost, through exposure or due to alteration to an insoluble form, the greater portion of its tannin.

This layer, which is always a dirty grey in colour, can be separated and scraped off without difficulty, using as a guide the fact that as the useful tannin bearing zone is approached, the colour changes to a reddish brown. It is, therefore, suggested that when stripping for commercial purposes this useless outer portion should always be removed, since there is little loss of tannin. A similar portion is removed in America when stripping the Hemlock Bark *Tsuga canadensis.*

The greatest proportion of the bark by weight is that included under No. 2, containing water solubles consisting of red tannins and a small amount of non-tannins. The inner portion, No. 1, contains light coloured tannins almost similar in quantity to those found in No. 2, but with a much higher percentage of soluble non-tannins. Since the No. 1 zone is the living part of the bark, one would expect to find the majority of soluble carbohydrates and nitrogenous materials within this portion, and this is borne out by the analyses.

The leather-forming properties of these pine tannins are not the same as the wattle tannins. This difference is largely due to the tannins found in No. 2 layer. This layer contains from 60% upwards of the total tannin found in the pine bark, and those tannins which give a light, coloured solution when extracted from the inner layer now give a deep red coloured solution, showing that quite an important chemical change has taken place.

In order to obtain a comparison with wattle bark it was considered necessary to carry out a further series of duplicate tests in which the No. 1 zone was again divided into two, thus giving four layers:—1, 1a, 2 and 3; Nos. 2 and 3 corresponding to the same zones as in the first experiments.

	1.	1a.	2.	3.
Weight	11.5 grm.	15 grm.	61.3 grm.	12.2 grm.
Tannin	11.98 „	26.06 „	17.43 „	3.14 „
Non-Tannin	10.63 „	7.43 „	4.46 „	2.8 „
Insolubles	65.89 „	55.01 „	66.61 „	82.56 „
Water..	11.50 „	11.50 „	11.50 „	11.50 „
	100.00 „	100.00 „	100.00 „	100.00 „
Tannin	16.61 „	34.51 „	24.75 „	5.23 „
Non-Tannin	10.64 „	8.72 „	4.60 „	2.74 „
Insolubles	61.25 „	45.27 „	59.15 „	80.53 „
Water	11.50 „	11.50 „	11.50 „	11.50 „
	100.00 „	100.00 „	100.00 „	100.00 „

It is seen from these results that the portion nearest the cambium (No. 1), in which the tissues might be considered to be most actively engaged in the transportation of food materials, contains a comparatively low tannin content with a fairly high soluble non-tannin figure, when compared with zone 1a. This result is to be expected, and corresponds

exactly with what was found to obtain in the wattle barks (c.f. Welch, McGlynn and Coombs). The insolubles are higher in No. 1 than in No. 2, but this is accounted for by the fact that, owing to the considerable increase which occurs in the size of the parenchymatous tannin bearing cells, the relative amount of cell wall to cell content would be decreased. Similarly the proportion of volume occupied by thick walled fibres is considerably lowered.

In the third layer, zone No. 2, there is a very definite drop in the soluble non-tannins in comparison with zones 1 and 1a, and also a decrease in tannin with respect to No. 1a.

It is suggested that this reduction in tannins would be accounted for by the decrease in solubility towards the outer limit of this zone reducing the average; the decrease in soluble non-tannins corresponds with that found in the first series of tests, but is more pronounced.

It is interesting to compare the comparatively low figure for non-tannins, averaging approximately 9% for zones 1 and 1a, with the high figure of 15.56-19.47% obtained in the first series; it seems probable that the difference in the time of collection might account for this.

Zone No. 1 gave a very cloudy solution which responded to the iodine test for starch, whilst that from zone No. 1a was less cloudy and contained a smaller amount of starch. By raising the temperature it was possible to reduce the suspended matter to a considerable extent in both these solutions. The filtered solutions did not respond to the iodine test. Layers No. 2 and 3 contained less suspended matter and did not respond to the iodine test before or after filtering.

