A PRELIMINARY INVESTIGATION OF THE STRUCTURE OF GREEN WATTLE TANNIN (ACACIA DECURRENS WILLD).

by

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SUMMARY.

Green wattle tannin extracts were separated by lead salt precipitation into a phenolic tannin fraction and a non-tannin fraction. The combustion analysis of the tannin fraction corresponded to an empirical formula $C_{15}H_{14}O_6$. Methoxy and acetyl values showed four oxygens were hydroxy groups while the residual oxygens could either be ether linked or carbonyl groups. Diazomethane methylation produced a white product of high methoxyl value which indicated that the four hydroxyl groups were phenolic. Chromatography of the lead salt purified tannin showed an indistinct non-fluorescent trail. Ether extraction of the tannin removed the associated phenolic bodies. The ethereal extract, unlike black wattle extracts, contained no fisetin and evaporation of the ethereal solutions yielded a gummy non-crystalline residue. Alkaline fusion of the purified tannin produced for the first time a variety of acidic and phenolic compounds i.e. resorcinol, pyrogallol, phloroglucinol, β-resorcylic acid, gallic acid and protocatechuic acid. From the high yields of resorcinol (6%) and gallic acid (3%), these units appear to predominate. These degradation productions, coupled with the analytical figures, indicate a possible C_{15} unit with resorcinol and pyrogallol nuclei as a basis. The non-tannin fraction failed to reveal any compound which might cause the excessive redness in green wattle extracts. Chromatography of this non-tannin fraction showed the presence of sucrose.

INTRODUCTION.

The green wattle (Acacia decurrens Willd) is closely allied to black wattle (Acacia mollissima Willd) and until recently some authorities regarded them as varietal forms of the same species. The trees, although similar in appearance, have certain distinctive differences. Amongst the most important are :-

(1) The foliage and trunk of a green wattle specimen is a a more vivid green than in the case of black wattle.

(2) The pinnules are longer and narrower and also not so closely crowded on the pinnae.

(3) Green wattle has a more profuse influorescence than black wattle and has a compact flower head with about thirty florets.

(4) The flowers of green wattle are a rich golden colour and the main flowering period is about July or August while black wattle flowers two or three months later.

The afforestation of the green wattle tree has been carried out in South Africa since 1929. Although closely allied to the black wattle tree which has been successfully cultivated since 1864, the first trial plantations did not find a ready market, and it was only during the war period with the increased demand for tanning materials, that black wattle tannin mixed with green wattle was accepted by manufacturers. Today green wattle trees are not grown to an appreciable extent for commercial purposes, and are mainly found in the drier areas of the wattle belt where the incidence of bagworm is high.

The main disadvantage of green wattle tannin lies in the fact that leather tanned with its extracts exhibits an undesirable red colouration and is not so closely grained as leather tanned from black wattle tannin (1). This reddish colouration is evident in leather tanned with many tannins. It is present also in black wattle tanned leather but to a far lesser extent. Owing to this handicap green wattle plantations have been gradually replaced by black wattle plantations and green wattle trees are now found only where conditions are unsuitable for black wattle growth. A comparison of the colour darkening in black and green wattle is shown in the following table of Williams (2) :-

Age of Trees	Acacia Decurrens		Acacia Mollissima	
	Red	Yellow	Red	Yellow
	Units	Units	Units	Units
3 years	3.5	4.3	2.5	3.8
4 "	4.0	5.2	2.9	3.9
5 "	4.8	5.5	3.5	4.6
6 "	6.8	8.1	3.2	3.5
7 "	5.4	7.4	3.5	4.9
8 "	6.0	8.8	3.9	5.1
9 "	5.7	8.1	3.9	5.0

In spite of these handicaps, green wattle has many advantages, the most important of which are (2, 3) :-

(1) It grows faster on poorer sites than black wattle.

(2) It produces a greater volume of timber under the same conditions as black wattle.

(3) It continues to strip freely in the dry season when

black wattle does not.

(4) The timber produced is straighter than that of black wattle.

(5) Seeds ripen within four or five months of flowering whereas black wattle seeds require fourteen months, thus the chance of pod-shedding is much greater in black wattle than in green. In addition, the seeds from green wattle have been found to have a higher percentage germination than those of black wattle.

(6) The green wattle tree recovers more easily from hail and drought damage and is more resistant to insect attack. The chief insect pest, the wattle bagworm (acanthopsyche junodi), causes intense defoliation and subsequent stunted growth in black wattle whereas green wattle is but slightly affected.

(7) From a comparative study of the tannin contents of trees of different ages from black and green wattle plantations, it has been shown that the tannin contents are nearly the same.

The value of the species as a tannin producer can be obtained from the following table by Williams (2) in which barks from trees of the same age are compared.

Age of Trees	Acacia decurrens	Acacia Mollissima
	Percentage Tannin	Percentage Tannin
3 years	23.5	32.6
4 years	29.0	28.4
5 years	31.6	32.3
6 years	30.4	29.3
7 years	32.0	33.9
8 years	37.7	34.5
9 years	37.0	35.2

It is evident, therefore, that apart from one major handicap, green wattle has many desirable characteristics. The utilization of green wattle plantations is economically important to South Africa where wattle, unlike most agricultural industries, is dependent on an international market where the demand for tanning materials is increasing while reserve supplies of tannins (e.g. quebracho) are dwindling. The timber, which is straight and hard, has also found a ready market as mine props.

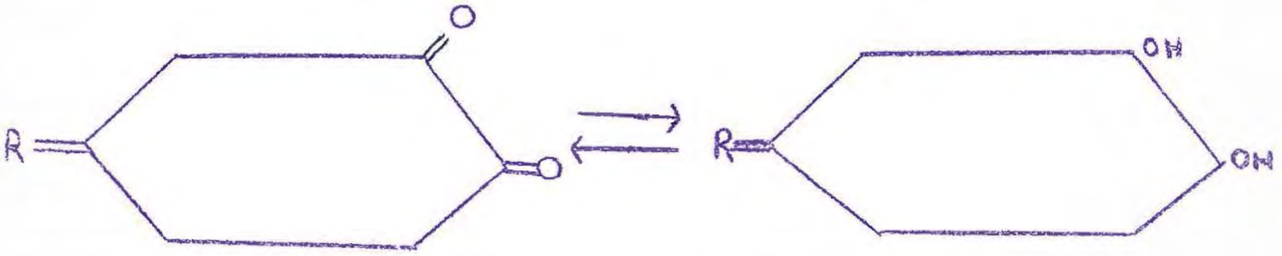
At present, in an effort to utilize green wattle, attempts are being made to hybridize black and green wattle trees (4) to give progenies which combine the vigour, timber-straightness and resistance to insect attack of green wattle with the bark qualities of black.

DISCUSSION.

Previous research on green wattle tannin has been limited to a chemical and physical comparison of methanol extracted green and black wattle tannins by Roux (5). These analytical results, combined with the oxidative degradation products, all indicated that the two tannins were closely allied. Green wattle tannin had slightly lower acetyl and methoxyl values which indicated a lower percentage hydroxyl groups, and thus possibly a higher proportion of catechol units.

A comparison of refractive indices, optical rotation, redox potentials, spectrophotometric analyses, and potentiometric titration curves was also carried out. The refractive indices appeared identical over a concentration range 0 - 30% and spectrophotometry showed only slight differences between the two tannins.

The deeper red colour present in green wattle extracts was believed to be due to some inherent factor in the tannin structure and not to any associated phenolic substance. Rice (64) showed that colour darkening in black wattle was due to oxidation and in the absence of oxygen very light extracts were produced. The oxidation most likely produced a conversion of ortho hydroxy groups from the phenolic to the quinonoid form (Roux. 5).



Watson (63) showed the presence of peroxidase activity in fresh bark. Roux (5) found this peroxidase activity, which accounted for much of the colour darkening, was greater in green wattle tannin than in black, thus partly explaining the deeper colour present in green wattle. When the enzymatic activity was destroyed and oxidation inhibited, a light extract was obtained which, however, still darkened more rapidly than similarily treated black wattle extracts. Two factors were therefore considered to account for the oxidative colour darkening :-

(1) A rapid darkening due to enzymatic activity.

(2) A slower subsequent darkening due to slight constitutional differences.

Empirical molecular weight determinations (6) indicated that green wattle tannin has a higher molecular weight than black wattle. Green wattle has also been reported to be more astringent (1) than black wattle tannin, a property usually associated with high molecular weight. The darker colour produced in green wattle when the tannins were submitted to the same degree of oxidation could possibly be accounted for by Nietzki's Rule which states that increase in molecular weight or complexity of organic molecules, deepens the colour from yellow through red and blue to yellowish green. Alternatively, since oxidation of catechol units produces a reddening effect in solution, an increase in the amount of catechol units present might also increase the red colouration of the solution. All the above experiments were carried out on methanol extracted tannin which Roux (38) has since shown to be 80% pure.

In the present investigation the tannin and non-tannin fractions were separated by Roux's (38) lead salt precipitation method. The tannin purified by this method was found to be 93% pure by the official method of tannin estimation of the International Society of Leather Trades' Chemists (39).

From the degradation products of permanganate oxidation, the tannin fraction was suspected to consist of at least two phenolic bodies since both veratric and trimethoxygallic acids were identified. Chromatographic analysis was thus applied to the problem because of its effectiveness in resolving components of complex mixtures and its ability to separate the components without chemical change.

Grassman and Lang (33) chromatographed quebracho, black wattle, pine, oak and chestnut tannins on an alumina column by developing with methanol-ethyl acetate mixture. Ultra-violet light was used to reveal the various bands. When applied to green wattle tannin, indistinct separation occurred and the bands fluoresced faintly compared with black wattle tannin. Alumina appeared an unsatisfactory absorbent for the phenolic fraction and because of its basic nature, it was found impossible to elute the tannin effectively.

Filter paper partition chromatography, developed by Consden Gorden and Martin (34) for amino acid separations and identification, and by Partridge (28) for sugars, was therefore attempted. This technique was first applied to quebracho tannin by White (27). After developing the chromatograph he was able to distinguish a variety of fluorescent bands under ultra-violet light thus proving the complexity of the quebracho extract.

Lead salt purified green wattle tannin was chromatographed using the butanol-acetic acid-water (4:1:5) mixture of Consden, Gordon and Martin (34). Under ultra-violet light an indistinct brown trail was visible. Since no definite bands were evident, and to compare the extracts with commercial black wattle tannin which is subject to heat treatment, the green wattle solutions were heated on a waterbath for 16 hours before spotting on the paper. This treatment was more drastic than that of commercial black wattle tannin. A dull brown streak R_F 0.0 - 0.21 and two spots (R_F .41, .62) were visible under ultra-violet light. Spot R_F .41 was weakly fluorescent and spot R_F .62 gave a faint blue fluorescence.

The fresh bark extract was ether extracted to remove associated small phenolic bodies. Evaporation of these ether extracss yielded a gummy residue which was chromatographed, and under ultra-violet light three spots were visible (R_F .41, .61, .92). In the presence of ammonia vapours the spot R_F .41 gave a slight fluorescence, spot R_F .61 a yellow fluorescence and spot R_F .92 a blue fluorescence. Lead acetate was added to an aqueous solution of the gummy residue. The precipitate was centrifuged, washed with water, acidified, filtered and ether extracted. Evaporation of the ethereal solution produced a gum which was chromatographed. In the presence of ammonia spots R_F .61 and R_F .41 were visible on the chromatograph. None of these compounds could be identified although spot R_F .92 was similar to phloroglucinol since it was a meta hydroxy phenol and gave a similar blue fluorescence in ammonia vapours (35).

The residual solution gave a single continuous brown streak. Roux (57) suggested the following factors could be responsible for overlapping spots in chromatographs of polyphenols.

(1) A mixture of polymers of varying molecular size formed by the condensation of a single unit to different chain lengths.

(2) Stereoisomers with slightly different R_F values could overlap giving a continuous streak. Bate-Smith (36) calculated R_F values of catechin stereoisomers and found they differed slightly e.g. d-catechin R_F .76, d-l-catechin R_F .74, l-epi-catechin R_F .65, d-gallocatechin R_F.57 and l-gallocatechin R_F .47.

(3) The mixture consisted of substances of R_F values sufficiently close to preclude their separation by one dimensional chromatography.

Lead salt purified tannin, its acetylated and methylated derivatives, were combusted and the acetyl and methoxyl values estimated. Combustion analysis agreed with an empirical formula $C_{15}H_{14}O_6$ while acetyl and methoxyl values indicated the presence of four hydroxyls per C_{15} basic unit and the original tannin therefore corresponded approximately to an empirical formula $C_{15}H_{10}O_2(OH)_4$. The remaining two oxygens thus were either ether linked or carbonyl groups. X

Since the methoxyl value increased from 32% to the maximum of 34.71%(using dimethyl sulphate) with difficulty, one of the hydroxyl groups might possibly be aliphatic. As in black wattle, (7) this difficulty in increasing the methoxyl content was attributed to one or more of the following factors :-

(1) The fission of labile bonds.

(2) The presence of aliphatic hydroxyl groups.

(3) Steric effects caused by ortho-hydroxy groups.

Evidence in favour of the completely phenolic character of green wattle tannin was found in the following observations :-

(1) Perkin (81) methylated catechin with dimethyl sulphate until no colour change occurred between acid and alkaline conditions. At this stage all the phenolic groups were satisfied. Green wattle tannin in methanol solution still exhibited a colour change between acidity and alkalinity when nearly fully methylated.

(2) Part of the nearly fully methylated tannin was irreversibly absorbed on an alumina column causing an increase in methoxyl content and a reduction in colour. This tenacious absorption on an alumina column is characteristic of phenols, particularly ortho-hydroxy phenols.

