A PRELIMINARY INVESTIGATION OF THE CHEMICAL NATURE OF WATTLE TANNIN.

by

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A C K N O W L E D G E M E N T S:

Thanks are due to THE NATIONAL RESEARCH BOARD and COUNCIL for a Scholarship without which this work could not have been undertaken.

Also to PROFESSOR W.F. BARKER, B.Sc., Ph.D., F.I.C., F.R.S.(S.A.), and DR. A.S. GALLOWAY, B.Sc., Ph.D., for their interest and suggestions during the course of the work.

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C O N T E N T S.

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EXPLANATORY NOTE.

In 1940 research on the Chemistry of Leather and Tanning processes was being carried out at Rhodes University College in the Tanning, Hides and Skins Research Department attached to the Department of Chemistry. Among the problems for investigation that of the chemical nature of the tannin in black wattle bark extract (Acacia mollissima) naturally figured. At Professor Barker's suggestion the writer undertook some of the preliminary work in this field, and in the period of one year it was not to be expected that the research would do more than indicate lines for further investigation.

Circumstances arose which made it impossible for the writer to proceed immediately with the compilation of this thesis and during the period between the cessation of the experimental work and the present time, when the first opportunity of writing up the work has arisen, the Tanning, Hides and Skins Research Department has grown into the Leather Industries Research Institute. For a period of three and a half years members of its staff have been fully occupied in specialised study of the chemistry of wattle bark extract. The work herein reported has therefore been largely duplicated, extended and to some extent superseded, but is presented as an account of pioneer work in a field which has since been extensively investigated and which is in consequence now much better understood.

At the end of this thesis some of the later results obtained at the Leather Industries Research Institute are summarised briefly.

- I N T R O D U C T I O N -

Although the tannins of wattle bark extract are generally considered to be of the class known as condensed tannins, a review of the general chemical nature of the tannins is given for the sake of completeness. This constitutes Part I of the thesis.

Part II describes the preliminary investigation of the chemical nature of black wattle extract.

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- PART I -

THE CHEMICAL NATURE of the NATURAL TANNINS.

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THE CHEMICAL NATURE OF THE NATURAL TANNINS.

INTRODUCTION:

The members of the group of naturally occurring compounds known as the "tannins" have a very wide distribution in the vegetable kingdom and vary greatly in their chemical nature.

Plant physiologists have been unable to attribute definite physiological functions to these substances notwithstanding quite extensive investigation. A few instances in which they have been found to fulfil a useful and necessary function in the plant have been reported, but such cases are very limited in relation to the widespread occurrence of tannins as constituents of plants. Their production in rapidly growing parts such as unfolding buds, germinating seeds, developing fruits and galls, their abundance in many barks, a part of the plant usually excluded from the nutritional system, as well as their association with secretory organs and pulvini, support the conclusion arrived at by Sacks that the tannins are results of metabolism rather than links in its mechanism.

CLASSIFICATION:

Several schemes of classification have been suggested (1). Earlier ones such as those based on the pathological or physiological origin of the tannin or on the colours which they produce with ferric salts are now generally considered obsolescent. Of the later schemes there are two in general use, that of Freudenberg and that of Perkin and Everest (2).

The Freudenberg classification divides the tannins into two classes, namely "hydrolysable tannins" and "condensed or phlobatannins". The description of the great majority of the known tannins as condensed tannins is perhaps hardly justifiable, as a large number of them have not been fully investigated. Maitland (3) suggests the addition of a third group, the "unclassified tannins", in which he includes for the time being the majority of natural tannins.

Russell (4) considers that eventually the phlobatannins (i.e. phlobaphene forming tannins), which constitute the overwhelming majority, will come to be regarded as the only true natural tannins, the others being regarded simply as plant products which also possess the property of tanning leather.

The Perkin and Everest classification distinguishes between hydrolysable tannins which are related to the depsides, i.e. gallotannins, and those which are related to diphenyl-dimethylolid, i.e. ellagitannins. Theoretically this distinction is quite logical, as these groups are structurally quite different, but practically the distinction is complicated in its application by conflicting evidence presented by various workers.

HYDROLYSABLE TANNINS.

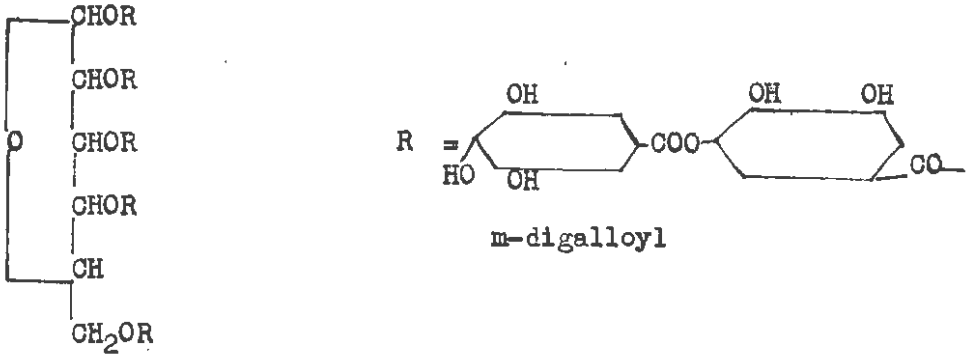
These are amorphous in character, and their purification is invariably accompanied by hydrolysis, making the determination of their exact constitution a very difficult task.

Hydrolysable tannins include the gallotannins, ellagitannins and caffetannins.

GALLOTANNINS.

Quite often distinction is made between Chinese gallotannin from Chinese galls and Turkish gallotannin, but Russell (4) and Karrer (7) suggested that these tannins may be mixtures of compounds of the same type, differing only in detail.

A number of workers, amongst them Strecker (5) and E. Fischer (6) showed that gallotannins are really glucosides. By considering the products of hydrolysis Fischer suggested penta m-digalloyl glucose (I) as the ideal formula for Chinese gallotannin and penta galloyl glucose as the ideal formula for Turkish gallotannin. He compared the synthetically prepared α and β penta m-digalloyl glucoses with natural Chinese gallotannin and the comparison, although difficult owing to the amorphous nature of the compounds, showed a satisfactory degree of agreement.



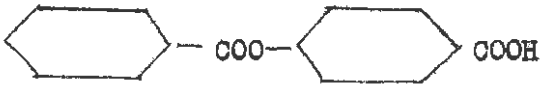
(I)

Natural gallotannins are usually associated with some free glucose, digallic acid, gallic acid and ellagic acid (II). Evidently m-digalloyl glucose is the possible origin of all these compounds. For example, it can give rise to ellagic acid on oxidation as follows :

m-digallic acid ellagic acid.

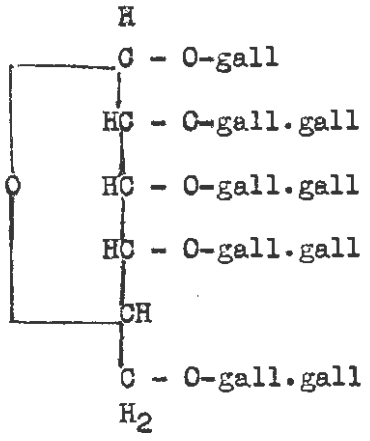
(II)

The gallotannins are related to the depsides, which are derivatives of p-benzoyl benzoic acid (III).

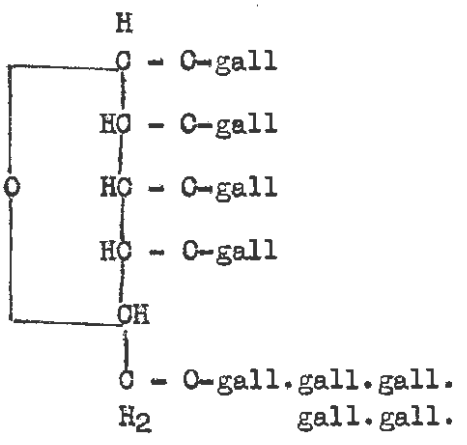


(III).

Experimental evidence has shown that gallotannins are mixtures of related compounds. Freudenberg has suggested that Chinese gallotannin is a mixture of glucosides with nine galloyl radicals, the alternative limiting possibilities of the arrangement of the galloyl groups being represented by formulae (IV) and (V).



(IV)

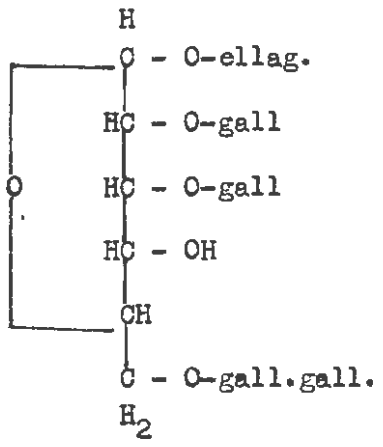


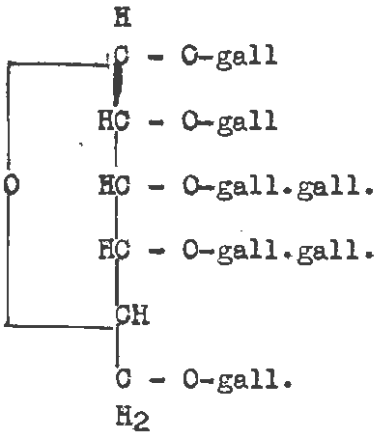
(V)

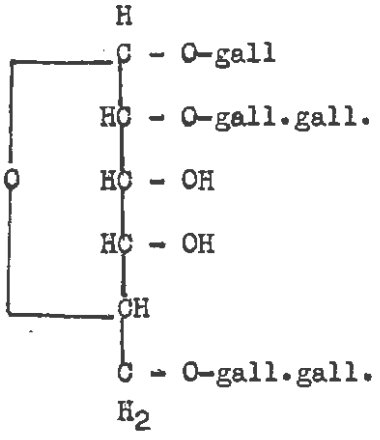
gall = galloyl

gall.gall = m-digalloyl.

Karrer (7) suggested that Turkish gallotannin is a mixture of glucosides, the formulae below representing possible types.



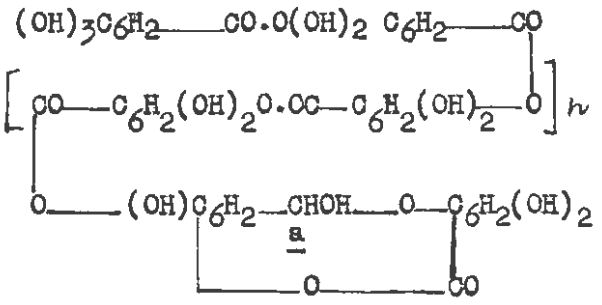




(VI)

ellag. = ellagic acid.