TANNIN VALUES OF PINE BARK SAMPLES.

Dunedoo, N.S.W., December, 1920.

Samples taken about 2 feet from ground.

	Diameter of tree.	Tannin.	Non-tannin.	Insolubles.	Water.
No. 1 ..	15 inches	21.34	6.62	60.54	11.50
„ 2 ..	12 „	20.93	6.21	61.36	11.50
„ 3 ..	12 „	22.63	9.09	56.78	11.50
„ 4 ..	12 „	25.41	6.90	56.19	11.50
„ 5 ..	9 „	18.24	18.14	62.12	11.50
„ 6 ..	7 „	20.45	6.38	61.67	11.50
„ 7 ..	5 „	34.11	8.27	46.12	11.50
„ 8 ..	4 „	26.41	7.52	54.57	11.50
„ 9 ..	4 „	36.37	10.18	41.95	11.50
„ 10 ..	3 „	18.58	7.25	62.67	11.50
„ 11 ..	3 „	32.27	11.46	44.77	11.50
„ 12 ..	1½ „	26.15	10.20	52.15	11.50

Cookamidgera, N.S.W., June, 1923.

Samples taken 2 feet from ground.

No. 1 ..	–	21.25	6.30	60.95	11.50
„ 2 ..	5 inches	27.28	9.18	52.04	11.50
„ 3 ..	11 „	22.19	5.53	60.78	11.50
„ 4 ..	5 „	19.57	6.38	62.55	11.50
„ 5 ..	6 „	36.29	7.92	44.29	11.50
„ 6 ..	–	21.31	7.24	59.95	11.50
„ 7 ..	8 „	26.12	5.40	56.98	11.50
„ 8 ..	14 „	32.55	5.22	50.73	11.50
„ 9 ..	10 „	33.67	7.09	47.74	11.50

These analyses were made to ascertain whether the diameter of the tree has any effect on the tannin content. In any one locality, diameter might be considered to vary approximately as the age of the tree, provided ecological conditions were similar.

It seems that in general the highest tannin content obtains in the smaller trees, although exceptions to this occur. From the results obtained from the Cookamidgera samples it seems that the highest yields are from trees

growing on level ground at the foot of the ridges or on the slopes.

It is obvious that many more analyses are necessary before any definite conclusions can be made, but our experience shows that the best results are obtained from well grown trees, and are probably independent of actual elevation.

The conditions of growth are important, e.g., whether the trees are in close or open stands, more especially since the closer stand produces fewer lateral branches, and is easier to strip; secondly the consequent greater height increment would produce trees yielding a greater amount of bark.

Comparative Tannin Value of Bark taken from bottom and top of Tree.

Samples collected at Dunedoo, N.S.W., December, 1920:—

No. 1 *Tree,* 50 *feet.*

Samples taken as follows:—

A at 2 ft. from ground—diameter 12 inches
B at 13 ft. „ „ „ 8 „
C at 23 ft. „ „ „ 5 „

	Tannin.	Non-tannin.	Insolubles.	Water.
A	20.93	6.21	61.36	11.50
B	21.38	8.74	58.36	11.50
C	20.21	9.71	58.58	11.50

No. 2 *Tree,* 30 *feet.*

	Tannin.	Non-tannin.	Insolubles.	Water.
A at 2ft.: diam. 7″	20.45	6.38	61.67	11.50
B at 10ft.: „ 5″	19.76	7.82	60.92	11.50
C at 18ft.: „ 4″	19.70	8.46	60.34	11.50

No. 3 *Tree,* 50 *feet.*

A at 3ft.; diam. 12″	22.63	9.09	57.4	11.50
B at 40ft.: „ 4½″	16.87	11.08	60.56	11.50

No. 4 *Tree,* 40 *feet.*

A at 3ft.: diam. 9″	18.24	8.14	62.13	11.50
B at 30ft.: „ 4″	19.75	12.10	58.84	11.50

These analyses were made to determine whether the percentage of tannin in the bark varied towards the top of the tree.

