

THE OXIDATION AND DEGRADATION  
PRODUCTS OF BLACK WATTLE TANNIN.  
(ACACIA MOLLISSIMA).

by

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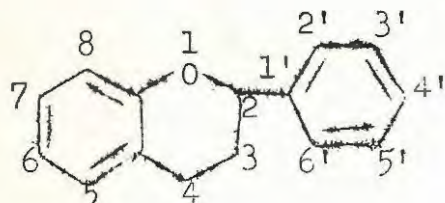
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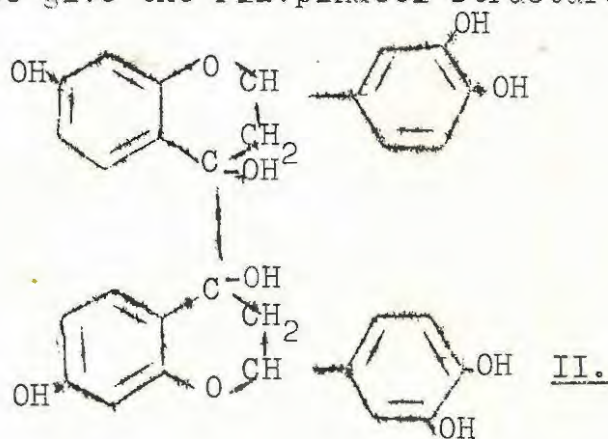
INTRODUCTION.

Black Wattle Tannin, obtained by the extraction of the bark of the Black Wattle (*Acacia Mollissima*), belongs to the group of tanning materials known as the phlobatannins. Reviews on the suggested structures of this group have been presented by Corbett (1), Stephen (2), and Silk (3). As an introduction, a brief summary of the extent of knowledge to date follows:

Russell (4) and Russell and Todd (5) suggest that the phlobatannins are built from catachin types of molecules I, these being linked through the 4 position to give the Flavpinacol structure II.



I.



II.

II was claimed to have been synthesised by them, and was shown in a series of qualitative reactions to be indistinguishable from typical natural phlobatannins. Carrying this further, they synthesised a number of other flavpinacols which were compared and found indistinguishable from individual natural tannins in qualitative reactions. Spectroscopic data obtained from comparison also favoured identity of the natural and synthetic products. These authors infer that whatever its structural make-up may be, phlobatannins are constituted in the same way as the synthetic product II, individuals differing only in the position and number of hydroxyl groups.



from carbon-hydrogen estimations. He supported this view from the fact that an increase in acetyl content was found in the polymer, showing that more hydroxyl groups had been made available.

Lowitt (8) however has ascertained that in the case of Black Wattle tannin, no such increase is found.

The above data does not exclude the possibility of a molecule of the Russell type resulting on HCl treatment, as such a condensation would occur without the loss of water. Moreover, if the pyran ring should open an increase in acetyl content would result.

The evidence at present available in support of these two theories does not justify their application to the whole class of condensed tannins. While they may be applied to some of the group, a general application must be made with some reserve if it is based merely on the similarity of the synthesised tannins to the particular phlobaphene being studied.

In order to elucidate the structure of Black Wattle Tannin, in recent years work has been carried out at the Leather Industries Research Institute in Grahamstown. Three main methods of experimental attack have been employed:—Hydrogenation (3); Conductimetric Titrations (9), making use of the fact that phenolic groups behave as weak acidic groups; and thirdly, General Chemical Degradations (2).

In the work on hydrogenation no distinctive compounds were isolated, but the work determined the best conditions for maximum yield of degradation products.

Shuttleworth (9) carried out conductimetric titrations on tannin, and on comparison with values obtained on other polyhydroxy

compounds has shown that the tannin gives high equivalent weights, suggesting that it exists in the form of a polymer. The values obtained are of the order of 250. Similar titrations performed on tannin prepared from fresh bark, precautions being taken to prevent polymerisation as far as possible, give values of 170 for the equivalent weight. This value is comparable with that for catechin, for which the value 160 was obtained.

These results are in accord with the chemical evidence on the nature of this extract, which polymerises rapidly on exposure to heat or air, and darkens in a short while.

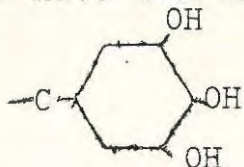
#### OXIDATION OF THE TANNIN FROM ACACIA MOLLISSIMA.

Corbett (1) performed a number of general oxidations on wattle tannin and its derivatives, Stephen (2) later repeating this work. While their work does not prove the essential building units of the tannin molecule, it provides a useful guide for later research.