On the other hand Nierenstein (8) maintained that Chinese gallotannin is a poly-digalloyl leucodigallic acid anhydride (VII), and that it may be a glucoside but is not necessarily so. The glucose would be attached to position "a":



(VII)

Various other hydrolysable plant products are reported under the name "tannin", although they have not been shown to be true tannins. Two such products appear to be related to gallotannin, namely Acer-tannin and Hamameli-tannin. They are both crystalline, and acer-tannin yields by acid hydrolysis one molecule of aceritol and two molecules of gallic acid (9), and Hamameli-tannin an undetermined amount of gallic acid and an unidentified hexose (10).

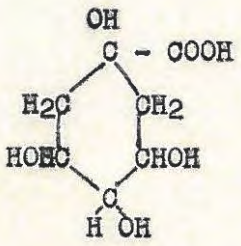
ELLAGITANNINS.

Here again the nature of the tannins shows them to be complex mixtures of compounds with ellagic acid (II) (a tetrahydroxy diphenyl-dimethylolid) as the essential constituent. It yields on hydrolysis ellagic acid and glucose and is therefore probably a mixture of glucosides of ellagic acid. However Nierenstein (11) considers that some specimens of ellagitannin are mono- or poly-galloyl derivatives of ellagic acid, and contain no sugar, while Reichel (12) considers that in some cases the gallic acid is carried in depsidic bond by the ellagic acid.

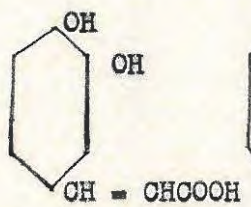
CAFFETANNINS.

The extracts of different coffee beans are called caffetannins, but they do not precipitate proteins and are thus not really tannins.

By the hydrolysis of caffetannins quinic acid (VIII), caffeic acid (IX) and a residue of unknown composition are obtained. The two acids arise from the hydrolysis of chlorogenic acid (X), the structure of which was established by synthesis.



(VIII)



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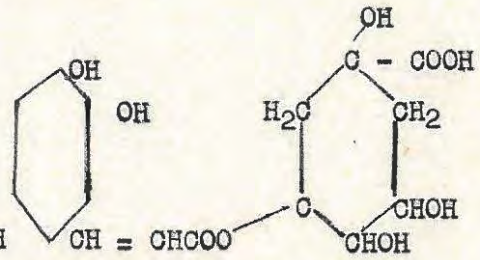


(X)

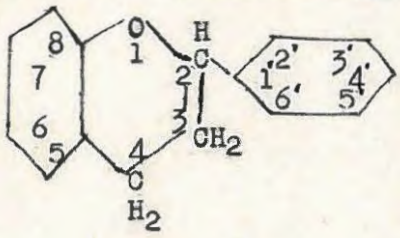
The tannins may thus be derivatives of chlorogenic acid or a mixture of several substances, amongst them chlorogenic acid.

Before going on to the discussion of the condensed tannins it is convenient to mention tea-tannin. It was reported by some workers as a hydrolysable tannin and by others as a condensed tannin. Tsujimura (13) suggested that the tannin is galloyl catechin, and Nierenstein (14) obtained a "well-crystallising" tannin from green Assam tea and found that it gave on hydrolysis three molecules of gallic acid and one of a catechin. The condensation of this catechin produces a substance which may well be reported as a condensed tannin. Careful work may reveal more tannins of this nature, thus leading to the explanation of apparently conflicting results.

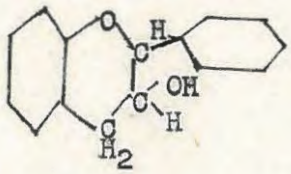
CONDENSED TANNINS.

Condensed tannins are related to the normal plant pigments of the benzopyrane type, as can well be expected from the occurrence of the two groups side by side in plant tissues.

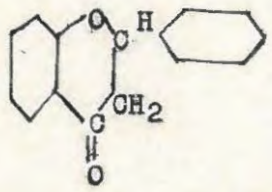
The benzopyrane group of plant compounds includes catechins, anthocyanidins, flavones, flavanones and flavonols, and a brief summary of the structures and relationships of these compounds is given.



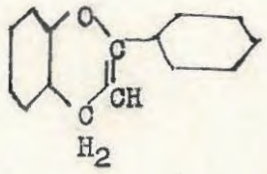
FLAVANE
(2-Phenyl Benzopyrane)



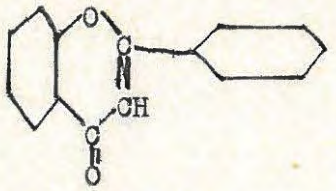
FLAVANOL 3 Hydroxy derivatives are known as catechins.



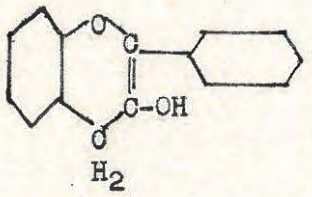
FLAVANONE Hydroxy derivatives are known as Flavanones.



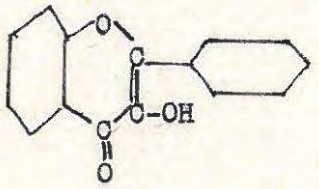
α FLAVENE



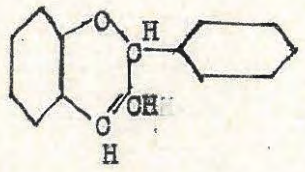
FLAVONE — Hydroxy derivatives are known as Flavones



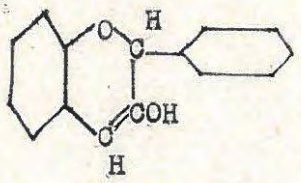
∝ FLAVENOL 3



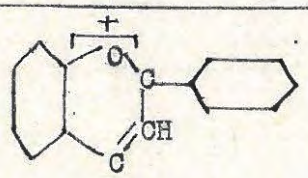
FLAVONOL — Hydroxy derivatives are known as Flavonols.



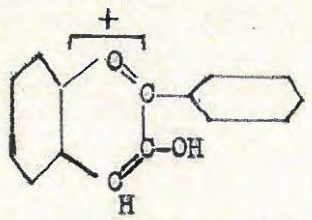
β FLAVENE



β FLAVENOL 3 — Hydroxy derivatives are known as Leucocyanidins.



FLAVYLIUM ION

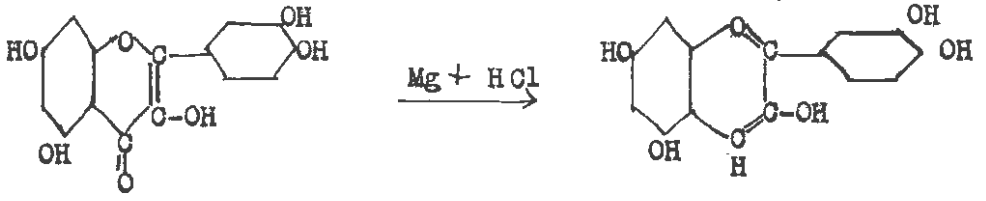


3 HYDROXY-FLAVYLIUM ION — Salts of hydroxy derivatives are known as Anthocyanidins.

The interconversions of all these groups correspond to reductions or oxidations of the heterocyclic ring, and the reduction of flavonols, flavonee and flavanones has been effected experimentally.

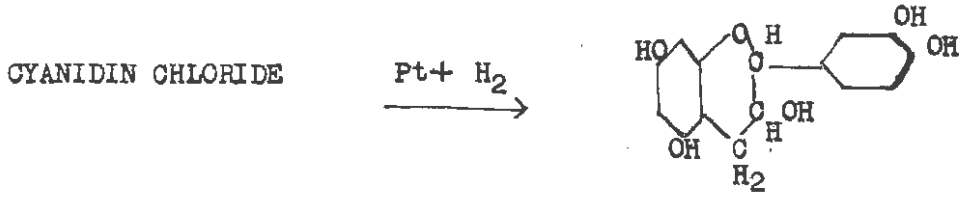
The reduction of flavonols:-

Quercitin was reduced to cyanidin chloride by Willstäter and Mallison in 1914 (15), as follows :



QUERCETIN CYANIDIN CHLORIDE

Cyanidin chloride was further reduced to cyanidol by Freudenberg (16) in 1925, using platinum black as catalyst



CYANIDOL
(= ordinary Catechin.)

By this method, Freudenberg in 1934 reduced fisetinidin chloride to fisetinidol and butinidin chloride to butinidol and in 1936 Oshima reduced delphinidin to delphinidol or teacatechin.

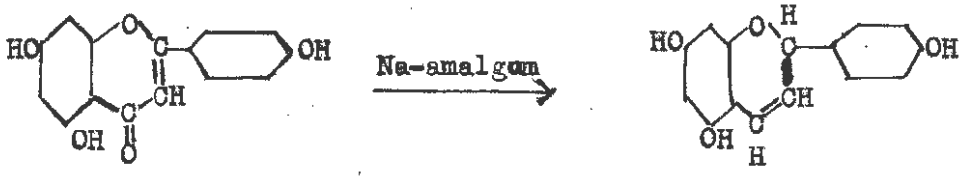
In 1927, Freudenberg found that by catalytic hydrogenation of quercetin pentamethyl ether it could be converted directly to epicatechin pentamethyl ether. Working on the interconversion of anthocyanidins and catechins he found that the hydrogenation of acetylated anthocyanidins led to the formation of intermediate compounds, which he called hydro-anthocyanidins, the de-acetylation of which produced amorphous "tannins".

He considered the occurrence of these amorphous products as constituents of natural tannins to be very possible.

The reduction of flavones and flavanones:-

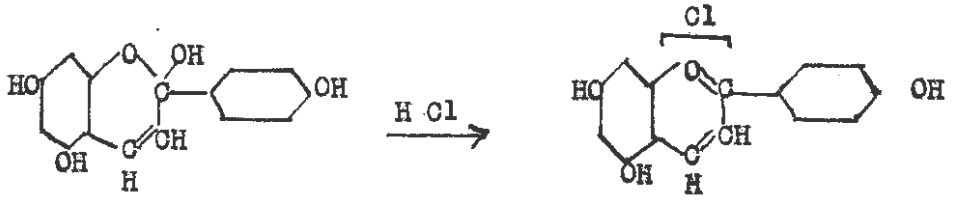
Asahina and Inubuse (17) carried out the reduction of flavones and flavanones to anthocyanidins by the use of sodium amalgam, in 1928.