With the exception of No. 3 in which there is a considerable decrease in the tannin content at the higher elevation, there is apparently little variation in the figures obtained. This is in very marked contrast to the Acacia barks, in which there is a very definite decrease as the bark becomes thinner, but is readily explained when it is understood that practically the whole of the Acacia bark consists of living cells of the secondary phloem and cortex, and the maximum tannin bearing zone is towards the outside; in the Callitris, however, the maximum tannin bearing zone is within a few mm. of the cambium (i.e., in the outer part of the living secondary phloem within the innermost periderm), outside which there is a progressive decrease in the soluble tannin.

Since this inner zone is only a comparatively small percentage of the whole bark, and does not vary greatly in thickness along the tree, it is evident that it cannot affect to any great extent the result obtained by analysing the complete bark.

In every case there is an increase in the soluble non-tannin, and a corresponding decrease in the "insolubles" towards the upper part of the tree. This would be accounted for by the fact that the proportion of inner living bark to dead tissues is highest in the uppermost portions, and it is principally in the living secondary phloem that the soluble non-tannins are present.

Commercial Samples.

The following results are important since they indicate the actual results likely to be obtained when stripping on a large scale.

	Tannin.	Non-tannin.	Insolubles.	Water.
(1) From a ten ton lot	23.34	9.21	55.95	11.5
(2) Sample taken from 10 cwt. of bark. Trees above 8in. diam.	25.29	8.53	54.68	11.5
(3) Sample taken from 10 cwt. of bark	25,36	8.27	54.87	11.5

Summary.—Callitris calcarata yields an important tannin-bearing bark available in large quantities. The bark or secondary phloem consists of two main portions—an inner living zone about 4 mm. in thickness, and an outer non-living zone, separated by a narrow periderm. These periderms are a few cells in thickness, and occur at intervals of from 225-1,200μ throughout the outer zone.

The secondary phloem consists of very regularly arranged layers of bast fibres, sieve tubes and phloem parenchyma. At irregular intervals are more or less concentric rings of tangentially anastomosing resin passages.

Tannin occurs principally in the phloem parenchyma, in the medullary ray cells, and in the epithelial cells lining the resin passages. It was not observed in the bast fibres or sieve tubes. The tannin is readily soluble in water in the inner living bark, but becomes progressively less soluble in the outer portion, finally becoming practically insoluble towards the outside; the cells being filled with a brownish amorphous phlobaphene-like body.

Starch grains were observed in the living cells of the inner zone in which tannin was present. No starch was found outside the innermost peridermal layer.

The extraction of the tannin on a practical scale gave results which showed that a considerable amount was destroyed. The reason for this destruction can be found by taking into consideration the methods of extraction for analysis. When the bark is ground to a fine powder, a large excess of water is used, and the temperature is con-

trolled so that only a small portion of the total tannin is exposed to higher temperatures. Working on a commercial scale, large particles of bark are used with a minimum amount of water and a longer exposure to high temperatures.

Experiments with fine and coarse bark particles showed that the resistance to water penetration and to tannin diffusion increases considerably with larger particles, in contradistinction to wattle. Therefore, for satisfactory commercial extraction, pine bark should be finely ground.

It is not considered that the presence of resin is a serious factor in the resistance to water penetration; rather is this due to the closely arranged impervious corky peridermal layers through which water cannot penetrate.

Pine barks are not sensitive to high temperatures, and some other factor must be sought to explain the loss of tannin in large scale extraction. It has been shown that starch is present, and this would be brought into solution at the high temperature used, and further, it has been proved that starch is able to destroy a considerable amount of tannin.

It is suggested, therefore, that since starch is extracted during the analytical process, prolonged exposure at a high temperature would bring about the removal of a greater amount of starch, and hence an increase in the amount of tannin destroyed.