Diazomethane methylation has been used to distinguish between phenolic and aliphatic hydroxyl groups in lignin (71, 77, 78, 79) and tannins (80). Although diazomethane reacted readily with phenolic and SLOWLY WITH aliphatic hydroxyl groups, it has been known to react with certain labile groups (85). Roux (7) thus compared the reaction of diazomethane on black wattle tannin with that of a variety of compounds and established the predominance of phenolic groups and virtual absence of aliphatic hydroxyls of the type present in d-catechin and brazilin. Green wattle tannin reacted readily with diazomethane giving a white unoxidized product of high methoxyl value thus verifying the predominance of phenolic hydroxyl groups as in the case of black wattle and pyrogallol (7). Steric hindrance due to ortho-hydroxy groups appeared responsible for the difficulty in obtaining a maximum methoxyl value.

Permanganate oxidation of methylated green wattle tannin produced trimethoxygallic and veratric acids. These acids were separated by Corbett's (10) silver salt method and equivalent weight determinations showed they were present in the proportion

4:1. The yields were increased from 5% (Roux. 7) to 20% by substituting an acetone-water mixture (Roux.57) for the aqueous medium. The increased yields can be attributed to three factors :-

(1) The temperature of oxidation was constant at that of boiling acetone.

(2) The two phase system present in an aqueous oxidation (since methylated tannin is water soluble) was replaced by a single phase system.

(3) A correct amount of potassium permanganate was used.

Besides increasing threefold the yields of acids, the proportion of trimethoxygallic acid was increased from 62% as found by Roux (7) to 80%. Trimethoxygallic acid is far less stable to permanganate oxidation than veratric acid (12), and the pyrogallol unit is probably in even greater predominance than these results indicate.

The isolation of trimethoxygallic and veratric acids from the permanganate oxidation of green wattle tannin indicated the presence of two types of end groups in the tannin structure i.e. gallic and catechol units with the gallic predominating. Kirby, (21,22) by fractional methylation, separated methylated black wattle tannin into two fractions. Permanganate oxidation of the one fraction gave only veratric acid, while the other fraction gave only trimethoxygallic acid.

Kirby's (21) fractional methylation technique was carried out on green wattle. Both fractions on permanganate oxidation yielded a mixture of trimethoxygallic and veratric acids, showing that no separation had been achieved. The combustion and methoxyl values of the two fractions showed little divergence (C = 66.96%

H = 6.112%, MeO = 34.6%; C = 65.72%, H = 6.145%, MeO = 33.6%.)

Fusion of green wattle tannin with mixed alkalis yielded 46% of a tarry product of which approximately 22% was phenolic and 23% acidic. After separation into various groups, chromatography of the gummy residues revealed the presence of the following substances when the chromatographs were sprayed with ferric chloride :

The phenolic fraction yielded :-

(1) Resorcinol in yields of 4 - 6%
(2) Pyrogallol in traces
(3) Phloroglucinol in 1% yield.

The acidic fraction yielded :-

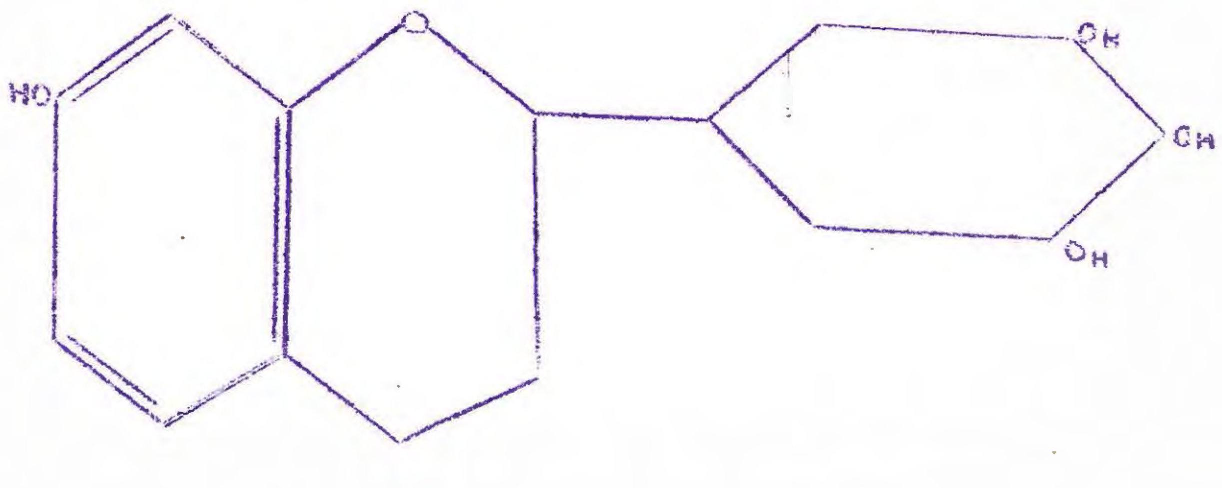
(1) Gallic acid in 3% yield
(2) Protocatechuic acid in traces
(3) β -Resorcylic acid in 1% yield.

Increase in temperature and length of fusion resulted in the complete elimination of β-resorcylic acid thus indicating that yields of β-resorcylic acid would be much higher if the acid was not converted to resorcinol. The thermal stability of these acids increases in the order: β-resorcylic acid, gallic acid, protocatechuic acid. β-resorcylic acid decarboxylates in boiling water (15) while heating to 160 - 165°C. also causes loss of the acidic group (16). Barth and Shreder (56) fused gallic acid with eight times its weight of alkali and decomposition occurred at 340°C. to pyrogallol, hexahydroxy diphenyl and 0.6 to 0.8% phloroglucinol (60). Under similar conditions protocatechuic acid slowly decomposes to catechol.

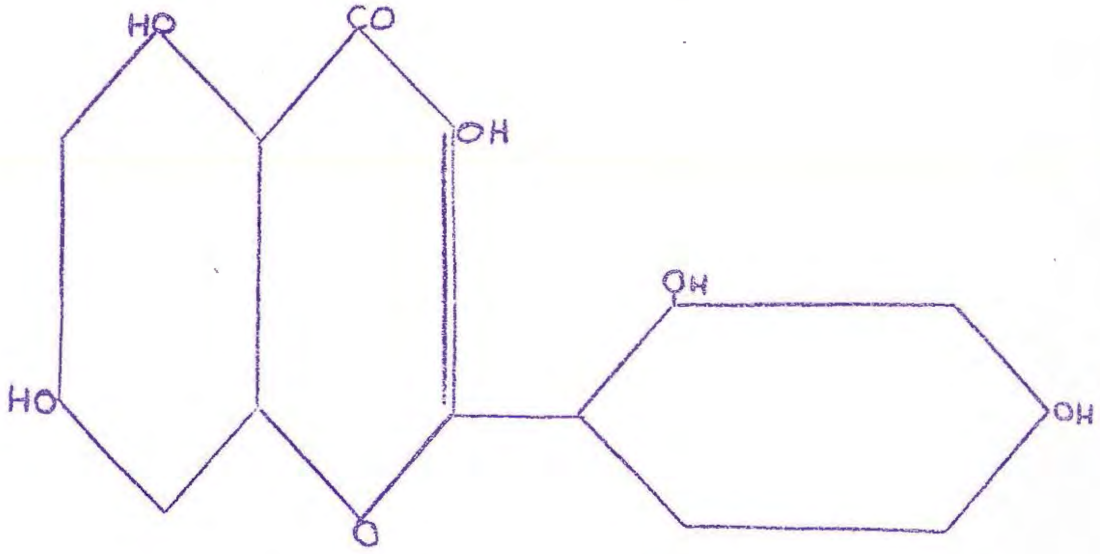
Alkali fusion of catechins and tannins produced similar phenolic and acidic compounds. Fusion of catechin gave phloroglucinol and protocatechuic acid (Etti. 45, and Perkin. 9); of quebracho gave the same products (Arata. 46) while cyanomaclurin gave phloroglucinol, β-resorcylic acid and resorcinol (Perkin. 59).

From the alkali fusion of green wattle extracts it is evident that resorcinol and pyrogallol nuclei predominate. Pyrogallol and resorcinol nuclei together have five hydroxyls. Assuming these nuclei are present in equal proportions, one of the five pyrogallol or resorcinol oxygens must be ether linked, as analytical results showed that only four hydroxyl groups are present in the hypothetical C_{15} structure. As oxidation of methylated tannin gave trimethoxy gallic acid, the three pyrogallol hydroxyls must be free and thus it is only a resorcylic oxygen which is possibly ether linked.

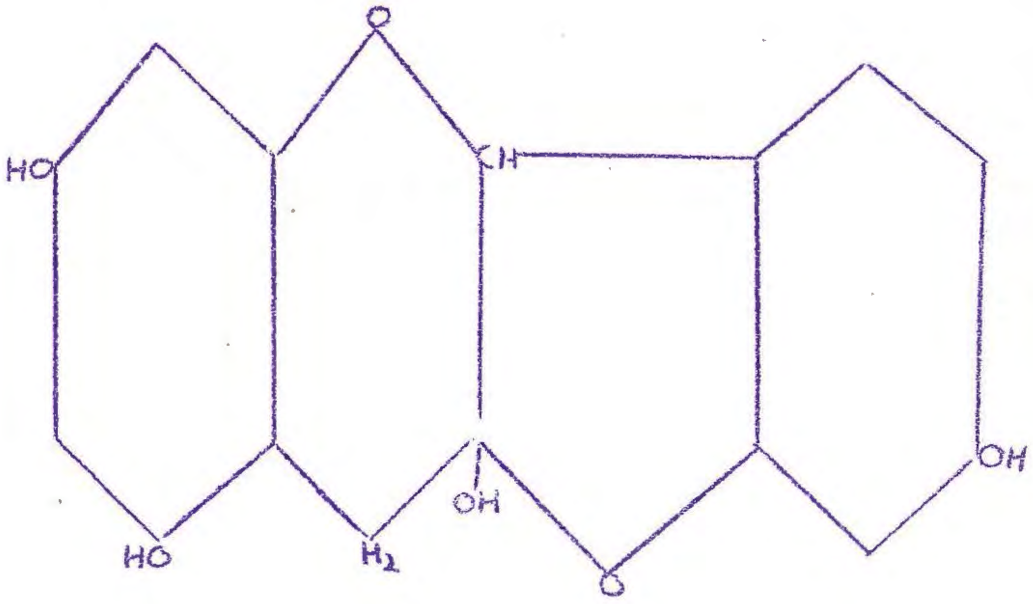
Since β -resorcylic acid has been identified in the degradation products of alkali fusion, the resorcinol nucleus appears to be free because β -resorcylic acid has not been found in units of the type



However, β -resorcylic acid and resorcinol have been found on alkaline degradation of morin (I) by Benedikt and Hazura (58) and cyanomaclurin (II) by Perkin (59).



(I)



(II).

The identification of β -resorcylic acid is thus not conclusive evidence of the absence of a ring structure in the tannin, and the reason it has not been found amongst the degradation products of catechin units by other investigators might possibly be due to decarboxylation caused by high fusion temperatures.

The reaction of green wattle tannin in acid media to form a phlobaphene is further evidence in favour of a pyrane ring structure. Baunschweig (19), summarizing the types of pyrane derivatives which condense in acid media, concluded that the following factors are necessary for phlobaphene formation :

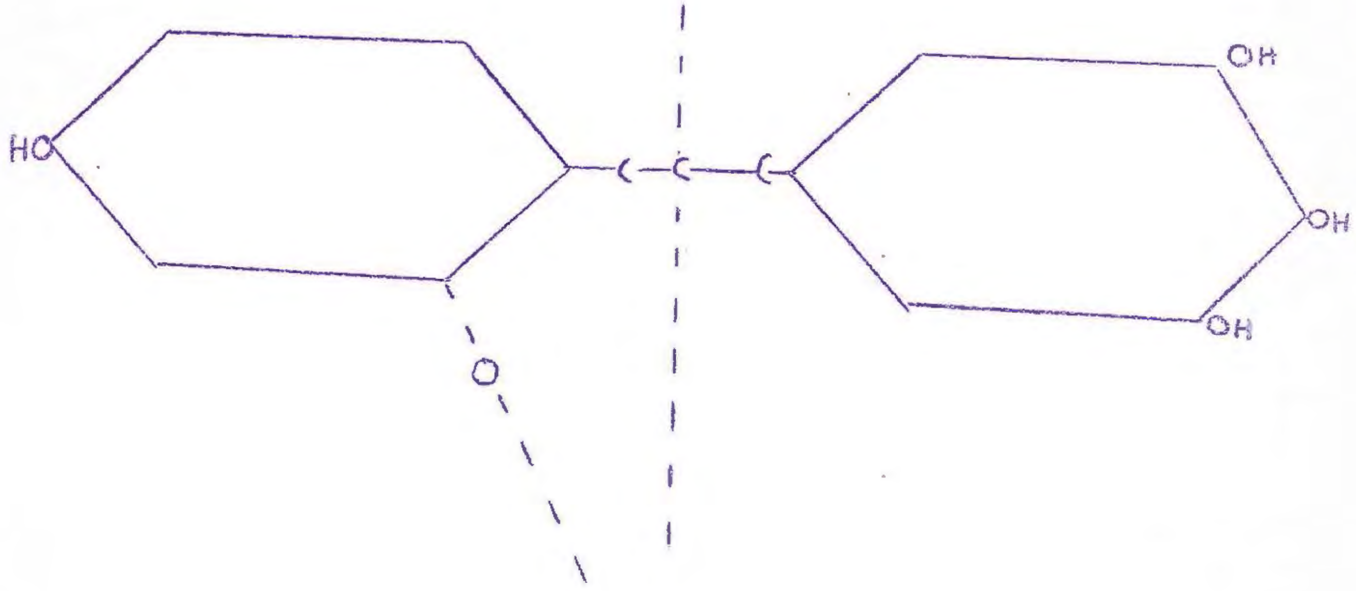
(1) The presence of a pyrane ring.