FLAVONE REDUCTION:



APIGENIN
(a flavone)

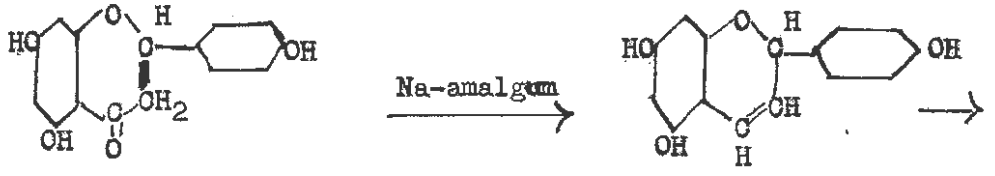
FLAVENOL
(a leucoanthocyanidin)



CARBINOL BASE

APIGENIDIN CHLORIDE
(an anthocyanidin)

FLAVANONE REDUCTION:

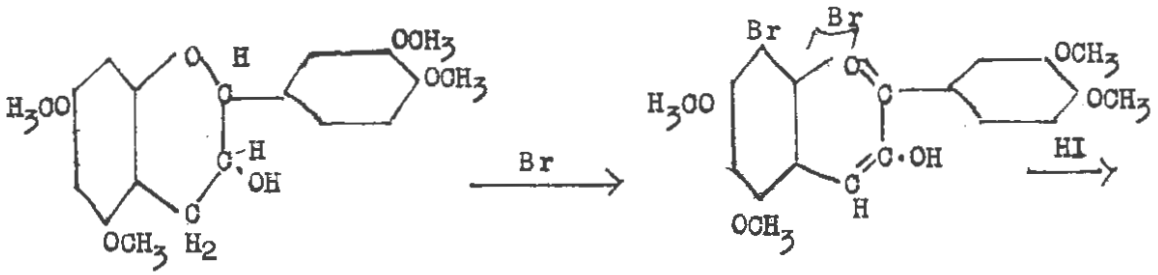


NARINGENIN
(a flavanone)

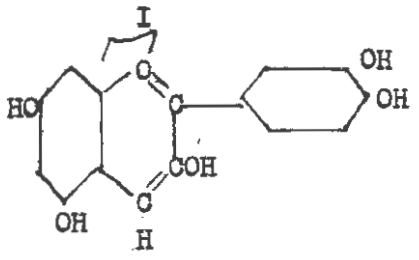
a flavenol

Carbinol base $\xrightarrow{H\,Cl}$ Apigenidin chloride.

The oxidation of a flavanol to an anthocyanidin was carried out by Appel and Robinson in 1935 (18), as follows :-



CYANIDOL TETRAMETHYL ETHER



CYANIDIN IODIDE.

As the physiological relationships had been by no means clarified, Reichel undertook the study of the interconversions of the benzopyrane group (19). Together with Burkart he was able to show that even such a powerful oxidising agent as hydrogen peroxide was unable to oxidise flavanones to flavones or flavonols, nor could he oxidise catechins to anthocyanidins. Attempts to reduce flavanones to anthocyanidins or flavanes phytochemically by means of yeast were also unsuccessful. On these grounds they concluded that these conversions were carried out in plants through the agency of a redox system as yet unknown or that the compounds were synthesised directly from simpler substances.

On investigation of the phytochemical reduction of anthocyanidins the first products obtained were flavenols(leucoanthocyanidins). On exposure to air the latter soon redeveloped the red colour of anthocyanidins, showing that reduction to this stage was easily reversible. The final products of the reduction were catechins in the form of amorphous

condensation products. Contrary to Willstätter's observations (20) they found that cyanidin chloride formed by reduction of anthocyanidins with hyposulphite or zinc and acetic acid was readily reoxidised to the original anthocyanidin by air.

From their work they concluded that anthocyanidins in the plant act as redox systems similar in character to flavines, halochrome, pyocyanine, etc., and that when the anthocyanidins are no longer of use as hydrogen acceptors they are transformed to catechins. The occurrence of catechins in the autumn leaves of the wild vine was adduced in support of this theory.

The exact relationship between the natural benzopyrane type of compounds and the condensed tannins is still an unsettled problem. The two chief investigators in this field of research, Russell and Freudenberg, hold different views.

Freudenberg's theory is that each phlobatannin is a polymer of that 3-hydroxy poly-hydroxy flavane which gives fission products corresponding to those given by the tannin itself. This theory was suggested by the following facts:

(a) Crystalline catechin, although it does not precipitate gelatin, easily polymerises or condenses to an amorphous tannin-like substance which could not be distinguished, qualitatively or quantitatively, from the amorphous tannins of catechu and gambier with which the catechin occurs in nature.

(b) Degradation products from these amorphous tannins and from crystalline catechin on the one hand, and from the related anthocyanidins and flavonols on the other, are identical.

(c) Some condensed tannins are accompanied by corresponding flavonols, e.g. quercetin accompanies catechin and the amorphous tannin of horse chestnut, all three giving the same fission products; and myrcetin accompanies maletto tannin. This suggests that any member of the benzopyrane group of compounds found accompanying a tannin affords an indication of the phenolic pattern of some of the units of the tannin.

For a more detailed study of this relationship Freudenberg chose the well-known extract of the wood of Quebracho colorado. In 1925 he suggested that the catechin which, when condensed, forms quebracho tannin is 3:7:3':4': tetrahydroxyflavane, since the fission products of the tannin are chiefly resorcinol and protocatechuic acid. Together with Maitland (21) he synthesised this catechin, which he called "quebracho catechin" or "fisetinidol", and found it to be like catechin in many respects, especially in that it coloured very slowly in the air and was easily converted into amorphous condensation products. It was actually more sensitive to condensation than catechin, which they suggested, may be due to the presence of an extra hydroxyl group in catechin which reduces the number of active condensation places or introduces more steric hindrance.

Knowing the properties of quebracho catechin they made a careful search for it in both the wood and leaves of Quebracho colorado, but could not detect even a trace of it.

The mechanism of condensation of the catechins was then considered. Bergmann and Pojarlieff (22), in investigating the so-called "phlobaphene reaction", i.e. condensation without the splitting out of water, found

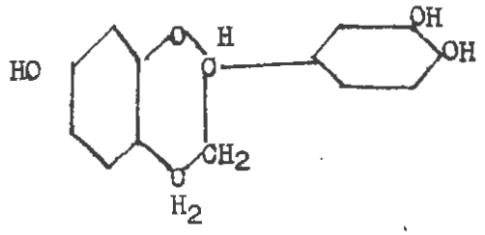
that three factors are necessary if the condensation is to take place, namely :

(i) a pyrane ring;

(ii) a double bond in a pyrane ring;

and (iii) an OH attached to the pyrane ring.

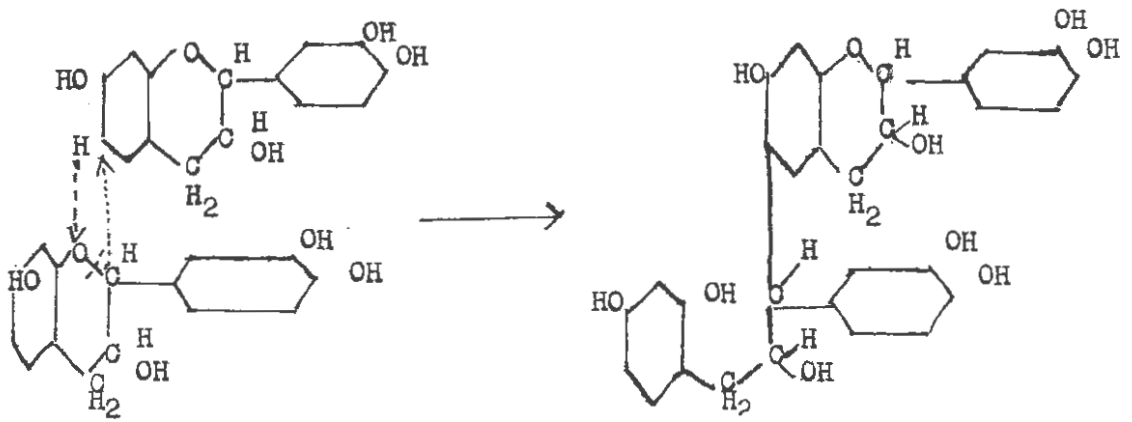
Freudenberg and Maitland therefore prepared 7:3':4'; trihydroxy-flavane (XI) to find out whether the OH group in the pyrane ring is essential for the condensation in question.



(XI)

They found this compound to be even more sensitive to condensation than quebracho catechin, and concluded that the bridging oxygen atom in the pyrane ring is the starting point of the condensation.

Examining the condensation further, they heated synthetic quebracho catechin for four hours with dilute acid until it was converted to a product insoluble in water. Comparison of the carbon, hydrogen and acetyl contents of acetylated products showed that no water had spilt out during the condensation. The mechanism proposed involves opening of the pyrane ring with simultaneous condensation, according to the following scheme:

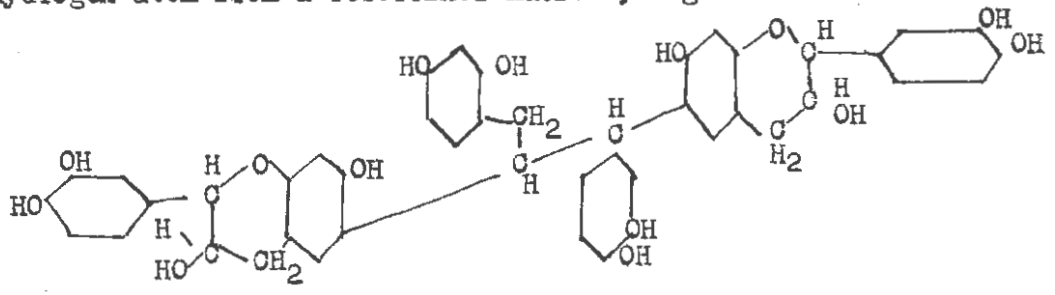


This condensation product has another bridging oxygen atom similar to the one just used in the condensation, and the molecule is thus capable of indefinite extension in this way. This may explain the high molecular weights obtained for quebracho tannin.

That the resorcinol and not the pyrocatechol nucleus was involved in the condensation was supported by the fact that oxidation of methylated synthetic quebracho phlobaphene gave only veratric acid, showing that the pyrocatechol was mostly unaffected.

Other instances of such a type of condensation are those of coniferyl alcohol, of glucals and also of dioxane with lignin.

When the action of dilute acid on the condensation product of the quebracho catechin was continued for twelve hours, there occurred a secondary condensation with the splitting out of water. They reported that "the reaction did not proceed like that in which a secondary hydroxyl splits off with a hydrogen atom from a neighbouring carbon", and suggested that a secondary hydroxyl group had split out along with a hydrogen atom from a resorcinol nucleus, e.g.