The specific surface of the bark would reach a high figure, but further investigation is required to show what influence adsorption would have on the resistance offered to extraction. Possibly adsorption is responsible for the high temperature required for the satisfactory extraction of the bark.

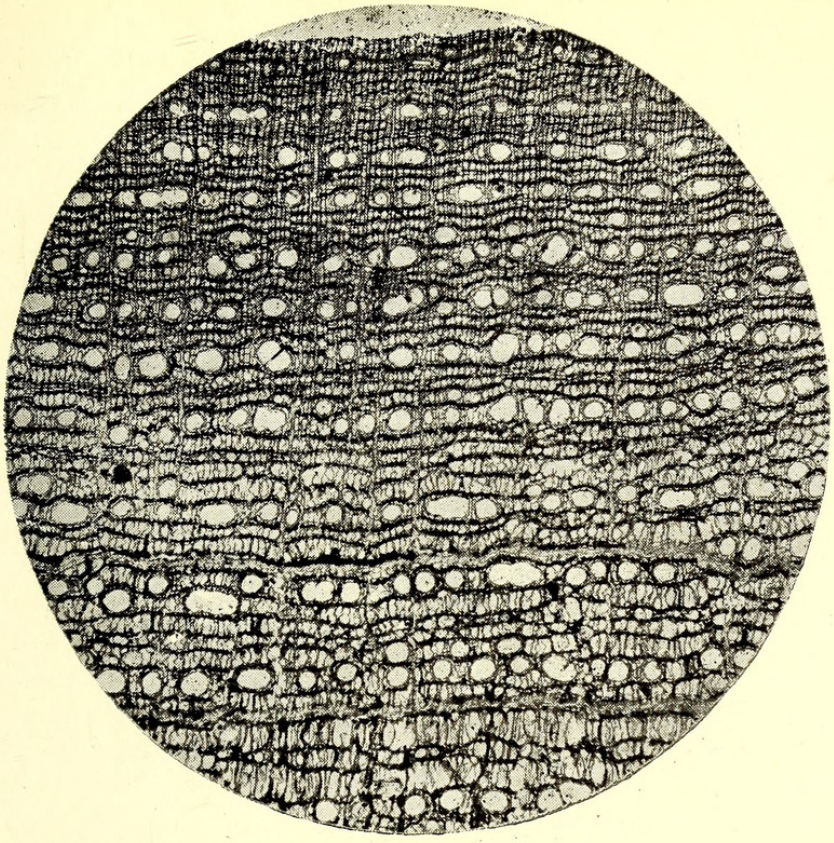

Fig. 1.