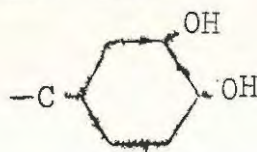
On permanganate oxidation of the fully methylated tannin, a mixture of acids was obtained. These were separated by means of their silver salts, and one was identified conclusively as trimethylgallic acid by a mixed melting point with an authentic specimen. The second was claimed to be veratric acid.

This second acid gave the correct carbon and hydrogen values, melting point, equivalent weight, and methoxyl values for veratric acid, and, when mixed with trimethylgallic acid, it melted over the same range as did the original mixture of acids. However, although the evidence is strong, it is by no means conclusive, and it must be accepted as veratric acid with reserve, as will be shown later.

We have therefore that one building unit is:-



and possibly



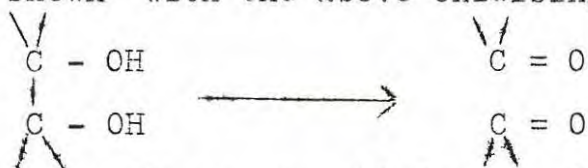
The permanganate oxidations in each case yielded very low percentages of acids - at the most 8% - and it seems as though a more thorough investigation of conditions might increase the yields.

The oxidation of tannin itself by Corbett was unsuccessful, and he suggests that the phenolic groups in the molecule render it too unstable to oxidise. An attempt to stabilise the molecule by acetylation before oxidation, thus rendering the benzene nuclei more resistant to complete disruption, was also shown to be fruitless, as was the oxidation of brominated methylated tannin.

Stephen (2), on oxidising the tannin with warm concentrated nitric acid, obtained a reasonable yield of styphnic acid (trinitro-resorcinol) and oxalic acid dihydrate. He showed that these products are identical with those obtained on nitration of a mixture of resorcinol, pyrogallol and catechol under the same conditions, and suggested that the tannin molecule is built up of these units. His oxidation of tannin with bromine, suggested by the work of former researchers (10), resulted in an anthocyanin type of molecule which, however, failed to give typical anthocyanin colour reactions. The colour of the pigment in acid solution was red, but no blue colour was produced in alkaline media, and no ionic chloride could be detected in the isolated pigment.

Stephen also attempted to show a flavpinacol unit according to the Russell theory, by an oxidation with periodic acid and lead

tetracetate. It has been shown that the 1-2 glycol linkages are split as shown with the above oxidising agents (11).



No such evidence was obtained. It should also be noted that Russell himself has never proved the glycol linkage.

Stephen (2) found that fusion of tannin with alkalis yielded resorcinol and gallic acid. A small quantity of acids which were not identified was also obtained.

The nature of the hydroxyl groups in tannin were studied by Corbett (1) and Stephen (2) independently, by means of acetylations and methylations. Corbett found that, on an average, approximately 4.6 hydroxyl groups were acetylated. The only concrete result obtained was that tannin proved to be acetylated more easily than it could be methylated, a higher acetyl content being obtained on acetylation than methoxyl value on methylation. It was also shown that to obtain a fully methylated product, it was necessary to methylate with dimethyl sulphate and KOH at least three times. These factors induced Stephen to state that "The reactivity of the hydroxyl groups in tannin is clearly far below that of those in Phloroglucinol".

The actual highest percentages of methylations and acetylations obtained were as follows:-

Acetylation:-	Maximum percentage average	39.3%
	By partial precipitation	41.0%
Methylation:-	Dimethyl sulphate	35.8%

Lowitt (8) has continued the studies on acetylations and methylations, and has recorded some interesting facts. He

exhaustively acetylated tannin, and then replaced the acetyl groups with methoxyl groups by means of diazomethane. It was found that whereas the majority of the groups were easily replaced, the last replaceable group proved much more difficult. Studies on related compounds induced him to suggest that this phenomenon is due to steric hindrance. This is important in relation to the position of one hydroxyl group of the tannin molecule.

Finally, on bromination, Stephen (2) found that tannin combined with 57% bromine, of which 10.5% could be removed as silver bromide in alcoholic solution.

It should be emphasised that all the above work, with the exception of Lowitt's, was carried out on tannin extracted with acetone from the commercial extract. The tannin samples had thus been exposed to all the factors known to facilitate polymerisation, and were almost certainly highly polymerised before use.

Williams (12) has since repeated much of the above work, and has used tannin extracted from the fresh bark with ethyl acetate. By a specialised method of extraction, he reports an extract containing 95.8% tannin, thus increasing Stephen's yield by as much as 10%. The tannin, he reports, is extracted in an unpolymerised state, but polymerises rapidly on exposure to heat and air. This is accompanied by a darkening of the product to a red-brown.