In the case of ordinary catechin, the secondary condensation with loss of water took place much more readily than in the case of quebracho catechin. This again might be explained by the presence of an extra hydroxyl group in catechin.

Comparison of the results of analysis of the condensed quebracho catechin and natural quebracho tannin showed fair agreement in the hydrogen content and acetyl number, but the carbon content of the natural tannin was found to be 3% lower than that of the synthetic product. This was also found to be the case when condensed catechin and gambier tannin were compared. To explain this they suggested the presence of sugar-like substances in the natural products or the occurrence of oxidation during the condensation in nature of the catechins. Further they observed a small decrease in the acetyl number on prolonged heating of the tannin with acid, corresponding with the secondary condensation with loss of water undergone by the synthetic products. Summarising, they say: "The properties of the synthetic quebracho catechin and its condensation product resemble those of quebracho tannin so markedly that this tannin can be regarded as derived chiefly from quebracho catechin."

The application of this theory to other condensed tannins, such as the tannin of wattle extract, because of their similarity to quebracho tannin, must however be made with reserve. There are however indications, that the theory can be applied to some of the other members of the class of condensed tannins. Amongst these indications are the following :-

(1) Freudenberg examined an amorphous tannin from Dicanopteris glauca which he suspected was a condensation product of pelargonidol. By direct hydrogenation of pelargonidol chloride a tannin of this type was obtained, but not even a trace of the highly sensitive pelargonidol itself could be found in nature.

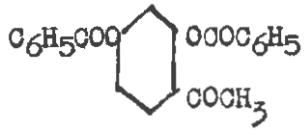
(2) For a long time the only member of the catechin group which could be isolated from natural products was cyanidol, but recently

gallocatechin (delphinidol, i.e. 3:5:7:2':3':4': hexahydroxyflavane) has been isolated from the bark of Casuarina equistifolia (23) and from tea leaves. In both these cases it is accompanied by tannins some of which probably result from its condensation.

(3) The conversion of certain vegetable tannins into anthocyanidins by oxidising them with bromine in dioxane was studied by W.J. Chater (24). The products from condensed tannins such as quebracho, mimosa, chestnut and gambier showed colour reactions similar to those of anthocyanidins. The hydrolysable tannins gave rise to no such products.

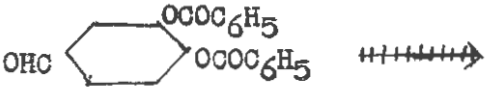
(4) As has already been noted, Reichel (25), by phytochemical reduction of anthocyanidins to catechins, obtained the latter in the form of amorphous "tannin-like" compounds.

However the evidence at present available in support of the Freudenberg theory does not justify its application to the whole class of condensed tannins, and A. Russell (26) has suggested that these may be derivatives of 4-hydroxy polyhydroxy flavane instead of 3-hydroxy polyhydroxy flavane. He prepared a number of products with this basic structure, taking the phenolic patterns suggested by the fission products of natural tannins. The following synthesis is given as an example:



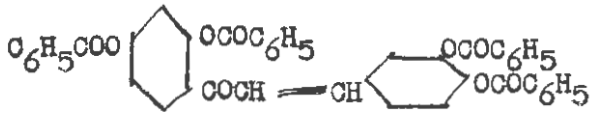
resacetophenone dibenzoate

+

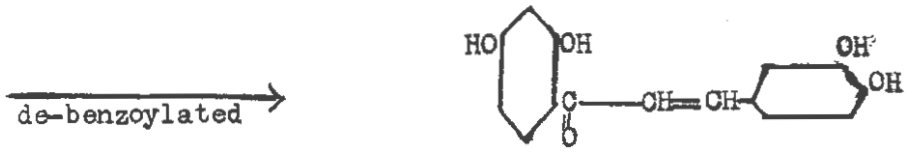


protocatechualdehyde dibenzoate

condensed with dry HCl in dry EtAc.

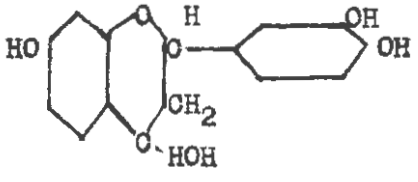


2:4:3':4': tetrabenzoyl oxychalcone

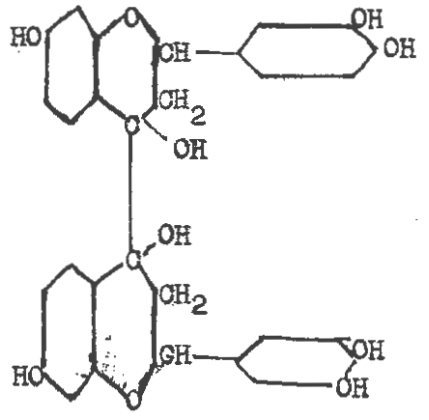


2:4:3':4': tetrahydroxy chalcone

This chalcone, by reduction with zinc dust and dilute alcoholic acid gave a pink amorphous compound which, by its mode of formation Russell considered to be either the tetrahydroxyflavane (XII) or the corresponding bis (7:3':4':trihydroxy) flavpinacol (XIII).



(XII)



(XIII)

By comparison with analogous known reactions and by consideration of the amount of hydrogen required for the reduction he concluded that the flavpinacol was actually formed. Various flavpinacols, when prepared, were found to be indistinguishable from typical phlobatannins, and Russell concluded that phlobatannins are phenolic hydroxy derivatives of flavpinacol.

On this theory quebracho tannin would be bis (7:3':4':trihydroxy) flavpinacol, and the numerous tannins which give as fission products phloroglucinol and protocatechuic acid would be bis(5:7:3':4':tetrahydroxy) flavpinacol. The individual differences between members of the latter group are probably accounted for by alkylation of some of the hydroxyl

groups, and the presence of various substituent groups in the nuclei; these would disappear during the alkali fusion.

The absorption spectra of the flavpinacols showed good agreement with those of the phlobatannins and marked differences from those of the gallotannins and ellagitannins.

Freudenberg, Karimullah and Steinbrunn (27) criticised Russell's interpretation of his work. They were not convinced that his products were flavpinacols, nor did they consider his comparison of these with the phlobatannins convincing. They attempted a simpler synthesis of a pinacol from flavanone itself instead of from chalcone, but found that complex condensation products were formed. They therefore suggested that what Russell claimed to be the synthetic production of phlobatannins was in reality the production of compounds of high molecular weight by condensation or polymerisation, since hot alcoholic HCl is known to be a reagent which changes substances such as chalcones into highly complex condensation products. Further they considered it doubtful whether the amorphous synthetic products, whatever their constitution, were identical with natural tannins, although they produce the same KOH fusion products and have the same colour reactions and absorption spectra. They found the absorption spectrum of the so-called synthetic hemlock tannin to correspond almost exactly with that of gallotannin, and concluded that the 3 hydrogenated carbon atoms between the benzene nuclei are of little or no importance in the production of characteristic bands in the absorption spectra.

They considered it premature to describe the constitution of a phlobatannin by a single constitutional formula, and while admitting that

some tannins might be derived from 4:hydroxy flavanes they did not consider the evidence adduced by Russell to be convincing. Further, they pointed out that pinacols have never been found in nature. In a later paper (28) Russell stated, regarding these amorphous products: "On various grounds it is probable that the amorphous products are flavpinacols hydroxylated on the pattern of the parent chalcones; for the sake of simplicity they are formulated as of the bis type, although their structure may be more complicated."

The Russell theory may well be applicable to some condensed tannins but here again, as was stated in regard to Freudenberg's theory, its application to a condensed tannin must be made with reserve if it is based merely on the similarity of the tannin to others which have been subjected to careful investigation.

- PART II -

THE PRELIMINARY INVESTIGATION OF THE CHEMICAL NATURE OF BLACK WATTLE EXTRACT.

- C O N T E N T S -

THE PRELIMINARY INVESTIGATION OF THE CHEMICAL NATURE OF BLACK WATTLE EXTRACT.

INTRODUCTION:

The bark of *Acacia mollissima* (black wattle) constitutes the chief source of the South African tanning material known as "Wattle extract" or "Wattle tannin". There are, however, quite a number of other species of *Acacia* found in South Africa, some of which are used for the production of wattle tannin, two of the most important being green wattle (*A. decurrens*) and silver wattle (*A. dealbata*).

Acacia is an important genus of leguminous trees and shrubs included in the family *Mimosaceae*, to which also belongs the genus under which the true mimosa falls. This explains the application of the name "Mimosa tannin" to wattle bark extracts. "Mimosa tannin", as used in the literature, cannot be taken to indicate the extract of any particular species of *Acacia*, and in the few reports on work done on the chemical constitution of mimosa tannin no mention of the species of *Acacia* from which the samples were obtained is made, and in consequence much of the value of the work is lost.

Einbeck and Jablonski (29) and Schwabe (30) found that the oxidation of mimosa tannin with concentrated HNO_3 led to the production of some (1 - 2%) dinitro -β-resorcylic acid and styphnic acid, thus showing that there were resorcin nuclei present in the molecule of the tannin.

Werner Eggert (31) found that the oxidation of methylated mimosa tannin with $KMnO_4$ gave rise to a mixture of acids. He was unable to separate this mixture into its component acids, but suggested that it might consist of veratric acid and trimethylgallic acid.

Russell (32) found that the extract of wattle bark from S. A. (whether of A. mollissima or not is unknown) gave, upon fusion with alkali, phloroglucinol, pyrocatechol and protocatechuic acid.

These facts formed a valuable background for the study of the extract of Acacia mollissima, to which the writer's investigation was confined. So far as can be ascertained this is the pioneer investigation of this particular extract as a definitely specified material.

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I. PREPARATION OF SAMPLES FOR ANALYSIS.

PRELIMINARY EXAMINATION.

The investigation was conducted on a commercial sample of black wattle bark extract. This is usually prepared in the factory by the extraction of disintegrated green bark packed in a battery of diffusers, using water preheated to 90° - 100°C. After the sludge has been allowed to settle the evaporation is carried out in double- or multiple-effect evaporators under reduced pressure, and is completed in a "finishing pan" or in a single-effect evaporator, producing a viscous extract which solidifies to a brittle solid on cooling (33).

The actual sample investigated was stored in tightly stoppered bottles with the addition of a few drops of chloroform to prevent the growth of moulds.

Free phenols and phenolic acids were first tested for by extracting a 5% aqueous solution of the solid with ether, and also by treating the solid with benzene in a Soxhlet extractor. On evaporation the ether gave 0·04 gm. and the benzene 0·06 gm. of a brown solid, per 100 gms. of original extract. This substance would not crystallise from water, alcohol or acetone, in which it was found to be fairly soluble, or from ether, in which it was less soluble. It was readily soluble in NaOH, and gave a greenish-blue precipitate with ferric chloride. This was taken to indicate that there was no appreciable quantity of free phenols or phenolic acids in the extract.