(2) The presence of a double bond on the pyrane ring.

(3) The presence of a hydroxyl group whether alcoholic or phenolic.

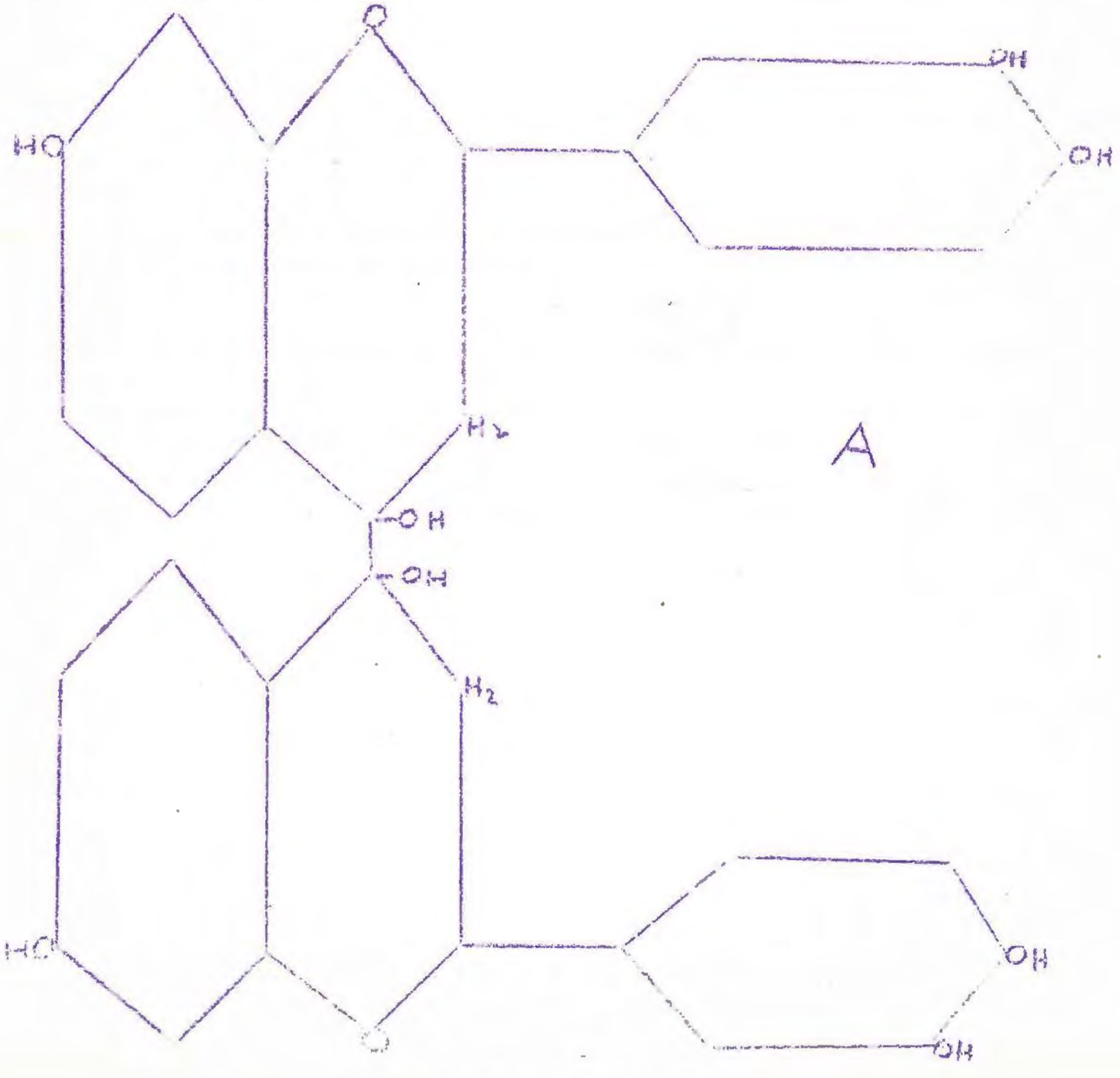
This statement, however, must be treated with caution because many phenols are known to condense in acidic media.

From the evidence obtained so far, it appears that the structure of green wattle is of the type



Although not yet conclusively proved, the phlobaphene forming ability of green wattle, coupled with the analytical results, favour a pyrane ring structure.

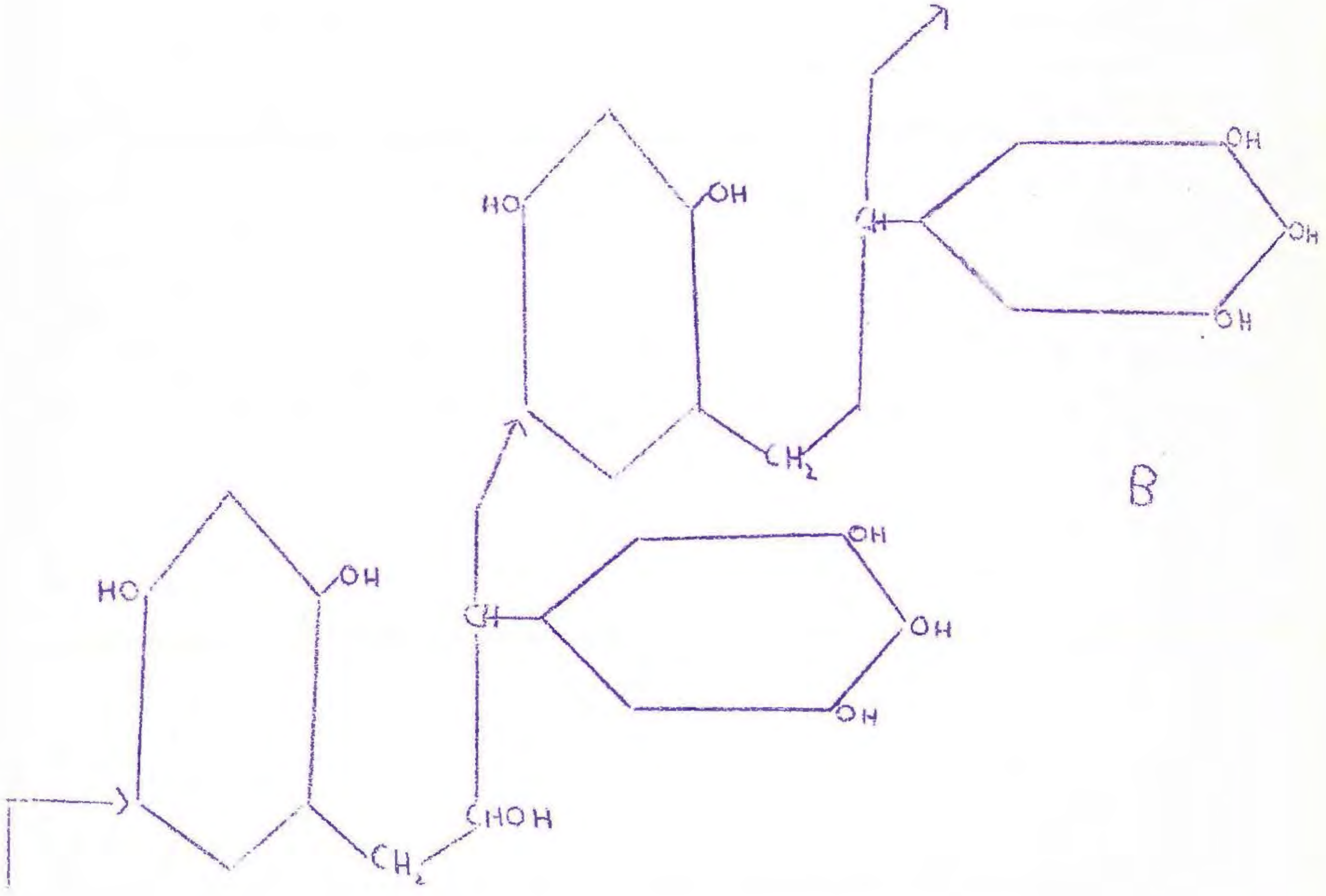
Green wattle tannin with its high molecular weight and amorphous nature is evidently a highly polymerized substance. The polymerization mechanism of polyphenols is still a subject for speculation. Russel (20) and Freudenberg (12, 14) put forward conflicting theories regarding the condensation mechanisms of polyphenolic compounds to form tannins. Russel postulated a flavpinacol structure and claimed (83) to have synthesised compound A. Absorption spectra (84) and colour reactions (83) showed A was indistinguishable from natural tannin



A

Freudenberg (82) contested this dimer structure and argued that owing to the complexity of the reactions by which Russel prepared this dimer, it was unlikely that a single product could have resulted. Freudenberg maintained the spectroscopic evidence was insufficient in view of the fact that hydrolysable tannins behave similarily, and in any case there was no reason to suppose that the pinacol nucleus only occurred in natural tannins.

Freudenberg (13) considered the pyrane ring opened and in polymerization linkage occurred through the resorcinol units as in structure B.



B

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Freudenberg's formula does not agree with the analytical figures for green wattle which indicated four hydroxyl groups per C_{15} unit whereas his formula requires six hydroxyl groups.

It appears that neither Russel's nor Freudenberg's theory can explain the condensation mechanism in green wattle which probably polymerizes as a result of enzymatic activity.

Many tannins are known to contain carbohydrates. The non tannin fraction of quebracho (27) contained the sugars glucose, arabinose and xylose. Tarboton (52) found sucrose, glucose and possibly maltose present in black wattle tannin while Roux (11) and Stephen found sucrose and only traces of reducing sugars.

Green wattle non-tannin fraction gave the characteristic burnt sugar odour and thus sugars were suspected.

The similarity of sugars in physical and chemical properties made it difficult to separate the mixtures especially when small quantities were present. The most successful method of identifying small quantities of sugars was by paper chromatography developed for sugars by Partridge (28, 29) and later by Horrocks (51) Forsythe (32) and Hugh, Jones, and Wadman (30, 31).

Spraying the chromatograph with resorcinol and naptharesorcinol (32) revealed the presence of sucrose. This was confirmed by hydrolysing the solution with hydrochloric acid, The product was chromatographed and sprayed with anisidine hydrochloride (73) aniline hydrogen phthalate (29) and naptharesorcinol. The identification of glucose and fructose confirmed the presence of sucrose in the original solution.

A COMPARISON OF BLACK AND GREEN WATTLE TANNIN.

The economic importance of utilizing the resources of green wattle has already been discussed and green wattle, except for the deeper red colour of its extracts, has many advantages compared with black wattle tannin. A comparison of the two tannins is important in order to find an associated compound or slight constitutional difference which might account for the deeper red colour of green wattle extracts.

Paper chromatography of fresh bark extracts of green and black wattle showed marked differences. The developed chromatograph of black wattle tannin (Roux. 57) indicated the presence of at least four substances. These were represented by (a) a dull brown streak $R_F = 0 - 0.47$ (b) a weakly fluorescent spot R_F 0.53 (c) a bright yellowish green fluorescent spot $R_F = 0.72$ which proved to be fisetin $R_F = 0.72$ (d) a blue spot $R_F = 0.90$ which gave evidence of being a phloroglucinol derivative. The chromatograph of green wattle tannin, however, gave a brown streak $R_F =$ 0 - 0.43 in daylight. When observed under ultra-violet light an indistinct non-fluorescent trail was observed.

A comparison of analytical results for green wattle with Roux's (23) for black are given in the following table :

	Green Wattle	Black Wattle
Combustion Analysis	C=61.55%, H= 4.73%	C=60.9%, H= 4.83%
Methoxy Values with Dimethyl Sulphate	34.61%, 34.70%	36.47%
Combustion Analysis of Methoxy deriv.	C=66.92%, H= 6.11%	C=65.85%, H= 6.14%
Methoxy Values with Diazomethane	30.26%, 30.15%	32.45%, 32.35%
Acetyl Values	36.80%, 36.75%	37.55%, 37.48%
Combustion Analysis of Acetyl deriv.	C=59.20%, H= 4.47%	C=60.08%, H= 4.74%

The analytical figures for both tannins correspond to a $C_{15}H_{14}O_6$ empirical formula with four hydroxyl groups. Diazomethane methylation showed that in both cases the hydroxyl groups were phenolic. The lower methoxyl and acetyl values of green wattle could be due to a higher proportion of catechol units.

Empirical molecular weights studies (6) showed green wattle has a higher molecular weight than black wattle tannin.

When fused with alkali both tannins gave approximately 22% acids and 20% phenols. The following acids and phenols were isolated in similar yields from the fusion of both tannins :

<u>Phenolic fraction</u>

(1) Resorcinol

(2) Pyrogallol

(3) Phloroglucinol

Acidic fraction

(1) Gallic Acid

(2) Protocatechuic Acid

(3) β-Resorcylic Acid

Since both tannins gave similar yields of the same products, they are obviously derived from the same basic units.

Permanganate oxidation of both methylated tannins produced approximately 20% yields of a mixture of trimethoxygallic and veratric acids.

Sucrose was the only sugar present in the non tannin fractions of green and black wattle.

Green wattle tannin contained no associated phenolic compound which might cause the excessive redness of the extracts.

From the aspect of colour darkening the most important differences between the two tannins were in molecular weight and hydroxyl content. As mentioned previously, a higher molecular or greater proportion of catechol units might account for the deeper red colour in green wattle extracts.