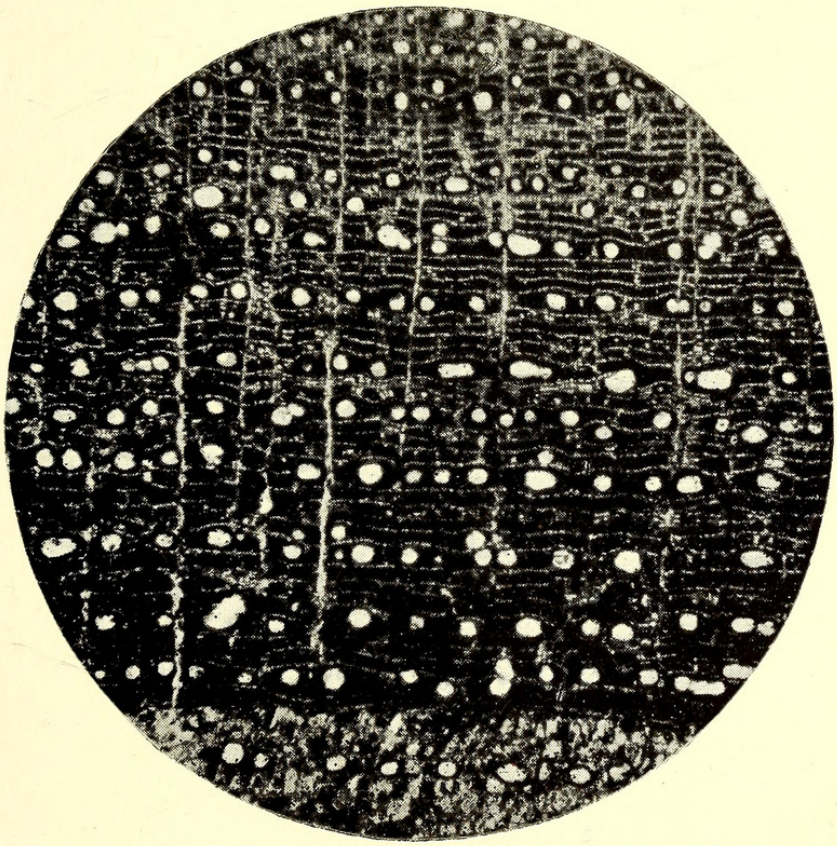

Fig. 2.

Callitris calcarata.

Analyses of different layers of the bark showed that the maximum tannin content occurred towards the outside of the inner zone, whereas the "soluble non-tannins" reached a maximum near the cambium. Outside the inner periderm there was a decrease in the tannin, which reached a minimum at the periphery. The tannins in the ross of pine bark, however, represent 60% of the total, and it is the predominating influence of these red tannins which give the distinctive features peculiar to a leather obtained with a straight pine tannage.

Analyses made on bark samples from different sized trees seem to show that the maximum tannin content is found in small, well-grown trees. There is practically no variation in the tannin content of the bark removed at different heights on the tree, in this respect differing considerably from wattle. Although analyses of individual barks have shown up to almost 37% tannin, the figure obtained from commercial samples is in the vicinity of 20-25% tannin.

In conclusion we wish to record our thanks to the Forestry Commission, N.S.W., Mr. A. R. Samuels, District Forester, Dubbo, and Messrs. Fitzpatrick and Greatrex for assistance in obtaining samples for investigation.

Explanation of Plates.

PLATE XVII.

Fig. 1. Transverse section of inner bark of *Callitris calcarata* R.Br. (cambium at top) after removal of the tannin, showing the regular structure. Near the bottom are portions of two concentric peridermal bands which occur at more or less regular intervals throughout the non-living secondary phloem. Numerous resin canals are clearly shown. The considerable increase in size of the phloem parenchyma cells can be traced from the cambium until they reach a maximum outside the second periderm. x 14.

Fig. 2. Transverse section of portion of bark of *Callitris calcarata* R.Br. (cambium at top) showing distribution of tannin.

Near the bottom of the microphotograph there is an apparent marked difference in tannin content between the upper living and the lower non-living zones. This is due to the fact that the contents of many of the cells of the latter zone have been broken away in sectioning. The tannin is seen to occur right to the edges of the resin canals, which appear as rounded openings. The verical clear marks are due to the non-occurrence of tannin in all cells of the medullary rays. It is evident that comparatively little tannin occurs in the tissues nearest the cambium. x 17.

PLATE XVIII.

Fig. 3. Transverse section of portion of inner living secondary phloem of *Callitris calcarata* R.Br. The regular arrangement of the alternating layers of bast fibres, sieve-tubes and phloem parenchyma is apparent. The epithelial cells lining the resin canals can be seen. At right angles to the bast fibre layers are several uniseriate medullary rays. x 110.

Fig. 4. Transverse section of portion of bark of *Callitris calcarata* R.Br., showing distribution of tannin, under a greater magnification. No tannin is seen in the sieve-tubes or bast fibres, and little in the medullary rays. The occurrence in the phloem parenchyma and in the epithelial cells of the resin canals is strongly marked. It is important to note the presence of starch grains, seen as small rounded, almost clear bodies in the tannin bearing cells. x 120.