The following two tests for the presence of gallotannin were then applied:

(1) 5 gms. of the extract were boiled for three hours with 250 ml. of water and 10 ml. of conc. HCl. The bulky red-brown precipitate was

filtered off and washed, and the filtrate, together with the washings, was concentrated by evaporation. No gallic acid could be detected in the resulting solution by qualitative tests.

(2) 5 gms. of the extract were refluxed with 100 ml. of water, 20 ml. of 40% formaldehyde solution and 5 ml. of conc. HCl for one hour. After the light coloured precipitate had been filtered off no gallic acid or gallotannin could be detected in the filtrate.

The isolation of a crystalline member of the benzopyrane group from wattle bark would not only be of interest, but might also be expected to give some indication of the phenolic pattern of the molecules of the accompanying tannin. Since the commercial preparation of the extract might have led to the alteration or condensation of any such substances present, the bark of a tree identified as Acacia mollissima was stripped, disintegrated, and extracted with cold water. The extract was then evaporated under reduced pressure to the desired concentration.

The tannin from an extract containing 20% of total solids was precipitated by saturating with salt, and the filtrate was extracted with ether and with ethyl acetate. No crystalline products could be obtained, however, from these extracts.

A 5% extract was then treated with gelatin to precipitate the tannin, but the filtrate, after concentration, gave no precipitate on treatment with formaldehyde and HCl at room temperature. Any appreciable amount of catechin would give a precipitate under these conditions, since it is not precipitated by gelatin.

The preparation of a sample of "pure wattle tannin" was attempted as the next step. The difficulties involved in purifying amorphous plant products are universally appreciated, especially where drastic methods lead

to decomposition as is the case with wattle bark extract. The methods applied were chosen to avoid, as far as possible, such drastic action.

1. SOLVENT EXTRACTION.

The method employed was, in outline, that used by Russell (34). The extract was dissolved in water with as little heating as possible to form a 15% solution which was then saturated with NaCl, when some of the extract precipitated out as a solid. This precipitate was thus rendered free from readily soluble impurities such as sugars, etc. It was then dried under vacuum and then extracted with acetone to remove inorganic impurities as far as possible. On removal of the acetone by evaporation the residue became viscous and was then placed under vacuum, when it puffed up and dried rapidly. The flaky product was then washed with ether, in which tannin is insoluble, to remove any phenols or other ether-soluble organic compounds which may have been present. The final product (Sample A) was fawn-pink in colour and very readily soluble in water, alcohols, ethyl acetate and acetone, while insoluble in hydrocarbons and chloroform.

As the NaCl precipitation was a source of much trouble it was omitted in preparing the next sample. The dried original extracted was simply extracted with acetone in a Soxhlet, and the resulting solution was treated as before. The product (sample B) gave analysis results closely similar to those of Sample A, and samples prepared by this method were used in the preparation of derivatives as reported in section II; these are the "tannin" referred to in that section. During the preparation of sample B it was found that about 30% of the original quantity of material remained in the extractor after 48 hours. This residue was not investigated further.

Sample B was analysed for non-tans by the official method of the

I. S. L. T. C. (35), and was found to return 5.7% as non-tans. Although this method is an empirical one, intended to gauge the efficiency of a material with respect to its ability to tan, and cannot be expected to give results as definite in their meaning as those of a quantitative ionic precipitation, it is the only method available by which the "purity" of a tannin can be estimated.

2. ULTRAFILTRATION.

Ultrafiltration and the related process of dialysis are methods frequently used to free amorphous and colloidal materials from crystalloid impurities. Douglas and Humphreys (36) have applied electrodialysis to the removal of impurities from tannins, while Browne (37) claimed to have attained a quantitative separation of tannins from non-tannins by the ultrafiltration of solutions of various tanning materials. He regarded ultrafiltration as a method by which the non-tannins, being compounds of relatively small molecular size, are simply sifted out from the tannins.

In the present work an apparatus without a mechanical stirring device was used. It consisted of a strong steel cylinder of about 300 ml. capacity with both ends removable. The lower end-plate was perforated to allow passage of the filtrate, and the upper one was fitted with an inlet tube for the application of pressure. As tannin solution in contact with steel produces a blue colour it was necessary to wax the inside of the apparatus very carefully, and to support the membrane on silver gauze.

For the ultra filtration a 0.4% solution was made by dissolving the original extract in cold water and filtering through quantitative filter paper.

The first membranes were prepared by soaking discs of filter paper (Whatman No. 40) in glacial acetic acid, then in 8% collodion in acetic acid and finally washing with water. They were found to retain only a very small

fraction of the extract. Following the method of Browne (37) three new varieties of membranes were then prepared by soaking similar filterpaper discs in 3, 6 and 9% solutions of collodion in ether-alcohol mixtures (1:1) respectively. These were allowed to dry for times ranging from $\frac{1}{4}$ to $\frac{1}{2}$ hour, in a place free from draughts. They were then immersed in distilled water and kept there until used.

The membranes prepared from the 3% collodion solution were found to be too porous; those prepared from the 6% solution gave a filtrate which was only very faintly coloured, showing the presence of only a small amount of tannin; while those from the 9% solution produced a colourless filtrate with no indication of the presence of tannin. Filtration through the latter, however, was found to be very slow. It was therefore decided to use membranes of the second type, since the main aim of the filtration was not to effect a quantitative separation of the tannins from the non-tannins, but to obtain a sample of the tannins for analysis as free as possible from non-tannins. The escape of a small amount of tannin through the filter indicated that the pore-size was sufficiently large to allow the escape of all the small non-tannin molecules.

There are, however, other factors which affect the permeability of the filters in addition to the strength of the collodion solution used in their preparation, e.g. the time and conditions of drying. It was therefore necessary to standardise the filters. Browne (loc.cit) used a method of standardisation depending on the number of ml. of water that pass through the membrane per minute at various pressures. The rate of filtration is then plotted against the pressure, when a straight line is usually obtained. The slope of this line serves to identify the performance of the membrane.

Two typical curves are shown in Figure I. The graphs, being straight

lines over most of their length, may be represented by the equation

$$v = mp + C$$

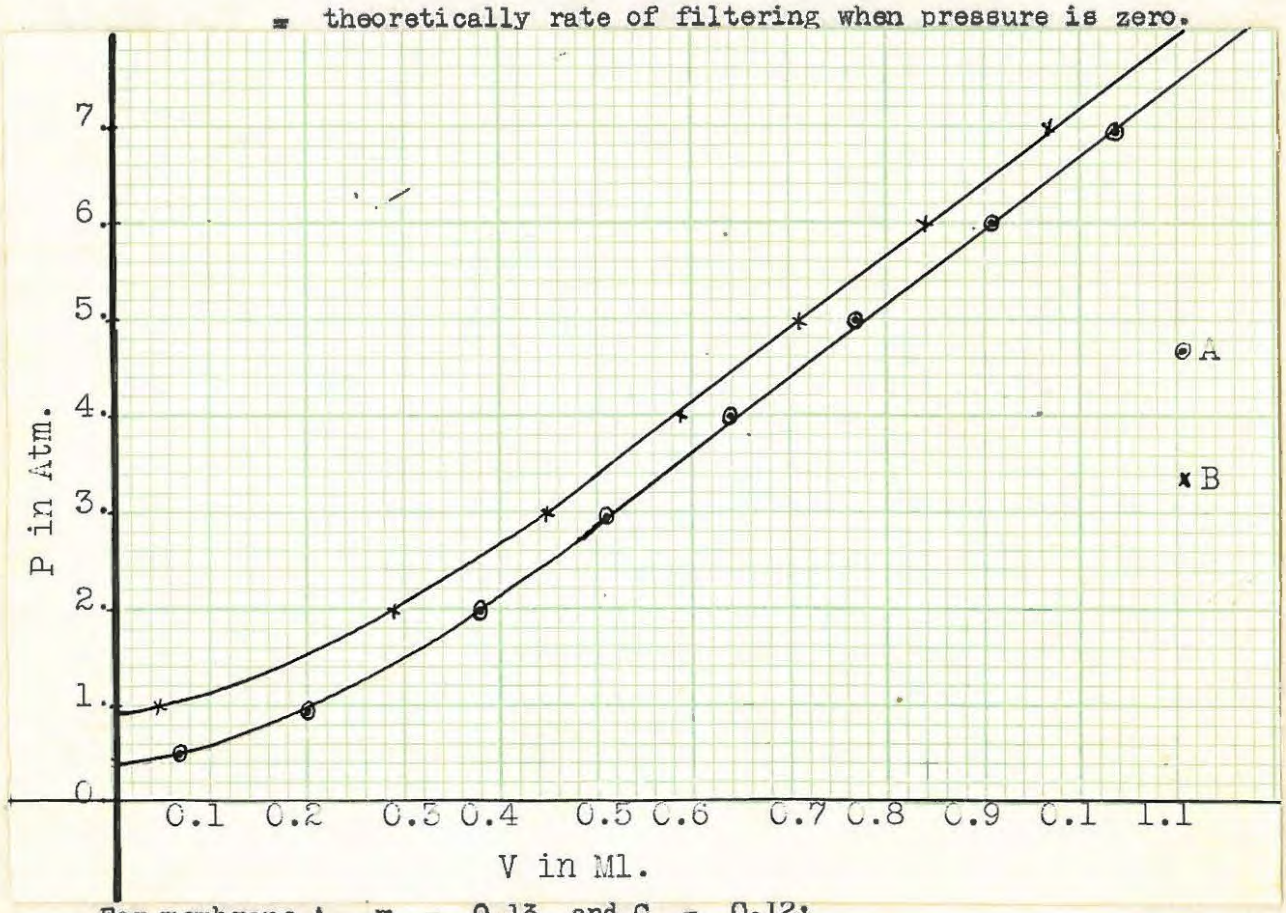
where v = volume of water filtered in ml. per minute.

p = pressure in atmospheres

m = increase in rate of filtering per unit increase of pressure, in ml. per min. per atm.

C = a constant for the given membrane

= theoretically rate of filtering when pressure is zero.



For membrane A, $m = 0.13$ and $C = 0.12$;

For membrane B, $m = 0.12$ and $C = 0.11$.

Only membranes with $m = 0.13$ were used in the preparation of sample C for analysis.

As an indication of the progress of the filtration the filtrate was collected in 50 ml. lots which were evaporated to dryness and weighed. In

a normal filtration the weights would be expected to reach a constant value a short time after the beginning of the filtration, but, as the set of results given in Table I shows, they actually decreased with time after reaching a maximum.