PART II

EXPERIMENTAL

PART II

EXPERIMENTAL

Purification and Preparation of Green Wattle Tannin

Bark, preserved in paraffin, was stripped of all oxidized areas, cut into fine slices with a stainless steel knife and leached with hot water. The aqueous extract was filtered and evaporated to dryness under reduced pressure giving a light brown, amorphous, powder.

Purification of Green Wattle Tannin

The method of purification was similar to that of Roux (38) for black wattle tannin.

15% lead acetate solution was slowly added with stirring to an aqueous tannin solution. A pale pink precipitate of lead tannate formed instantaneously. A slight excess of lead acetate was added, the precipitate centrifuged, and washed with water three times centrifuging after each washing. The final product was suspended in water and dilute sulphuric acid added to precipitate the lead sulphate and liberate the tannin. The lead sulphate was removed by centrifuging and 2 N sodium hydroxide added to the tannin solution until the pH was raised to 4.0 just below the natural pH of green wattle tannin (4.6 - 5.0). At this pH all the sulphuric acid was neutralized. The solution was evaporated to dryness under vacuum giving the light brown tannin which was extracted twice with ethanol to remove traces of salts, and again concentrated under reduced pressure.

Estimation of Purity of Green Wattle Tannin

The hide powder method of the International Society of Leather Trades Chemists (Proctor (39)) was adhered to in estimating the tannin content of the above purified tannin. Although this is an empirical method, a high degree of accuracy can be obtained. Found :

Tannins = 93.37%, 93.34%
Non Tannins = 6.63%, 6.67%

Analysis of Tannin and its Derivatives

(1) Green Wattle Tannin

The tannin used in analytical studies was purified by lead acetate precipitation and dried for 2 hours in an Abderhalden gun at 110°C.

Found :

0867 gms. tannin gave .1959 gms. CO_2 and .0366 gms H_2O
whence C = 61.62%, H = 4.72%
0981 gms tannin gave .2214 gms CO_2 and .0413 gms. H_2O
whence C = 61.55%, H = 4.73%

$C_{15}H_{14}O_6$ requires :
C = 62.07%, H = 4.83%

The affinity of tannin for retaining small amounts of impurities, its high resistance to combustion and the effects of atmospheric oxidation, could contribute to the slightly low carbon and hydrogen values obtained.

(2) Acetylation of green wattle tannin.

A vigorous acetylating agent is required to completely acetylate green wattle tannin because the steric effects of ortho-hydroxy groups makes complete acetylation difficult. Stephen (42) successfully acetylated black wattle tannin with acetic anhydride and anhydrous pyridine while Roux (23) obtained complete acetylation with acetyl chloride and pyridine.

5 gms. lead acetate purified, dry, green wattle tannin were dissolved in 20 ml. pyridine and cooled in an ice salt mixture. 10 gms. acetyl chloride were added in small portions with shaking. A vigorous reaction occurred precipitating a white amorphous product. The mixture was left in contact with ice for thirty minutes and then slowly poured, with stirring, into iced water. The insoluble acetylated compound was sucked off in a Buchner funnel, washed with iced water and air dried. The light brown product was dissolved in acetone, filtered, and evaporated to dryness under vacuum.

Acetyl estimation of green wattle tannin

(a) Trans Esterification method

This method of Matchett and Levine (53) has been successfully used by Stephen (42) to estimate the acetyl content of acetylated black wattle tannin.

The technique was standardized against acetyl salycylic acid which had been recrystallized twice from a mixture of water and acetic acid (1 : 1).

Found :

.1332 gms. acetyl salycylic acid required 4.65 ml.

.1226 N NaOH whence acetyl = 23.58%

.0965 gms. acetyl salycylic acid reouired 4.45 ml.

.1226 N NaOH whence acetyl = 23.71%

$C_7H_5O_2(OCOCH_3)$ requires :

Acetyl = 23.9%

The acetylated tannin was dried in an Abderhalden gun for 2 hours at 110°C. The acetylated product was reacetylated twice to ensure maximum acetyl values.

No. Acetylations	Value
1	35.67, 35.87
2	36.16, 36.56
3	36.80, 36.75

Combustion analysis of maximum acetylated tannin

Found :

.0854 gms. tannin gave 11737 gms. CO_2 and .0340 gms. H_2O

whence C = 59.07%, H = 4.43%

.0800 gms. tannin gave .1849 gms. CO_2 and .0321 gms. H_2O

whence C = 59.20%, H = 4.47%

$C_{23}H_{22}O_{10}$ or $C_{15}H_{10}O_2(OAc)_4$ requires :

C = 60.53%, H = 4.84%

Acetyl = 36.67%

(b) Valentins Method

Valentin (54) devised an alternative shorter method for acetyl estimations. The basis of this method was the catalytic esterification of the substance with acetic anhydride under anhydrous conditions. The excess acid was titrated against standard alkali and deduction from a blank gave the equivalent hydroxyl. Fluoboric acid which was the catalyst, was specific for esterification.

Resorcinol found :

.2272 gms. resorcinol required 4.2 ml. .9150 N NaOH

whence acetyl = 28.54%

.3294 gms. resorcinol required 4.77 ml..9150 N NaOH

whence acetyl = 2834%

$C_6H_4(OCOCH_3)_2$ requires :

acetyl = 29.03%

Pyrogallol found :

.1981 gms. pyrogallol required 4.32 ml. .9173 N NaOH

whence acetyl = 34.08%

.1929 gms. pyrogallol required 4.01 ml. .9173 N NaOH

whence acetyl = 33.5%

$C_6H_3(OCOCH_2)_2$ requires :

acetyl = 40.08%

Green wattle tannin found :

.3613 gms. tannin required 4.70 ml. .9173 N NaOH

whence acetyl = 20.2%

.5338 gms. tannin required 5.25 ml. .9173 NaOH

whence acetyl = 19.3%

Although the method gave satisfactory results for resorcinol, low values were obtained with pyrogallol and tannin. These low values were attributed to steric effects of ortho-hydroxy groups and this method appears unsuitable when these groups are present.

(3) Methylation of green wattle tannin

Stephen (42) obtained a highly methylated derivative of black wattle tannin by methylating three times with dimethyl sulphate. 30 gms. potassium hydroxide in methanol were slowly added with thorough stirring. A vigorous reaction occurred and after the reaction subsided, 50 cc. more dimethyl sulphate were added and the methylation repeated twice. Towards the end of the methylation the solution heated to boiling point.

After the third methylation was completed, the cold solution was poured into a litre of water slightly acidified with sulphuric acid. The white precipitate which formed was sucked off in a Buchner funnel, washed with water, and dried. Finally the methylated tannin was dried in an Abderhalden gun at 110°C for 2 hours.

Found :-

.0766 gms. tannin gave .1891 gms. CH_3I

whence OCH_3 = 32.06%

.0917 gms. tannin gave .2146 gms. CH_3I

whence OCH_3 = 32.09%

To obtain a maximum methoxyl value three methylations were necessary before constant results were obtained. Furthermore, after each subsequent methylation, the methylated tannin was passed through an alumina column using this technique. Kirby (21) obtained highly methylated black wattle tannin because partially methylated hydroxy groups were retained on the column while the fullymethylated tannin was eluted off.

Found :-

No. Methylations	Methoxy Content
1	32.06, 32.09
2	33.60, 33.22
3	34.58, 34.60
4	34.61, 34.70

Combustion analysis of maximum methylated tannin

Found :-

.0562 gms. tannin gave .1380 gms. CO_2 and .0307 gms H_2O

whence C = 66.97%, H = 6.11%

.0620 gms. tannin gave .1487 gms. CO_2 and .0341 gms H_2O

whence C = 66.92%, H = 6.11%

$C_{19}H_{22}O_6$ or $C_{15}H_{10}O_2(OCH_3)_4$ required :-

C = 65.9% H = 6.36%

OCH_3 = 34.8%

Methoxy and acetyl values therefore indicate the presence of four hydroxyls per C_{15} unit.

Methylation with diazomethane

Since it was difficult to increase the methoxyl content from 32% to 34.65% using dimethyl sulphate, one of the four hydroxyls per C_{15} unit might possibly be aliphatic as aliphatic hydroxyls are not easily methylated by dimethyl sulphate. In black wattle tannin (7) a similar difficulty in increasing the methoxy content was attributed to the presence of aliphatic hydroxy groups or steric effects caused by ortho-hydroxy groups. Either or both these factors could account for the difficulties found in methylating green wattle tannin.

Diazomethane methylation has been used to distinguish between aliphatic and phenolic hydroxy groups. Roux (7) thus compared the reaction of diazomethane on black wattle tannin with that of a variety of compounds and established the predominance of phenolic groups and absence of aliphatic hydroxyls of the type present in d-catechin and brazilin.

Diazomethane was prepared from nitrosomethylurea by Arndts method (55). From 42 gms. nitrosomethylurea used in each case, 10 gms. diazomethane was produced.

2 gms. lead salt purified tannin was dissolved in 100 ml. methanol, cooled in ice and added to the ethereal diazomethane solution. An immediate vigorous reaction occurred. After remaining in an ice bath overnight, the ether was evaporated and the alcoholic solution added to 600 ml. water. On salting out the solution, a pink precipitate formed which was filtered, washed and dried. The dry product was dissolved in ethanol, filtered and evaporated to dryness.

No. Methylations	Methoxyl Value
1	29.9%,30.06%
2	30.26%,30.15%

From the high methoxyl content of the product, green wattle, similarity to black wattle, appears to have a predominance of aliphatic hydroxyl groups.

Chromatography of Green Wattle Tannin

(a) Absorption Chromatography

Light coloured fresh bark extracts were chromatographed on an alumina column (10" x 1½") as described by Grassman and Long (33). The column was developed with methanol-ethyl acetate mixture but indistinct separation occurred. Other eluents were tried, e.g. pyridine and acetone, but the strong basic character of alumina made it difficult to develop the column successfully.

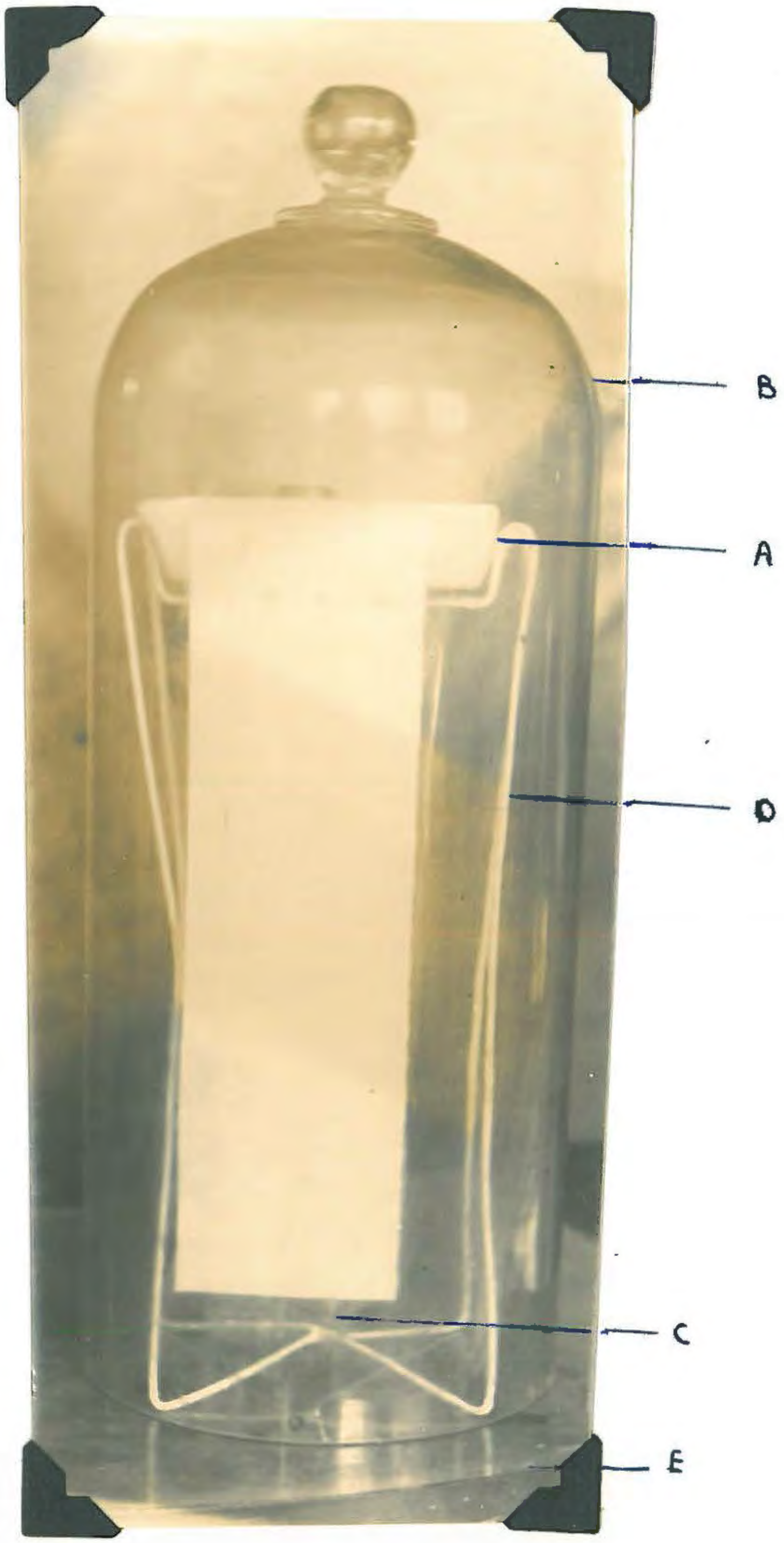
(b) Partition Chromatography

Consden, Gordon and Martin (34) technique of filter paper chromatography was then used. Originally an apparatus similar to that described by them was used. Difficulties were encountered in making a glass trough and finally a glazed earthenware trough was found to be satisfactory. This apparatus was later replaced by a simpler structure illustrated on page 34.

A glass trough, A, was supported on a steel structure D, and contained the organic solvent saturated with water. Tray C contained water saturated with the organic solvent. The filter paper was suspended from A and the apparatus covered by a bell jar. The surfaces between the bell jar B and the glass sheet E were made air tight with plasticine so that the atmosphere inside the bell jar could become saturated with the mixture in C.

The solutions were spotted on six inches from the top of the strip and the spots were approximately 5 mm. in diameter.

Chromatography Apparatus



The chromatographs were developed from12 hours at 22°C.

Consdon, Gordon and Martin (34) maintained that the reproducibility of R_F values depended on six factors.

1) type of paper,
2) temperature. Equilibria in terniary mixtures such as butonol-acetic acid-water are sensitive to temperature fluctuations with the possibility of slow esterification occurring.
3) quantity of material employed.
4) distance between starting point and solvent source,
5) degree of saturation with water.

In the present investigation various precautions were taken. Whatman No. 11 paper was used throughout and the solutions were always spotted on 6" from the end of the paper. The apparatus was kept at a constant temperature of 22°C. Airtightness was ensured by ground glass surfaces and plasticine on the outside, as variations in the degree of saturation through leakages produce uneven development of the chromatograph.

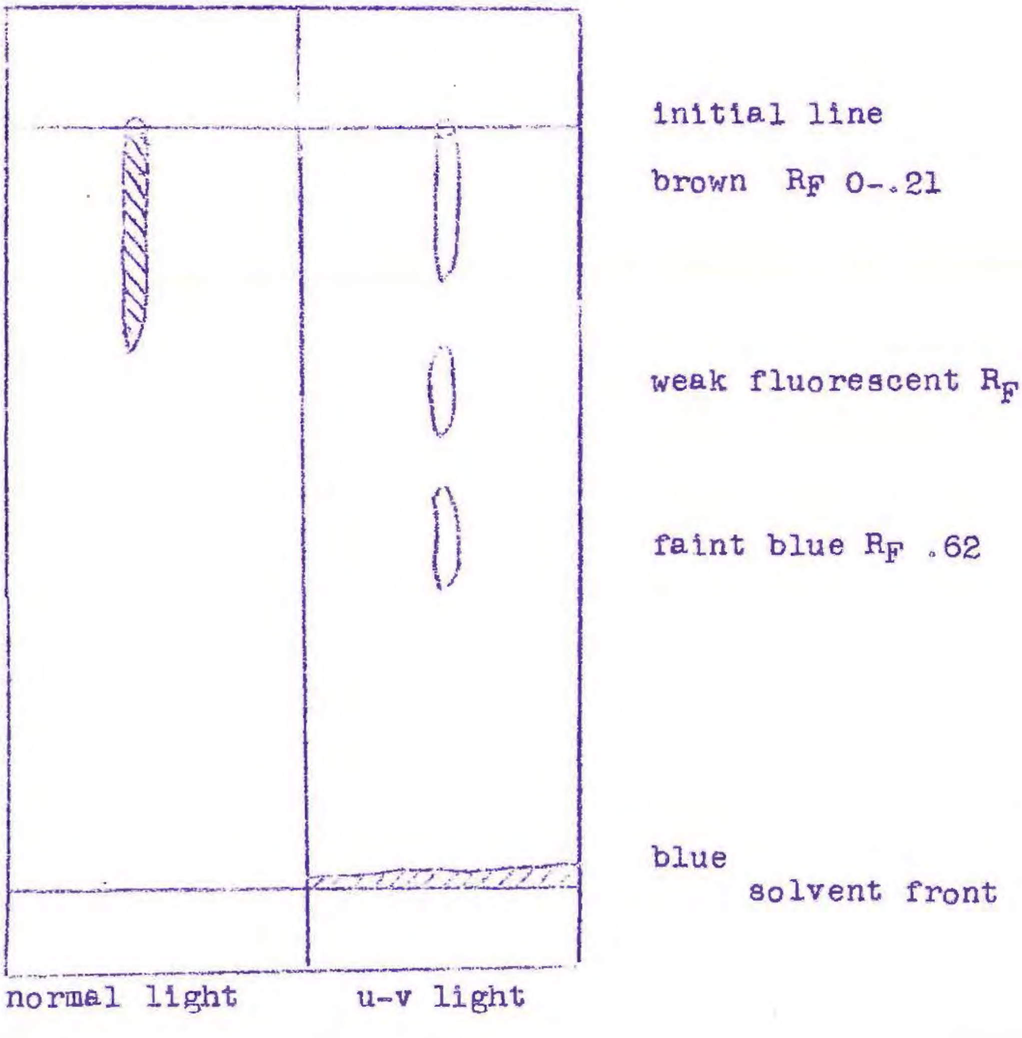
Chromatography of green wattle tannin.

A concentration range of 5 - 25% was tried out and finally 15% was found to be most suitable. Daylight showed a brown trail R_F0 - 14 while ultra violet light revealed an indistinct, weakly fluorescent streak which showed no separation.

Since no definite spots were visible, and to compare the extracts with commercial black wattle tannin extracts which are subject to heat treatment, the green wattle solutions were heated on a waterbath for 16 hours before spotting on the paper. In daylight a brown trail was visible from the point of application to a point R_F .31, while under ultra-violet three spots could be detected.

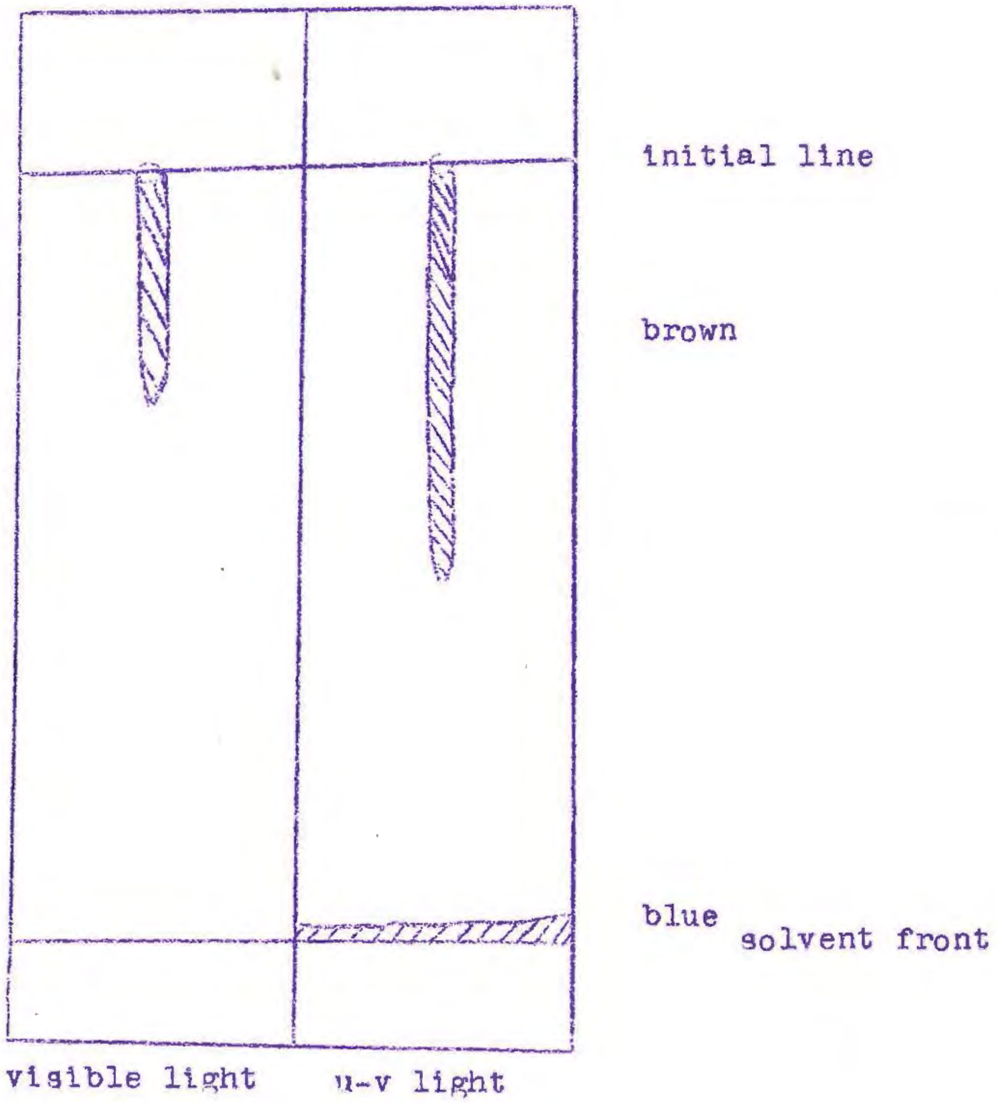
<u>R_F values of spot</u>	
	Spot I R_F .21
	Spot II R_F .41
	Spot III R_F .62

Spot I was dull brown, spot II weakly fluorescent and spot III was a faint blue. The spots were present on chromatographs of purified and unpurified extracts. In spite of the precautions taken, R_F values varied considerably. When sprayed with ferric chloride the chromatograph gave a continuous blue streak which showed no definition of the various spots.



The tannin was shaken up twelve times with ether to remove associated small phenolic bodies. The mixture was difficult to separate and had to be centrifuged. A small proportion was ether soluble and the insoluble residue was chromatographed.

Chromatography of Ether extracted tannin



The ether soluble extracts were evaporated to dryness (yield 3%) and chromatographed. Under normal light a reddish brown streak was visible while ultra-violet light distinguished three bands.

1) Spot R_F .41 of slight fluorescence gave a purple ferric chloride reaction.
2) R_F .60. Blue Colour. In the presence of ammonia gave a yellow fluorescence. With ferric chloride gave a green reaction.
3) R_F .92 yellow fluorescence which turns blue in the presence of ammonia vapours. Gave a grey ferric chloride reaction.

Lead acetate was added, and the precipitate acidified, and ether extracted. Evaporation of the ethereal extract produced a gum which was chromatographed and showed spots (1) and (2) had been precipitated. Spot (3) was meta-hydroxy and gave a blue fluorescence similar to phloroglucinol but the R_F value was much greater (0.92 compared with 0.78). The phloroglucinol produced from alkali fusion could possibly be a degradation product of this compound. The compound (R_F .92) was therefore fused with mixed alkali at 195°C for 2 hours. Ether extraction and subsequent chromatography failed to yield any phloroglucinol although the amount of material used was very small (.1 gm) and intimate mixture was difficult to achieve due to the gummy nature of the material.

Attempts to Isolate Small Phenolic Bodies by Fractional Precipitation

Fractional precipitation techniques have been used to precipitate large molecular weight bodies while small molecular weight compounds remained in solution (48). In this way tannins could be salted out and relatively small units, e.g. catechins, flavones etc., left in solution.

A cold concentrated green wattle extract was salted out and filtered. The filtrate was extracted three times with ethyl acetate and the extracts dried over sodium sulphate. The extracts were concentrated and shaken up with chloroform when a whitish precipitate formed. The precipitate was filtered, the filtrate extracted twice with water, and the aqueous extracts concentrated to 25 ml. The concentrate was left in an ice box for 10 days but no crystals formed.

Ether extraction of this aqueous solution yielded a small gummy residue which gave a green ferric chloride reaction. Paper chromatography of this solution produced two spots which coincided with the spots of R_F value (.05, 162) in the green wattle chromatograph indicating that these bands represented low molecular weight species.

The chromatograph was sprayed with

1) Ferrous tartrate (68), giving a purple streak.
2) Benzidine (70), giving a streak similar to tannin.

The yield of gummy residue was too small for further identification.

DEGRADATION STUDIES

Alkaline Fusion of Green Wattle Tannin.

In these fusions the lowest possible temperature was desired because of the thermal instability of some of the degradation products. A phase diagram of the fusion of potassium and sodium hydroxide (72) showed that at the eutectic temperature (167°C.) the proportions of the alkalis were 3:2. These proportions were therefore used in all the fusions.

10 gms. tannin were mixed with 50 gms. of a mixture of 60% potassium hydroxide and 40% sodium hydroxide which had been ground up in a mortar. The mixture was placed in a sealed bomb and fused for one hour at 195°C.

The cold mixture was dissolved in water and ether extracted three times. The ethereal extracts contained only the neutral fusion products, and on evaporation yielded a trace of oily residue too small to identify.

The aqueous solution was acidified, ether extracted ten times and dried over sodium sulphate. The extracts were concentrated to a small volume, excess sodium bicarbonate added, and again extracted five times with ether. This fraction contained phenolic degradation products. The residual aqueous solution was acidified, ether extracted ten times and dried. This fraction contained acidic compounds.