Lots of 50 ml:	1st.	2nd.	3rd.	4th.	5th.
Wt. of residue, gms.	0·035	0·063	0·060	0·055	0·052.

TABLE I.

The abnormality may be explained on the assumption that some particles of non-tans were retained by adsorption and by the blocking of pores, i.e. by factors other than their size. The tannin retained by the filter was washed three times in order to minimise the effect of this on the purity of the final product.

The ultrafiltration of the solutions of the wattle extract proved to be a tedious process, 250 ml. of solution taking 80 hours to filter, and so only small samples were prepared by this method. (Sample C.)

A 0·4% solution of sample B was also ultrafiltered to obtain another sample for analysis (Sample D), for comparison with samples prepared by the other methods.

3. DIFFERENTIAL ADSORPTION.

W. Grassmann (38) made a detailed investigation of the fluorescence chromatograms produced by different tannins, and found them to be characteristic and easily reproducable. Each chromatogram consisted of a number of separate bands, and this suggested that adsorption analysis might be of use in the separation of wattle bark extract into its components. A very short preliminary investigation of the possibilities of this method was

therefore made.

Calcium carbonate of small grain and also aluminium oxide of granular character were tried as adsorbents. The adsorbent was contained in the tube of an apparatus of the conventional type, and was first washed with methyl alcohol. A solution of the wattle bark extract in 2 parts of methyl alcohol and 1 part of water was then poured in, but no suction was applied until the chromatogram had formed. Development was carried out with ethyl acetate, with a view to washing the various bands right through, but the process was found to be so slow that it was abandoned. An attempt was then made to isolate the tannin by removing the adsorbent in layers and boiling these with solvents such as methyl alcohol, ethyl acetate, and water. These, however, failed to remove any appreciable quantity of material from the adsorbent. It is thought, however, that the method may be successful if the correct adsorbent and solvent can be found.

4. LEAD "SALT" PRECIPITATION.

The tannin from a solution of the original extract in water was precipitated out in stages by the addition of lead acetate solution. The first and last portions of the precipitate were discarded, and the rest was thoroughly washed and then decomposed by precipitating the lead with H_2S. The "pure" tannin was then obtained by evaporation of the solution. (Sample E).

II. ANALYSIS OF SAMPLES.

Since amorphous substances retain the last traces of water very tenaciously, great care was taken in the drying of the samples for analysis. They were kept for 24 hours in a good vacuum at 95° - 100° over anhydrone.

The ash determinations were made by igniting slowly at first and then completing the ignition in a slow stream of oxygen.

The carbon, hydrogen and oxygen results were obtained by the usual combustion method, allowing for the ash content in the calculations.

The molecular weights were determined by Beckmann's cryoscopic method in aqueous solution.

The figures for the nitrogen content were obtained by the Kjeldahl method, and those for the methoxyl content by Zeisel's method.

DISCUSSION OF RESULTS.

Judging from the ash contents and the molecular weights of the samples, ultrafiltration was the most effective method of purification employed. The presence of 0·05% of ash presumably showed that the sample was not free from inorganic material, and this would probably have some effect on the molecular weight determinations.

No significant differences between the carbon and hydrogen contents of the various samples are evident, and average values were therefore taken.

TABLE II/ .....

TABLE II.

SAMPLE.	% Ash.	%C.	%H.	%N.	% Methoxyl.	Molecular Wts. in 0·5% Soln.	Molecular Wts. in 5·0% Soln.
Original Extract. (OE)	1·36	-	-	0·15	1·1	530	520
A. Acetone extract of salt pptd. OE.	0·14	61·2	4·56	0·0	1·0	735	730
B. Acetone extract of OE.	0·18	60·8	4·52	-	-	*	720
C. Ultra-filtered OE.	0·06	61·3	4·60	-	-	1050	1010
D. Ultra-filtered B.	0·05	61·7	4·53	-	-	-	980
E. Lead pptd. OE.	0.21	61.6	4.61	-	-	-	620

AVERAGE VALUES : C 61·3%

H 4·56%

O 34·1% (by difference).

Taking as a basis a unit containing 15 carbon atoms, this being the number present in the 2:phenylbenzopyrane molecule, a "unit empirical formula" may be calculated from the figures given above, and works out at

$$C_{15}\ H_{13\cdot4}\ O_{6\cdot3}$$

which corresponds to a molecular weight of 294.

In gauging the significance of this formula the following points should be borne in mind:

(1) It is very difficult to ascertain that an amorphous substance such as wattle tannin has been completely purified.

(2) The tenacity with which the last traces of water are retained by the tannin might lead to high results for the hydrogen content.

(3) Wattle tannin is very easily oxidised, which might lead to low carbon and hydrogen values.

This unit empirical formula, referred to later in this work as Unit W, has been adopted for convenience in the interpretation of the results of other experiments. The choice of a unit containing fifteen carbon atoms is based merely on analogy with tannins, the structures of which have been much more fully investigated, e.g. quebracho tannin and hemlock tannin, and may easily be in error.

The fact that nitrogen was present in the original extract but not in sample A indicates that it is present in an impurity which is removed by the process of purification used.

Russell (39) has suggested that the presence of methoxyl in phlobatannins may be due to the presence of organic solvents. This possibility may be discounted in the present case, since a Zeisel estimation on the cold-water extract of wattle bark itself gave 1.0% of methoxyl. The presence of one methoxyl group in the unit W would correspond to a methoxyl content of 10.1%, and it therefore seems either that there must be one methoxyl group per ten units W, or else that an impurity containing the group is present and is not removed by the methods of purification employed.

In considering the molecular weights obtained for the tannin, it should be remembered that the tannin in solution behaves as a negatively charged colloid and that the removal of crystalloid materials which might have acted as peptising agents for this colloid would have led to agglomeration of the particles. The

fact that the highest molecular weights were obtained with the samples which showed the lowest ash contents may be taken to support this, but on the other hand it can be explained equally well by the fact that a small quantity of impurity of low molecular weight may lower the result of such a determination by a very disproportionate amount.

The few parallel determinations of the molecular weight in 0.5% solution were carried out with the object of discovering whether any detectable dissociation of the colloid particles occurred on dilution. Evidently none did take place.

While the results obtained indicate a molecule containing three units W, the high result of 1800 reported for mimosa tannin by Douglas and Humphreys (36) corresponds to six units. They obtained their tannin, however, by electrodialysis, and here again agglomeration might be expected to cause high results.

III. PREPARATION OF SOME WATTLE TANNIN DERIVATIVES.

The preparation of a number of derivatives of wattle tannin was carried out with the object of finding some compound which could be more easily purified than the tannin itself. It was also expected to yield some information on the number and nature of hydroxyl groups present in the molecule.

1. ACYLATION OF THE TANNIN.

Both acetyl and benzoyl derivatives of the tannin were prepared.

ACETYLATION. The acetylation was carried out by means of fused sodium acetate and acetic anhydride as follows:

12 gms. of tannin were refluxed for 30 minutes with 50 ml. of freshly distilled acetic anhydride and 18 gms. of fused sodium acetate. The reaction mixture was then poured into $2\frac{1}{2}$ litres of distilled water, when the acetylated tannin was precipitated in the form of a light-coloured amorphous solid. The product was filtered off and washed thoroughly with water, and while still damp weighed 23 grams.

The acetylated tannin was found to be insoluble in water, ether and benzene, but dissolved readily in solvents such as acetic acid, acetone and ethyl acetate.

The determination of the acetyl content of the substance presented some difficulties, as it proved very resistant to acid hydrolysis, and when hydrolysed with alcoholic KOH it took on a dark brown colour which made the detection of the end point by means of colour indicators impossible. In order to obtain a rough estimate of the acetyl number of the tannin, a number of 1 gm. portions was weighed out and acetylated with corresponding quantities of acetic anhydride and fused sodium acetate. After reaction the

mixtures were poured into 200 ml. lots of water, and just sufficient NaOH to leave the solution slightly acid was added to each, in order to minimise the solubility of the acetylated product. After allowing to stand for 24 hours the acetylated products were collected quantitatively, washed four times with 25 ml. of cold water and then dried at 80°C under vacuum. Some difficulty was experienced in drying the products to constant weight without at the same time inducing decomposition. An average of 1·65 gm. of acetylated product was obtained from each gram of original tannin. This corresponds to 40% of acetyl, and this in turn corresponds to 4·6 hydroxy groups capable of acetylation by this method per unit W.

The amorphous product, being difficult to dry, did not seem to possess any advantages over the tannin itself for purposes of analysis.

BENZOYLATION.

The benzoylation of the tannin was carried out by treating 10 gms. of tannin with 28 gms. of benzoyl chloride and 80 ml. of 10% NaOH in three stages with thorough shaking. The reaction mixture was then poured into 2 litres of water, when the benzoylated tannin was precipitated in the form of a light orange coloured amorphous solid, which was filtered off after standing for 24 hours.

The product was only slightly soluble in cold alcohol, acetone and benzene. When heated in benzene, partial decomposition took place, and a sticky, partially de-benzoylated mass remained. Benzoic acid crystallised out from the benzene on cooling. The benzoylated product was found to hydrolyse very readily with alcoholic KOH.

Like the acetylated tannin, it was considered useless for analytical purposes.

2. METHYLATION OF THE TANNIN.

The methylation of the tannin was attempted by three well-known methods.

METHYLATION BY MEANS OF METHYL ALCOHOL AND DRY HCl. 5 gms. of tannin in 20 ml. of cold dry methyl alcohol were run drop by drop into alcoholic HCl, made by passing dry HCl gas into 20 ml. of dry methyl alcohol over a period of 30 minutes. Dry HCl gas was then passed into the mixture for 30 minutes at such a rate that the mixture did not boil. After this it was refluxed for $4\frac{1}{2}$ hours, filtered hot, and the residue washed with 40 ml. of hot methyl alcohol, the washings being united with the filtrate and evaporated to dryness on a water bath. The solid black product was taken up in ethyl alcohol and precipitated in water containing H_2SO_4. The final product appeared to be a modified tannin, insoluble in water, but it was found to contain only 1.5% of methoxyl.

Phloroglucinol can be converted into its mono- and di-methyl ethers by this method, and it was therefore concluded that the reactivity of the OH groups in the tannin is below that of the OH groups of phloroglucinol.