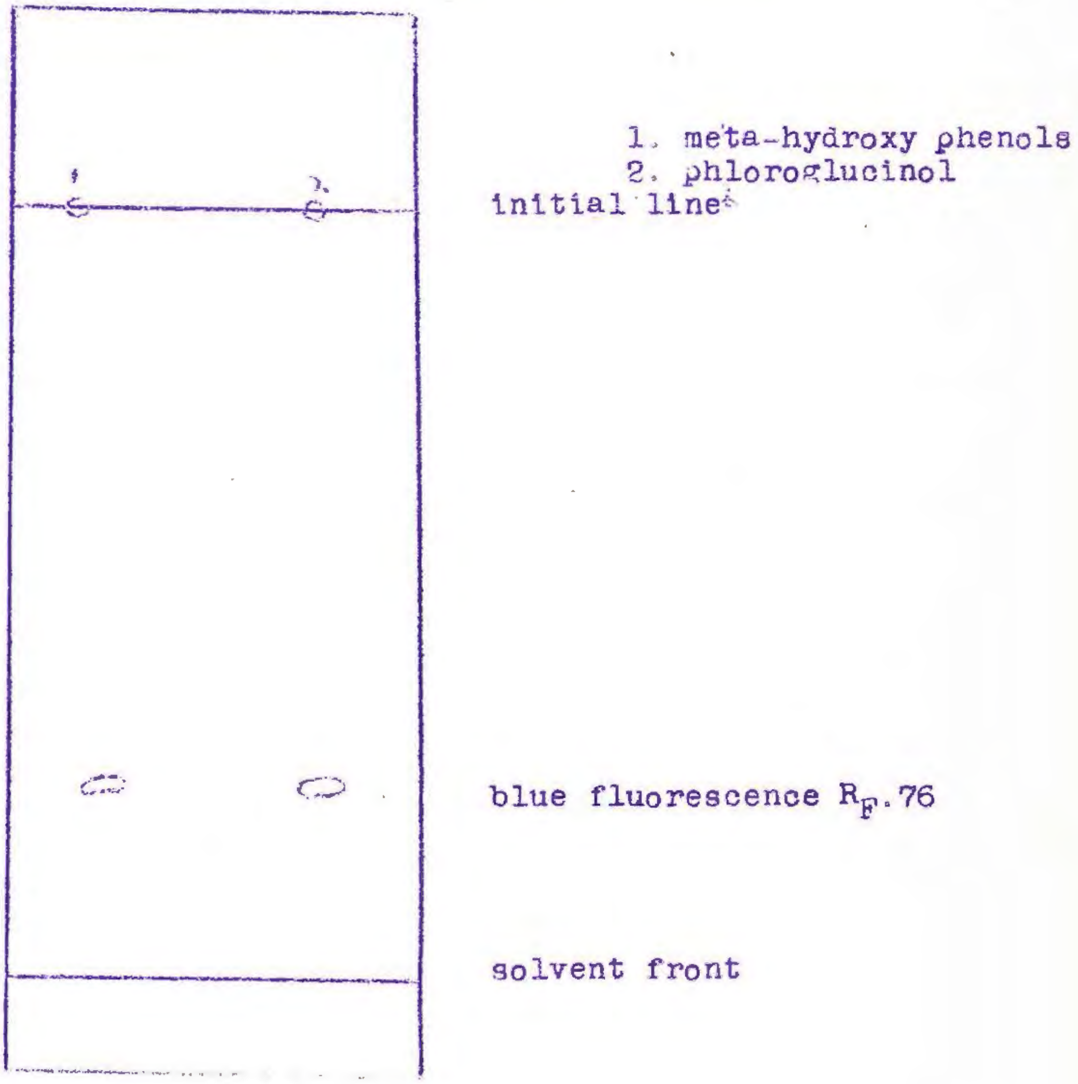
Phenolic Fraction.

Evaporation of the phenolic fraction yielded a dark gum (22%). This gum was dissolved in water, excess lead acetate added, and the precipitate centrifuged. The precipitate was washed twice with water, acidified, and ether extracted. This fraction contained ortho hydroxy phenols. The filtrate was united with the washings, acidified, and ether extracted five times giving the meta- and para-hydroxy phenolic fraction.

Evaporation of the meta-hydroxy fraction yielded a brown gum (12%). The gum was chromatographed in conjunction with phloroglucinol for 12 hours using butanol-acetic acid-water (4:1:5) as the developing mixture.

In daylight only a brown trail was visible, but under ultra-violet light, in the presence of ammonia vapours, a blue fluorescent spot was observed. This bright blue fluorescence is characteristic of phloroglucinol (35) and has the same R_F value .76.

Chromatograph of meta-hydroxy phenols as observed under ultra-violet light and in the presence of ammonia vapours.



Preliminary fusions indicated the presence of resorcinol in the phenolic gum. The resorcinol and phloroglucinol were separated by fractional sublimation. The gum was heated to 80 - 100°C under vacuum and at this temperature yellow white crystals sublimed (4%). The sublimate was recrystallized twice from benzene yielding colourless crystals MP 108°C. which gave a violet ferric chloride reaction. Mixed melt with RESORCINOL MP 108°C.

The temperature of sublimation was slowly increased to 110°C. and when all the resorcinol had sublimed increased to 150 - 175°C. At 150°C. crystals began subliming (1%) which gave a blue-violet ferric chloride reaction. The crystals were resublimed M.P. 207°C. Mixed melt with PHLOROGLUCINOL 207°C.

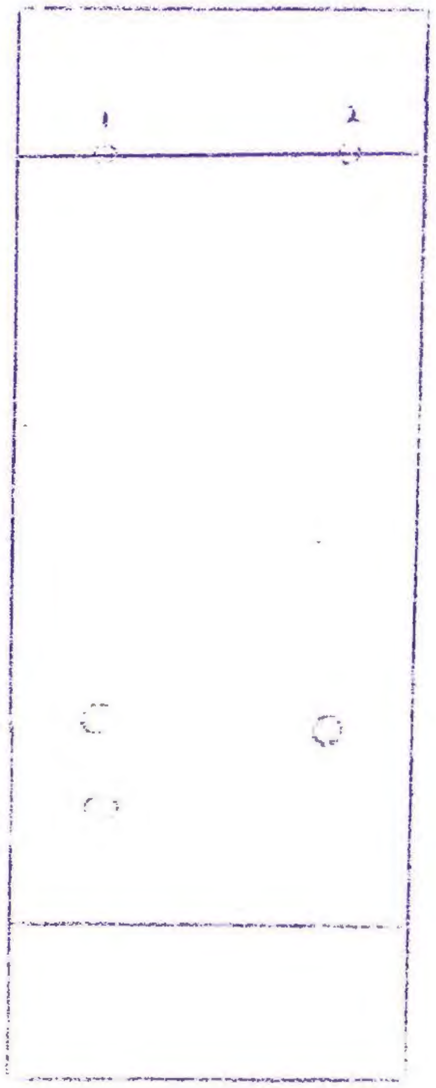
Evaporation of the ortho-hydroxy ethereal extracts yielded a brown gum (8%). This gum was chromatographed for 12 hours in butanol-acetic acid-water (4:1:5). Under normal light only a brown streak was visible. Spraying reagents revealed :-

(1) A purple streak of greatest intensity at R_F .77 when the chromatograph was sprayed with 1 gm. ferrous tartrate (Mitchell. 68).

(2) Similar dark purple spots at R_F .77 and R_F .88 when the chromatograph was sprayed with ferrous tartrate and 10% ammonium acetate buffer (Glasstone. 69).

These reactions were indicative of gallic groupings and when a chromatograph was run in conjunction with pyrogallol, spot R_F .77 agreed in R_F value with pyrogallol. Since the yield of gum was low and pyrogallol appeared present, a more drastic fusion was carried out at 200°C. The ortho-hydroxy gum sublimed and on standing for a week the oily sublimate crystallized. The crystals M.P. 126 - 129°C. gave a transient blue colour which turned yellow with ferric chloride in aqueous solution. Mixed melt with PYROGALLOL 128°C.

Chromatography of ortho-hydroxy fraction when sprayed with ferrous tartrate and 10% ammonium acetate buffer.



1. ortho-hydroxy fraction
2. pyrogallol

yellow R_F .76

R_F .86

Acidic Fraction

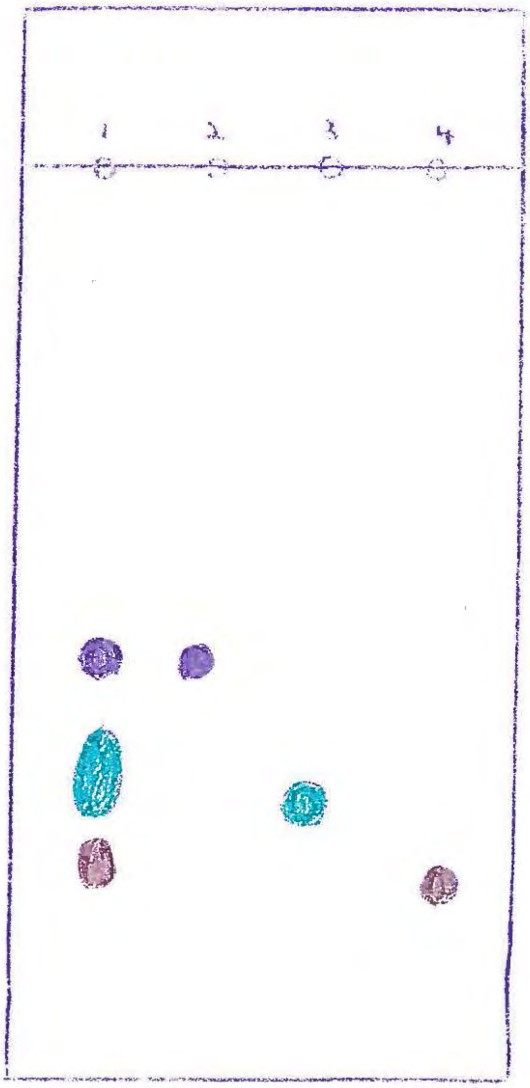
Evaporation of the acidic fraction yielded 23% of a brown gum. The gum was chromatographed for 12 hours in butanol-acetic acid-water (4:1:5). Under normal light a brown streak was visible.

Treatment of Chromatograph.

(1) Spraying the chromatograph with bromo-cresol-green (35, 37) revealed three distinct yellow acidic regions.

(2) Spraying the chromatograph with ferric chloride (35) revealed three spots corresponding to the three acidic regions. These spots were blue, green and red and agreed in R_F value and colour with gallic acid (R_F .68), protocatechuic acid (R_F .85) and β -resorcylic acid (R_F . 93) when the chromatograph was run in conjunction with these acids.

Chromatography of Acidic Fraction after spraying with ferric chloride.



1. acid mixture
2. gallic acid
3. protocatechuic acid
4. β -resorcylic acid

R_F .68

R_F .85

R_F .93

Since gallic, protocatechuic and β -resorcylic acids were suspected, the gum was divided into the ortho-hydroxy and meta-hydroxy acids by lead salt precipitation.

The precipitate of ortho-hydroxy groups was acidified, ether extracted, and evaporated to dryness. The oily gummy residue (10 gm) was dissolved in methanol and methylated with 10 gms. diazomethane in methanol-ether solution. After remaining in an ice bath overnight, the solution was evaporated to dryness and refluxed for ½ hour with 150 ml. 4% potassium hydroxide. The hydrolysed solution was acidified, ether extracted eight times, and dried. Evaporation of the extracts gave a gummy residue which was sublimed at 160°C. The sublimate was recrystallized twice from water giving colourless crystals M.P. 166°C. Mixed melt with TRIMETHOXYGALLIC ACID, prepared by the method of Bogert and Coyne (49) 166°C. Protocatechuic acid, which was identified on the chromatograph, appeared absent. The protocatechuic acid which was in much lower proportions than trimethoxygallic acid, was possibly completely decomposed during hydrolysis.

The aqueous filtrate containing meta-hydroxy acids was extracted six times with ether. Evaporation of the extracts yielded a yellow gum (1%) which was twice recrystallized from water giving colourless crystals M.P. 205°C. Mixed melt with β -RESORCYLIC ACID 203°C. The crystals gave a violet ferric chloride reaction.

The fusion was repeated at 240°C. for 2 hours. The increased temperature and length of fusion increased the yields of phenols (27%) due to decarboxylation of the acids and a maximum of 6% resorcinol was obtained. However, the total yield of phenolic and acidic fractions 46% was not appreciably affected by increasing the temperature and length of fusion.

Catalytic Alkaline Oxidation of Methylated Green Wattle Tannin.

Pearl (24) oxidized lignin in the presence of copper salts as catalysts and obtained vanillin and syringaldehyde. Lignin resembles green wattle tannin in certain aspects, and since lignin is a highly methylated substance, Pearl's methods of oxidation were employed on methylated tannin.

(a) Pressure Oxidations.

80 gms. copper sulphate were mixed with 16 gms. methylated tannin in 350 cc. water. 70 gms. sodium hydroxide in a litre of water were added and heated to boiling with stirring. The mixture was then sealed in an autoclave and heated for six hours at 160°C.

The cold solution was acidified to pH 3, ether extracted and the extracts evaporated to dryness. The gummy residue was extracted with benzene and the extracts shaken with 20% sodium bisulphite and left for a week. No crystalline aldehydic or ketonic precipitate formed. The benzene layer was shaken with potassium hydroxide, separated, dried, and evaporated to dryness. This fraction contained only neutral compounds and on evaporation gave a trace of oily residue too small to be identified.

Sodium bicarbonate was used to separate the phenolic and acidic fractions. These fractions were ether extracted, acidified, dried, and evaporated to dryness. Both fractions yielded traces of oily residues insufficient for further investigation.

(b) Atmospheric Oxidation.

In a two litre flask 100 gms. sodium hydroxide in 700 cc. water were mixed with 125 gms. copper sulphate in 500 cc. water. 20 gms. methylated tannin were added with shaking and refluxed for 16 hours.

The same extraction and separation procedure was used as for the pressure oxidation. This oxidation yielded only traces of oily residues.

Since these experiments failed to produce any positive results, it seemed that the types of linkages in green wattle are not the same as in lignin.

Fractionation Attempts on Methylated Green Wattle Tannin.

Kirby (21) separated methylated black wattle tannin into two fractions, one giving only veratric acid on oxidation while the other fraction gave only trimethoxygallic acid. The method was followed as closely as possible using green wattle tannin, but no separation occurred.

100 gms. acetone soluble tannin were dissolved in 500 ml. methanol and 65 ml. dimethyl sulphate slowly added. 50% aqueous potassium hydroxide was introduced dropwise with stirring. The solution was remethylated twice with the same quantities of dimethyl sulphate and potassium hydroxide and finally made just acid

by the addition of more dimethyl sulphate.

Towards the end of the reaction a brown resin separated out together with insoluble salts and after standing most of the methanol could be decanted. More methanol was added, filtered into the first solution, and the product precipitated by pouring these solutions into 5 litres water. The product was a brown amorphous powder.

This methylated tannin was filtered, dried and allowed to stand with absolute ethanol. The insoluble portion was filtered and the filtrate evaporated to dryness.

The portion which separated out of the methanol solution was mixed with 500 ml. water and 300 ml. chloroform when it all dissolved. The aqueous layer was re-extracted with chloroform, the combined chloroform extracts dried, filtered and evaporated to dryness giving a dark brown powder. This powder was allowed to stand with 400 ml. benzene and filtered. The filtrate was evaporated to dryness and known as Fraction B (50 gms.).

Purification of Fraction A.

5 gms. fraction A were dissolved in 100 ml. benzene and poured through an alumina column (2 x 15 cm.). Elution was effected with 250 ml. benzene-ethanol solution (100:2). The eluted mixture was evaporated to dryness under reduced pressure and the procedure repeated three times when practically nothing remained on the column after elution. Evaporation gave a light brown amorphous powder. The tannin was dried for one hour at 110°C. in

an Abderhalden gun.

Found :

.0684 gms. tannin gave .1640 gms. CO_2 and .0379 gms. H_2O

whence C = 65.70% H = 6.15%

.0652 gms. tannin gave .1576 gms. CO_2 and .0369 gms. H_2O

whence C = 65.92% H = 6.29%

.0934 gms. tannin gave .2379 gms. CH_3I

whence MeO = 33.7%

.0697 gms. tannin gave .1790 gms. CH_3I

whence MeO = 33.9%

Fraction B.

Fraction B was dissolved in 250 ml. benzene and absorbed on an alumina column (3 x 30 cm). Elution with benzene-ethanol (100:3) yielded 14 gms. light powder. The absorption and elution was repeated three times as before. Fraction B was dried for 1 hour in an Abderhalden gun at 110°C.

Found :

.0793 gms. tannin gave .2073 gms. CH_3I

MeO = 34.61%

.0658 gms. tannin gave .1780 gms. CH_3I

MeO = 34.74%

.0562 gms. tannin gave .1380 gms. CO_2 and .0307 gms. H_2O

whence C = 66.97%

H = 6.11%

.0640 gms. tannin gave .1570 gms. CO_2 and .0349 gms H_2O

whence C = 66.90%

H = 6.06%

Oxidation of Fraction A.

Methylated black wattle tannin has been oxidized with permanganate by Kirby (21), Roux (57), Stephen (42), Heugh and Corbett (61, 10). Stephen, Heugh and Corbett oxidized the methylated tannin in an aqueous medium while Kirby used acetone. In all cases low yields of trimethoxygallic and veratric acid were obtained. Roux (57) improved the technique by oxidizing in an acetone-water medium. The increased yields were attributed to three factors :-

(1) Constant temperature of oxidation.

(2) Two phase system in an aqueous oxidation was replaced by a single phase.

(3) A correct amount of potassium permanganate was used.

5 gms. fraction A was dissolved in 600 ml. acetone-water mixture (1:1) and 65 gms. $KMnO_4$ added in three equal portions. After each addition, the solution was refluxed until the permanganate colour disappeared before adding the next portions of $KMnO_4$. The manganese oxide was centrifuged, washed with acetone, and the combined acetone extracts concentrated under reduced pressure. The aqueous solution was acidified and ether extracted. The ether extract on evaporation gave a gummy crystalline residue (20%) which was sublimed and twice recrystallized from water

M.P. 145°C. showing that the two fractions had not been separated.

Oxidation of Fraction B.

The same procedure was adopted with fraction B and sublimation and recrystallization of the gum also gave a mixture of acids M.P. 153°C.

Proportions of Acids.

.1 gm. samples were titrated against .04 N NaOH prepared from CO_2 free water.

Wt. Taken	Ml. 03943 N NaOH	Equivalent Wt.	Average
.0833	10.30	205.1	
.0670	8.21	206.9	206.1
.0358	4.40	206.3	

This equivalent wt. corresponds to

Veratric Acid = 19.7%

Trimethoxygállic Acid = 80.3%

Separation of Trimethoxygallic and Veratric Acids.

Corbett (10) separated the acids by forming the silver salts. .3 gms of the mixed acids were dissolved in 100 ml. water, slight excess ammonia added, and the solution boiled to neutrality. Hot dilute silver nitrate was added and solution cooled in ice. A white gelatinous mass formed which was filtered, washed, and the filtrate kept. The precipitate was acidified, boiled and filtered rapidly. Crystals formed in solution which were recrystallized twice from water M.P. 178°C. Mixed melt with VERATRIC ACID M.P. 178°C.

The filtrate from the original silver nitrate treated neutral acids was acidified, boiled, and filtered. The crystals which formed on cooling were filtered, dried and redissolved in 50 ml. hot water. Ammonia was added, the solution boiled to neutrality, and hot silver nitrate again added. The fractional precipitation was repeated once and white crystals were obtained M.P. 166°C. Mixed melt with TRIMETHOXYGALLIC ACID 166°C.

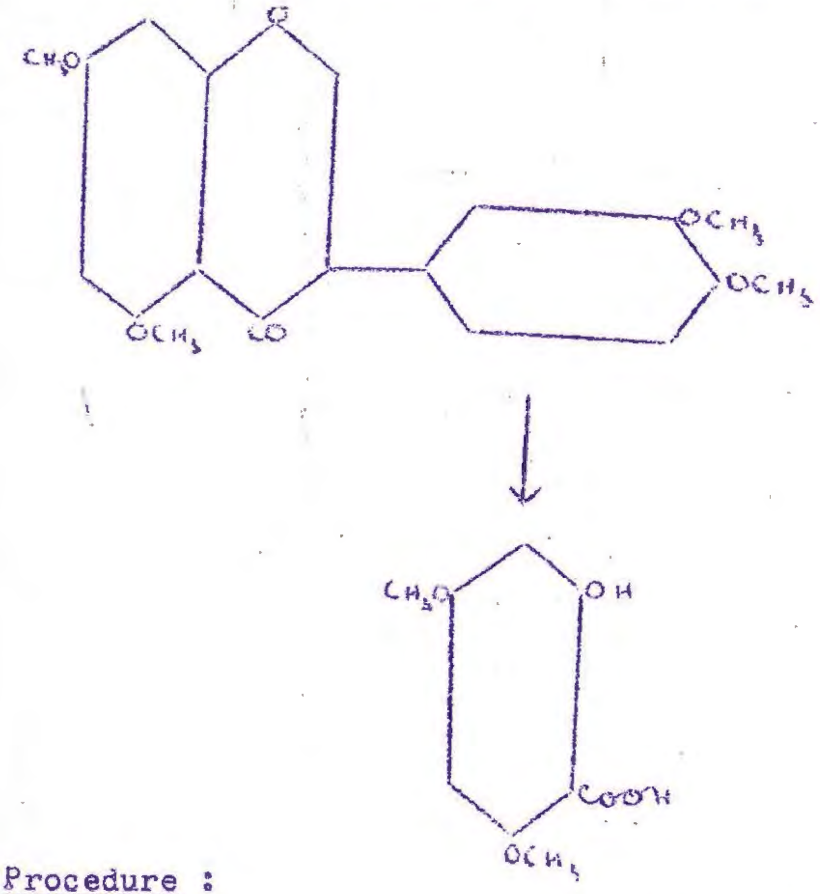
Ozonolysis of Methylated Green Wattle Tannin.

Gentler degradation techniques such as ozonolysis and zinc dust distillation were tried in an effort to obtain a derivative of a large molecular fraction and thus to ascertain the position at which polymerization occurs.

Acetylene compounds unite with ozone to form ozonides, The formation and properties of ozonides have been studied by Harries and his co-workers (74, 75). They used the formation of ozonides to determine the presence and, frequently, the position of a double bond and applied the process to pulegone and pinene. According to Harries, the fact that benzene formed a triozonide may be taken as evidence of the Kekulè formula. The action of ozone on aldehyde and ketone groups was to furnish one additional atom of oxygen and form a peroxide.

Ozone has also been used to split ring structures and

Robertson, Suckling & Whalley (4) opened the pyrone ring in trimethoxysantal (1) giving 2 hydroxy 4:6 methoxy benzoic acid (2).



(i)

(ii)

Procedure :

Ozonides are generally formed in an inert solvent at 0° C in order to diminish the risk of explosion as some ozonides are extremely explosive.

2 gms methylated tannin was dissolved in 200 ml chloroform and ozone bubbled through the solution for 2 hours at 0° C

The Chloroform was evaporated off under reduced pressure and the amorphous brown residue was ether extracted five times. Evaporation of the ethereal extracts yielded an oily gum which failed to recrystalize from benzene.

A pyr~~o~~ane ring is more resistant to ozone attach than a pyrone ring (41) as in trimethoxysantal and the ozonalysis was thus repeated for a period of 68 hours. A higher yield of ether soluble gum was obtained.

Found :-

Found :-

Ether Insoluble Fraction : $-OCH_3 = 23.9\%$ 24.04%

Ether Soluble Fraction : $-OCH_3 = 18.1\%$ 18.13%

Original Methylated Tannin : $-OCH_3 = 32\%$

The ether soluble gum gave no ferric chloride reaction but was soluble in alkali which indicated the presence of acidic groups. The acidic groups could either be a product of demethylation or of ring opening.

Zinc Dust Distillation of Green Wattle Tannin.

When certain aromatic oxygen compounds (47) i.e. phenols, naphthols, quinones are heated with zinc dust, they are reduced to the corresponding hydrocarbons. In this way phenol yielded benzene while anthraquinone (62) or alizarin yielded anthracene. Ellagic acid (43, 44) produced diphenyl and fluorene while purpuratannin gave diphenylene (26).

The method employed for green wattle was similar to that used by Cummings Hooper and Weeler (66) to obtain anthracene from anthroquinone.

A paste was prepared from 100 gms. zinc dust and 30 cc. of alcohol. Pieces of pumice were added and the mixture stirred to incorporate the pumice and paste. The alcohol was finally evaporated off by passing the pumice rapidly over a flame.

A hard glass tube 3 ft. 6" long and 6" in diameter was used. A 5 cm. layer of zinc dust was placed at one end of the tube next to it, a layer of 2 gms. tannin mixed with 20 gms. excess zinc dust, and finally a 30 cm. layer of pumice stone. The pumice zinc dust section was first heated and the heat increased from the front slowly backwards until finally the tube was heated as strongly as possible. The experiment was carried out in an atmosphere of dry hydrogen.

After one hour a small amount of yellow oil condensed at the top of the tube. The oil was ether extracted and gave a small quantity of a pale yellow gum which failed to crystallize from a variety of organic solvents.

Pyrolysis of Green Wattle Tannin.

Dry distillation of tannins have given low malecular weight phenols. Both quebracho (46) and catechin gave catechal (71) while under similar conditions cyanomaclurin gave resorcinol (59).

Three methods of pyrolysis of green wattle tannin were attempted :-

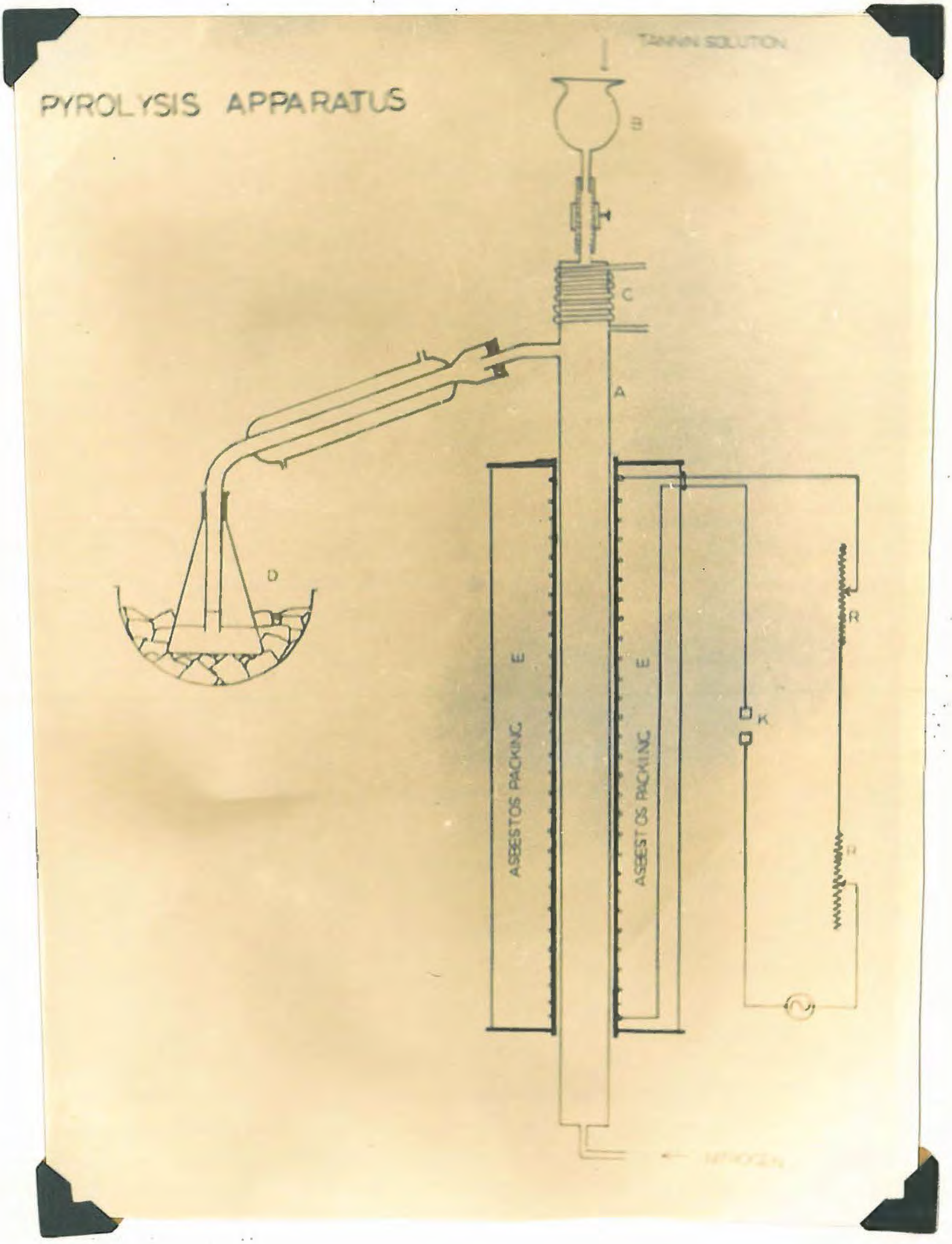
(1) Straight-forward heating of tannin on a metal bath.
(2) Heating under vacuum.
(3) Heating in a copper tube.