METHYLATION BY MEANS OF METHYL IODIDE AND SILVER OXIDE. 10 gms. of tannin in 60 ml. of dry methyl alcohol were treated with 25 ml. of methyl iodide added dropwise and 30 gms. of silver oxide added in correspondingly small portions. The reaction mixture was then refluxed for four hours on a water bath, filtered hot, and the residue washed with 50 ml. portions of hot methyl alcohol the washings being added to the filtrate. The combined filtrate and washings were taken to dryness in a vacuum, when a powdery brown solid weighing 10.2 grams remained. This was washed with water until the washings were colourless when there remained 4 gms. of a light brown product.

After drying in a vacuum at 70° - 80°C for 48 hours this product was found to contain 10·6% of methoxyl. However, when subjected to the action of dimethyl sulphate and KOH (see next section) it was found to contain 31.2% of methoxyl. This showed that methylation by means of methyl iodide and silver oxide did not produce a fully methylated tannin.

METHYLATION BY MEANS OF DIMETHYL SULPHATE AND KOH. 20 gms. of tannin were dissolved in 100 ml. of methyl alcohol, and 25 ml. of dimethyl sulphate were added, followed by 50 ml. of KOH solution (90 gms. in 100 ml. water). The reaction was somewhat violent, and thorough stirring was found to be essential. After stirring for 30 minutes the methylation was repeated, using the same quantities of reagents, and it was finally repeated again. The reaction mixture was then poured in a thin stream into 2 litres of distilled water, when the methylated product was found to precipitate out as a granular mass. It was noticed that if the reaction mixture was not slightly acid before pouring into the water, the product formed had a sticky nature and the supernatant liquid took on a dark brown colour. This fact might account for the failure by some workers to obtain methylated products of condensed tannins. The usual yield from 20 gms. of tannin was found to be 18 gms., i.e. 90% of the weight of the original tannin.

The methoxyl content of a sample of methylated tannin prepared in this way was found to be 34.4%. Subjection of a portion of the product to a further methylation with dimethyl sulphate showed that no further increase in the degree of methylation could be effected. On the other hand two methylations, by this method were found to be insufficient, as the product then contained only 28.1% of methoxyl. Reduction of the violence of the reaction by adding the required amounts of reagents in six portions instead of three and cooling the mixture in ice gave a smaller yield of methylated product (only 60% of the original weight of tannin), containing

only 30.3% of methoxyl.

The "Fully methylated" tannin appeared to be only sparingly soluble in ether, and so a sample for analysis was prepared by extracting 20 gms. of the substance with five lots of 250 ml. of boiling ether, and evaporating off the ether. The portion extracted by the ether weighed 1.5 gms. and had a feathery appearance, being almost white in colour. It was found to contain over 35% of methoxyl. This showed that the methylated tannin, as was expected, was not a homogeneous product.

The results of the methoxyl determinations on tannin methylated in various ways are collected in Table III.

A methoxyl content of 34.4% indicates the presence of 3.8 methoxyl groups in the unit W; while the rough estimation of acetyl groups in acetylated tannin indicated the presence of 4.6 acetyl groups per unit W. These results indicated that there were present in the tannin molecule hydroxyl groups which were acetylated but were not methylated by the methods employed.

With this in mind the methylated tannin, containing 34.4% of methoxyl, was subjected to the action of acetic anhydride and fused sodium acetate. The product proved to be very similar to the original methylated tannin, but was found to contain only 31.8% of methoxyl. When this product was methylated with dimethyl sulphate and KOH, a compound was produced which had a methoxyl content of 34.3%, i.e. almost exactly the same as that of the original methylated tannin. These results can be explained by either of two hypotheses:

TABLE III/.....

TABLE III.

SAMPLE AND METHOD OF METHYLATION.	METHOXYL CONTENT. %
A. HCl and MeOH.	1.53 1.46
B. MeI and Ag_2O.	10.78 10.52
C. B methylated with Me_2SO_4 and KOH	31.45 31.02
D. Me_2SO_4 and KOH, 3 times. (3 different samples)	34.66 34.40 34.14
E. Me_2SO_4 and KOH, 2 times.	28.16. 28.02
F. Me_2SO_4 and KOH, 4 times.	34.52 34.62
G. Me_2SO_4 and KOH, 6 times, with cooling.	30.31 30.25
H. Ether extract of D.	35.42 35.60 35.25

(1) There are present in the tannin molecule hydroxyl groups which can be acetylated with acetic anhydride and anhydrous sodium acetate, but which cannot be methylated by means of dimethyl sulphate and KOH.

(2) Some of the methoxy groups were actually replaced by $OCOCH_3$ groups, and these were later hydrolysed and replaced by methoxyl groups by the action of the alkaline methylating agent in the second methylation.

The former theory is obviously supported by the fact that 4.6 hydroxyl groups were found which could be acetylated, but only 3.8 which could be methylated.

Taking this theory as correct the decrease of 2.6% in the methoxyl content of the methylated tannin when acetylated would be accounted for by the presence of 0.7 acetyls per methylated unit W.

The methylation of acetylated tannin by means of dimethyl sulphate and KOH yielded a product with a methoxyl content of 33.3%. This indicated that primary acetylation had no effect on the limit to which the methylation could proceed.

The combustion analysis obtained on a sample of methylated tannin containing 34.4% of methoxyl and those calculated for unit W methylated to the extent of 34.4% methoxyl are compared below.

	OBSERVED.	CALCULATED.
% C	63.4	64.6
% H	6.25	6.10

While these figures do not agree too well, the agreement is possibly all that can be expected for the analysis of amorphous substances.

The molecular weight of the methylated tannin, determined by Rast's

method, was found to be of the order 600 to 650.

3. PREPARATION OF BROMINATED DERIVATIVES.

BROMINATION OF THE TANNIN. 20 gms. of tannin were suspended in 300 ml. of carbon tetrachloride and a solution of 15 ml. of bromine in 50 ml. of carbon tetrachloride was added over a period of three hours, refluxing the mixture meanwhile. This led to the disappearance of all the suspended tannin, and the product obtained from the carbontetrachloride solution on evaporation of the solvent weighed 28 gms. The granular product differed markedly from the original tannin, being insoluble in water but fairly soluble in alcohol.

BROMINATION OF METHYLATED TANNIN. 10 gms. of methylated tannin (containing 34.0% methoxyl) were dissolved in 50 ml. of chloroform and 30 ml. of bromine were added slowly to this with shaking. After evaporation of the chloroform there remained 3.9 gms. of red-brown product. This was redissolved in chloroform and precipitated by the addition of petrol ether, and the precipitate was then dried. The methoxyl content was found to be 19.8%. Calculation for the unit W with 3.8 methoxyl groups (corresponding to 34.0% OCH_3), shows that this content of 19.8% can be explained by the bromination of the unit to the extent of 4.2 Br atoms.

METHYLATION OF BROMINATED TANNIN. 5 gms. of the brominated tannin, after thorough washing and drying, were methylated with dimethyl sulphate and KOH, and the product, which was dark in colour, was purified by dissolving in chloroform and precipitating with petrol ether. The 4.5 gms. of product obtained gave a methoxyl content of 18.0%. Comparison of this result with the previous one shows that the primary bromination of the tannin does not affect its susceptibility to methylation to any great extent.

IV. OXIDATIVE DEGRADATION OF TANNIN AND ITS DERIVATIVES.

To gain an idea of the nuclei present in the tannin molecules oxidative degradations were made on the tannin and some of its derivatives.

1. Oxidation of Tannin.

Wattle tannin resembles the polyhydroxy phenols in that it undergoes autoxidation with the development of a dark colour, especially when in alkaline solution.

The oxidation was carried out with $KMnO_4$ as follows :-

25 gms. of tannin in 250 ml. of water were oxidised by adding 70 gms. of $KMnO_4$ in 5% solution in water, over a period of two hours. The oxidation took place in the cold. The MnO_2 suspension was dissolved by the addition of dilute H_2SO_4 and sodium bisulphite and the resulting solution was extracted with ether five times. The ether extract was shaken with saturated sodium bicarbonate solution, and this yielded on acidification a few small particles of yellow oily material, which appeared to be phenolic acids. The ether extract yielded 0.2 gms. of yellow oily material which was not examined further.

It was concluded that this oxidation was far too vigorous to yield any quantity of degradation products.

It was hoped that acylation or methylation of the hydroxyl groups would render the benzene nuclei in the molecule more resistant to complete disruption during oxidation. Some of the derivatives, the preparation of which is described under Section III, were therefore subjected to oxidation.

2. Oxidation of acetylated tannin.

10 gms. of powdered acetylated tannin were suspended in 200 ml. of water and oxidised with 30 gms. of $KMnO_4$ in 5% solution, in the presence of

$MnSO_4$. No oxidation took place in the cold, but it took place readily on heating. Dilute H_2SO_4 and $NaHSO_3$ were added to clear the suspension of MnO_2, and the solution was then extracted five times with ether. The ether solution, on extraction with sodium bicarbonate solution, gave no organic acids, and on evaporation it yielded only a small residue (0.1 gm.) of a sticky brown substance.

The acetylation of the tannin had obviously not had the desired effect on its stability.

3. OXIDATION OF METHYLATED TANNIN.

With $KMnO_4$ in cold aqueous solution.

10 gms. of methylated tannin were oxidised as described under 1, but without heating. After clearing the MnO_2 suspension about 3 grams of a material of a sticky nature remained. After this had been filtered off the solution was extracted five times with ether. The ether extract was shaken with saturated $NaHCO_3$ solution, and upon acidification with concentrated HCl this gave 0.4 gm. of crystals (Sample 1). The examination of these crystals is reported later.

With $KMnO_4$ in hot aqueous solution.

The residue of sticky material obtained in the process of cold oxidation suggested that the oxidation had not gone far enough, and it was therefore repeated with the application of heat. The mixture was placed on a water bath for two hours after the completion of the reaction at room temperature. 0.4 gram of crystals were obtained (Sample 2), and about 2 gm. of the partially oxidised sticky residue remained.

With $KMnO_4$ in acetone solution.

20 gms. of methylated tannin were dissolved in 600 ml. of acetone and refluxed gently on a water bath while 100 gms. of $KMnO_4$ were added slowly

over a period of three hours. The refluxing was continued until the mixture was decolourised (about five hours). The MnO_2 suspension was filtered off and dissolved in dilute H_2SO_4 and $NaHSO_3$. Treatment of this solution as before gave 0.25 gm. of crystals (Sample 3). The acetone was evaporated off, leaving a yellow, sticky product weighing 6 gms. This was extracted with hot water and then with dilute NaOH solution, and the combined extracts were filtered into dilute H_2SO_4 solution. After extraction with ether and further treatment as before this solution gave 0.30 gm. of crystals (Sample 4). The yellow sticky residue, after the extractions with hot water and dilute alkali, was again oxidised with 20 gms. of $KMnO_4$ in acetone solution, and gave, after treatment, a further 0.05 gm. of crystals (Sample 5), from the MnO_2, but no further crystals could be extracted from the residue left on evaporation of the acetone.