The first two methods failed to produce any appreciable yields. The apparatus finally used is illustrated on the next page.

A is a copper tube surrounded by an electric element E which is controlled by a series of resistances R. Copper Tube A is fitted with a side arm connected to a condenser leading to a flask D which is cooled in ice. C is a condenser to prevent the volatile phenols escaping through B. During pyrolysis nitrogen was passed continuously through the apparatus.

Dried tannin was dropped into the copper tube through B and large quantities of gas were evolved. A portion of the gaseous matter condensed to form a dark yellow oil which failed to crystallize.

The experiment was tried under reduced pressure to increase the yields but no apparatus was available that could withstand


PYROLYSIS APPARATUS
TANNIN SOLUTION
B
C
A
D
E
E
ASBESTOS PACKING
ASBESTOS PACKING
K
R
R
NITROGEN

the temperature and pressure required.

Since the lower phenols are steam volatile, the experiment was carried out with a 30% aqueous tannin solution. The tannin was allowed to drip slowly into the copper tube and steam was evolved. A gummy product condensed and was extracted with benzene. Crystals, which formed in solution, were recrystallized from benzene MP 108°C. Mixed melt with RESORCINOL 108°C.

No definite conclusions can be derived from the production of resorcinol which can be either "free" or produced by fission of ether linkages.

Oxidation with Nitric Acid.

15 gms acetone extracted tannin were dissolved in 75 ml water and concentrated nitric acid added with shaking. Brown fumes were evolved and the brown precipitate which formed was left to stand for 24 hours.

The precipitate was heated on a steam bath for 1½ hours and, on cooling, large crystals separated out. The crystals were recrystallized twice from water MP 102°C and addition of calcium chloride precipitate calcium oxalate. Mixed melt with OXALIC ACID 101°C.

Chromatography of the Non-Tannin Fraction.

Various tannins contain carbohydrates. The non tannin fraction of quebracho (27) contained the sugars glucose, arabinose and xylose. Tarboton (52) found sucrose, glucose, and possibly maltose present in black wattle tannin while Roux (11) and Stephen found sucrose and only traces of reducing sugars. The non-tannin fraction of green wattle also gave the characteristic burnt sugar odour. The similarity of sugars in physical and chemical properties made it difficult to separate mixtures especially in small quantities.

The most successful method of identifying any small quantities of sugars was by filter paper chromatography developed for sugar by Partridge and extended by Horrocks (51) Forsythe (32) and Hough, Jones and Wadman (28,29).

Various spraying reagents were used to reveal the positions of sugars on chromatographs. e.g.

(1) Ammoniacal Silver Nitrate.

Originally Partridge (28) used ammoniacal silver nitrate and in this way metallic silver was precipitated in the region occupied by reducing sugars and gave rise to black and brown spots. The ammoniacal silver nitrate reagent had the advantage of being effective for all reducing sugars but had the corresponding disadvantage of reacting with a very wide range of reducing substances other than sugars.

(2) Aniline Hydrogen Phthalate.

Partridge (29) found aniline hydrogen phthalate was a more selective reagent than ammoniacal silver nitrate. This reagent was very sensitive to aldo-hexoses and pentoses giving various shades of green and brown while ketoses gave no reaction. Furthermore, since it was dissolved in moist butanol, migration of the sugar spots during the spraying process was avoided.

(3) Anisidine Hydrochloride.

Hough, Jones and Wadman (73) found p-anisidine hydrochloride a very satisfactory reagent. Aldo-hexoses gave a green-brown colour and ketohexoses a brilliant lemon-yellow colour. The intensity of the coloured spots obtained with the reagent was considerably enhanced in ultra-violet light, thus providing a very sensitive means of detection.

(4) Resorcinol and Naptharesorcinol.

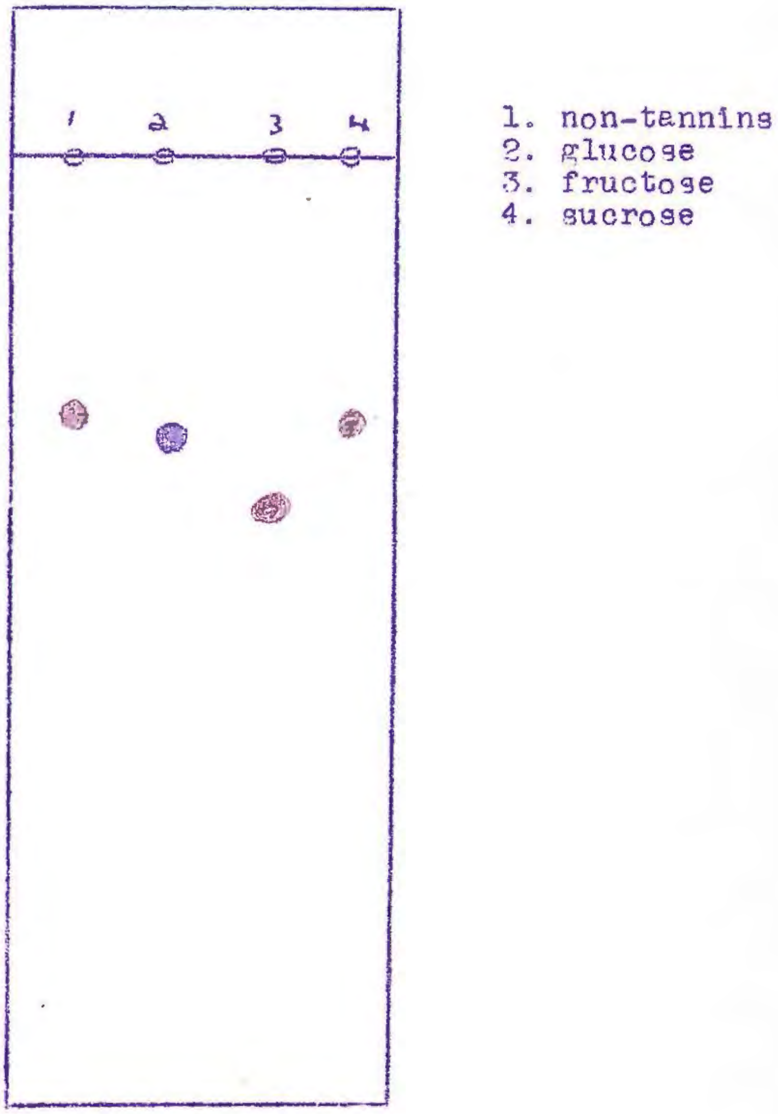
10 ml of a 1% alcoholic solution of resorcinol in 2 N Hydrochloric acid gave a characteristic red colour with sucrose

and fructose and only a faint colour with glucose. Naptharesorcinol also gave a red spot with sucrose and fructose and a faint blue spot with glucose.

Tannins reduce ammoniacal silver nitrate and Fehlings solution and the large excess present had to be removed from the solution before the sugars could be identified. Partridge (28) removed traces of tannins with base exchange resins but this could not be used where large quantities of tannin were present.

78 gms tannin in a litre of solution were shaken up with 8 portions of lightly chromed hide powder each of 25 gms dry weight. The resulting liquor was clear and gave an olive green ferric chloride reaction instead of the usual purple colour. Finally, the solution was concentrated under reduced pressure in the presence of carbon dioxide. The concentrated solution was chromatographed for 24 hours in butanol - acetic acid - water (4:1:5) in conjunction with sucrose, glucose and fructose. Spraying with resorcinol and naptharesorcinol indicated the presence of sucrose only.

Chromatograph of non-tannin fraction sprayed with naptharesorcinol hydrochloric acid reagent.

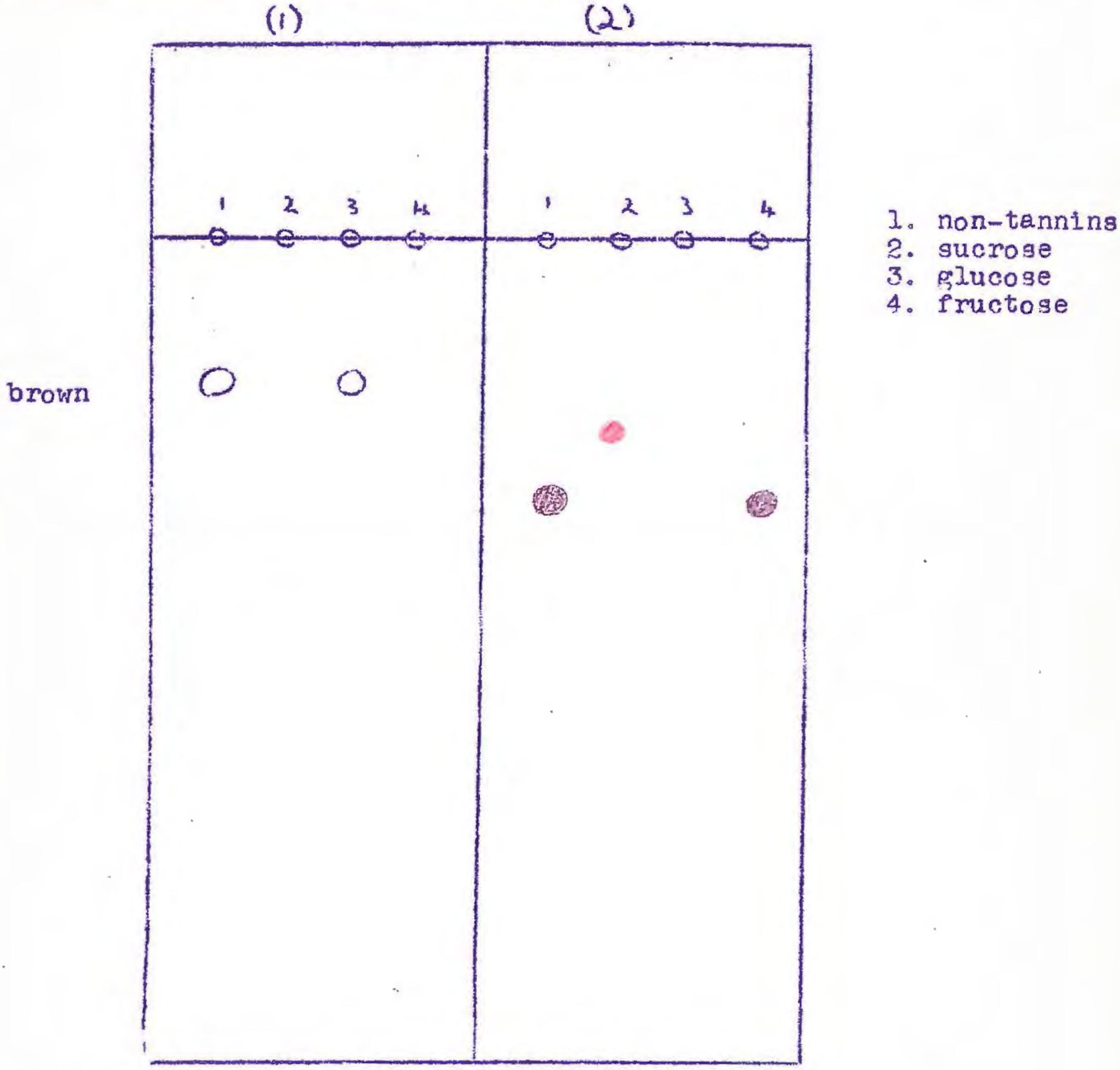


Hydrolysis of Sugars.

2 ml non-tannin solution and 1 ml. 5N Hydrochloric acid were placed on a water bath for 4 hours in a sealed capsule. The cold solution was filtered, centrifuged and neutralized with barium hydroxide. The neutral solution was evaporated to dryness and extracted with methanol in order to remove barium salts. The

The solution was chromatographed for 36 hours in butanol-ocetic acid (4:1:5) and sprayed with anisidine hydrochloride, aniline hydrogen phthalate and resorcinol. Anisidine hydrochloride and aniline hydrogen phthalate indicated the presence of glucose, while resorcinol showed fructose present, thus confirming sucrose alone constitutes the original non tannin solution.

Chromatograph of hydrolysed sugars.



(1) Sprayed with aniline hydrogen phthalate.

(2) Sprayed with resorcinol in hydrochloric acid.

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