Examination of crystal samples.

Samples 1 to 5 were examined separately after recrystallising from 40 parts of hot water and drying over P_2O_5. The equivalent weights were determined by titration with N/100 NaOH solution, and the melting points were determined, with the results set out below in table IV.

TABLE IV.

SAMPLE NO.	1	2	3	4	5
Melting point, °C.	142	142	145	143	142
Equivalent weight.	201	200	198	-	-

It was thus safe to assume that the crystals were of the same nature, and the residues were combined and used for a methoxyl determination, which gave 39.5% OCH_3.

The crystals were thought to be a mixture of veratric acid and trimethylgallic acid, on the grounds of the following comparison:

TABLE V.

	SAMPLES 1-5.	VERATRIC ACID.	TRIMETHYLGALLIC ACID.
M. Pt. °C.	142 - 145.	179 - 180	167 - 169.
Equiv. Wt.	198 - 201.	182	212
Methoxyl content	39.5%	34.07%	43.80%

According to Schrötter (40) silver veratrate is only slightly soluble in cold water, while Heffter reports (41) that the silver salt of trimethylgallic acid is easily soluble in water, and therefore the silver salt method of separation suggested itself. 5 gms. of the crystalline mixed acid was therefore prepared by a large scale oxidation of methylated tannin with $KMnO_4$ in acetone solution, and a sample of this mixture, when recrystallised from hot water, melted at 142°C. The mixed acid was dissolved in a slight excess of ammonia and boiled until neutral. To this 400 ml. of almost boiling solution a hot solution of 5 gms. of silver nitrate in 100 ml. of water was added, when a silver salt having a bluish colour at once separated out. When the mixture was cooled thoroughly a small amount of a cream-coloured silver salt also separated. The silver salt was filtered off and treated as one fraction (Fraction I), and the filtrate was treated as the second fraction (Fraction II).

Fraction I was suspended in 100 ml. of hot water, a slight excess of conc. HCl was added, and the mixture was heated almost to boiling point; the precipitate of AgCl was then filtered off and the filtrate was allowed to cool. When cold it was extracted with ether and the ether solution was itself extracted with saturated $NaHCO_3$ solution. On acidification of this solution with conc. HCl needle-like crystals separated out. They started to melt, however, at 143°C, which showed that they were still a mixture of the acids.

They were therefore subjected again to the above treatment, but this time the silver salt was washed twice with 25 ml. of water at 60°C. The crystals from the silver salt fraction were found to melt at 178° - 179°C. No specimen of pure veratric acid was available for comparison with this substance, but its identification as veratric acid was made reasonably certain by determination of the equivalent weight, the methoxyl content and the carbon and hydrogen contents as well as the melting point. The results are given in Table VI.

TABLE VI.

	ACID OBTAINED FROM TANNIN.	VERATRIC ACID.
M. Pt., °C.	178 - 179	181
Equiv. Wt.	183.2	182
% OCH_3.	33.77	34.07
% C	59.2	59.3
% H	5.59	5.49

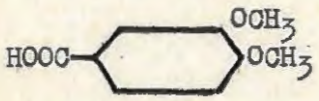
Fraction II was cooled, and some more silver salt which separated was filtered off. The acid extracted from the filtrate as before was found to melt at 145°C, and was apparently still a mixture. The crystals were therefore subjected to a further silver precipitation, and a small quantity of silver salt again separated on cooling in ice. The filtrate yielded 0.4 gram of a crystalline substance which, after recrystallisation from 40 parts of water, melted at 166°C.

For comparison with this substance trimethylgallic acid was prepared by methylation of gallic acid with dimethyl sulphate and KOH. After repeated recrystallisation from 30 parts of water the product melted at

167° - 168°. A mixture of this acid with that obtained from the tannin melted at 167°. The equivalent weight of the acid obtained from the tannin was determined as 211.2, as compared with 212 calculated for trimethylgallic acid.

The synthetic trimethylgallic acid, when mixed in equal quantity with the veratric acid from the tannin, melted at 142°C. (Different specimens of mixed acids from methylated tannin melted within the range 142° - 145°C).

The acid crystals obtained from the oxidation products of methylated tannin were thus shown to be a mixture of veratric acid (I) and trimethylgallic acid (II). The production of these presumably indicates the presence of catechol and pyrogallol nuclei in the molecule of the tannin.



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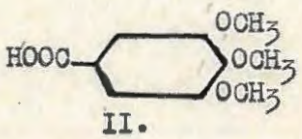

II.

The oxidation produced only small quantities of these crystals, but it is possible that oxidation under optimum conditions may give greater yields. It is equally possible, however, that the methylated tannin molecules are of such a nature that oxidative degradation requires the use of such powerful reagents that the majority of the benzene nuclei present are simultaneously disrupted. It is therefore considered probable that the amounts of veratric acid and trimethylgallic acid obtained represent only a small fraction of the corresponding nuclei present in the tannin molecule, the remainder having been disrupted in the oxidation process.

Oxidation with nitric acid.

5 gms. of methylated tannin in suspension in 20 ml. of water were

heated on a water bath with 10 ml. of fuming nitric acid diluted to 100 ml. with water. The methylated tannin, however, remained unaltered, and after washing gave a negative test for nitrogen. The use of concentrated nitric acid was therefore necessary.

20 gms. of methylated tannin in the form of fine powder were added gradually to 100 ml. of fuming nitric acid of specific gravity 1.52. In the ensuing reaction nitrous fumes were evolved. The mixture was then evaporated down to about 40 ml. on a water bath, and then poured into 1 litre of distilled water. The resulting cohesive yellow-orange precipitate was rubbed well until it became amorphous and was then filtered off. It was found to weigh 4.2 gms. while still slightly damp. It was soluble in ether, and the ether solution was extracted as before with $NaHCO_3$ solution. Upon acidification no crystalline product separated, but only a very small quantity of amorphous substance. All further attempts to obtain a crystalline product by this method also failed.

4. Oxidation of brominated methylated tannin.

So far no residues from metadihydroxy benzene nuclei had been obtained during the oxidation of methylated tannin. It was hoped that the introduction of bromine into the molecule would have a stabilising effect on these nuclei if present, as it is known to have this effect in some compounds. The brominated methylated tannin was therefore oxidised with $KMnO_4$ in acetone solution, but a thorough search by ether extraction yielded only a small amount of an amorphous product. It was then evident that the bromination had not had the desired effect.

In this section it has been shown that, while acetylation does not appear to increase the resistance of the nuclei to oxidation to any great

extent, methylation does appear to stabilise those nuclei with two substituent hydroxyl groups in ortho positions.

SUMMARY AND CONCLUSIONS.

The combustion analysis obtained for the "purified" tannin, when converted to a "unit empirical formula" on the basis of fifteen carbon atoms per unit gave $C_{15}H_{13.4}O_{6.3}$. It was shown that this unit contains approximately 4.6 hydroxyl groups which may be acetylated by means of acetic anhydride and fused sodium acetate, and 3.8 hydroxyl groups which can be methylated by means of dimethyl sulphate and KOH. This leaves 1.7 oxygen atoms per unit unaccounted for. This oxygen may be linked in such a way as to render it unavailable for purposes of acylation, but there is the possibility that some of it was present in the form of oxidation products, and not actually in the tannin itself.

Oxidative degradation has shown that catechol and pyrogallol nuclei are present in the tannin. A quantitative estimation of their amounts could not be carried out because of the apparent resistance of the tannin to this means of degradation.

BRIEF SUMMARY OF RESULTS OBTAINED SUBSEQUENTLY AT THE LEATHER INDUSTRIES RESEARCH INSTITUTE, mainly by MR. A.M. STEPHEN, M.Sc. (42).

Mr. Stephen has worked with extracts of the fresh bark of Acacia mollissima and with commercial extract. He has adopted different procedures for obtaining tannin preparations; two of the preparations have shown the following compositions:

$C_{15}H_{13}O_{6.5}$ and $C_{15}H_{13}O_{6.0}$

The average number of OH groups per unit which could be acetylated by various methods was found to be 4.25, and the number which could be methylated was found to be 3.7. It has been found that both partially and fully methylated tannins are capable of fractionation into parts of differing acetyl content, by making use of their solubility relationships is acetic acid of varying strength. The latter was taken to show that the tannin is not homogeneous.

The oxidation of the methylated tannin yielded veratric acid and trimethylgallic acid, thus confirming the results obtained in the writer's investigation. It has been shown that the methylated tannin does not behave like a methylated anthocyanidin towards hydrogen peroxide, nor does it behave as if it possessed the structure proposed by Russell for phlobatannins towards periodic acid, lead tetra-acetate and Pb_3O_4. Substances possessing the Russell structure (26) would be expected to give ketonic products after such treatment, and no notable quantities of such products were found.

Alkali fusion of the tannin has been found to result in the production of resorcinol in relatively large amounts, but no other phenols or phenolic acids could be isolated.

The tannin was shown to be unlike lignin in that it did not produce carbonyl compounds on boiling sulphited and methylated derivatives with strong caustic alkali for long periods.

On boiling the tannin with KOH it was discovered that a certain quantity of strong acid was liberated. This was compared with the amount of acid liberated by pyrogallol and by catechol under similar treatment, and was found to lie between the two. In the case of pyrogallol the acid is known to be a mixture of oxalic and acetic acids.

The action of various acids on the tannin has been studied, and among these nitric acid has been shown to give oxalic acid and styphnic acid.

The tannin was oxidised with bromine in dioxane, a procedure which, it has been suggested (24), should lead to the production of anthocyanidins. These were not found, however, and it was concluded that even prolonged action of the oxidant does not break down the tannin structure completely.

An attempt to determine the number of reactive positions in the tannin structure by the analysis of brominated derivatives gave results which varied between 4.8 and 3.5, depending on the solvent employed during bromination.

In his conclusion Stephen states:

"We know that the tanning material in wattle is built up predominantly of resorcinol, catechol and pyrogallol nuclei and in addition, aliphatic chains or rings which are fairly highly oxygenated. The mode of linkage of the aromatic rings is such that carboxylic acids related to catechol and pyrogallol, but not to resorcinol, may be liberated by oxidation after suitable stabilisation by conversion of the more reactive -OH groups to methoxyl, but that the low yields indicate a great complexity

of structure. The stability of resorcinol as compared with the other polyhydric phenols leads to its recovery in high proportion after vigorous acid or alkaline treatment, where catechol and pyrogallol are unable to survive. The points of attachment of resorcinol to the rest of the structure are as yet unknown, but should be capable of determination by degradation of suitable derivatives of the tannin."

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