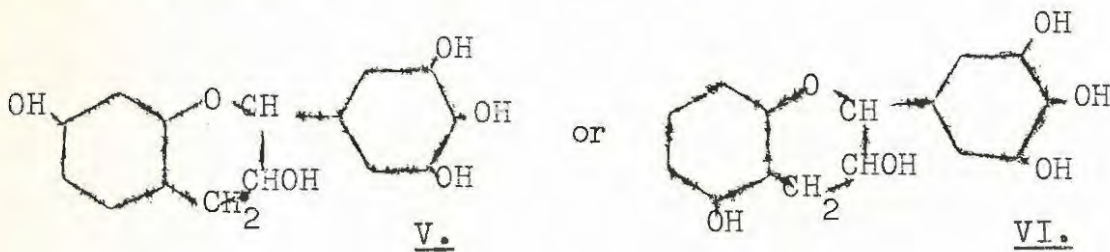
This tannin, unlike the commercial extract, was readily soluble in ethyl acetate and amyl acetate, but insoluble in benzene and other hydrocarbons. His combustion analysis agreed

with the  $C_{15}$  molecule of Stephen, and an alkali fusion gave resorcinol. On oxidation with bromine, anthocyanin pigments were produced which failed to crystallise and which could not be separated chromatographically.

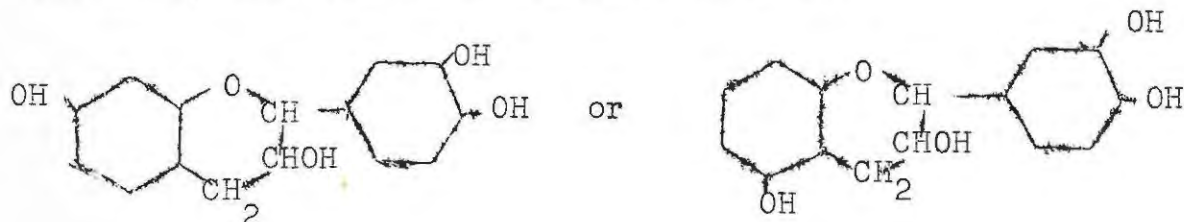
Williams' acetylations gave higher values than Stephen's for the fully acetylated material, viz. 41.5%, and he claimed that an acetylation only of phenolic groups was effected, giving a constant value for the partially acetylated material of 35.5%. Molecular weights of the fully acetylated material gave the values of 450, 433, 470. A chromatographic separation of the partially acetylated material was successful, but the two fractions obtained failed to crystallise.

THE STRUCTURE OF THE TANNIN BUILDING UNIT.

The tannin molecule is probably of the catechin type, as would seem to be borne out by the reported isolation of anthocyanin pigments on bromine oxidation of fresh bark extracts. Since resorcinol only was isolated on alkali fusions of both fresh bark extract and commercial acetone extract, one building unit in the molecule becomes immediately established as a resorcinol nucleus. Corbett (1), in isolating and identifying gallic acid established the other half of the molecule. It therefore becomes possible to postulate the structure of one of the building units as V or VI



If the other acid Corbett isolated on oxidation is veratric, and this must be accepted with due caution for reasons later outlined, then the other molecule would be:-



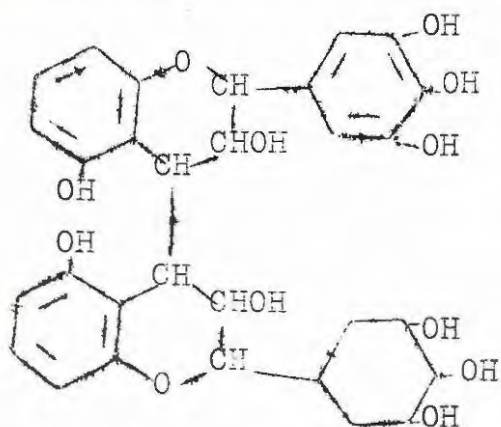
Stephen (2) suggests that the tannin is actually a mixture of these two types of molecules. This would seem to find support in Williams' chromatographic separation of the partially acetylated tannin. Lowitt (8) showed that if we assume these two molecules to be present, an acetyl content of 41%, which was obtained, would correspond to a mixture of 52.5% of the 3'4' dihydroxyphenyl 3.5 dihydroxypyran and 47.5% of the 3'4'5' trihydroxyphenyl 3.5 dihydroxypyran.

Lowitt's(8) studies on the acetylations and methylations of tannin and comparison with similar studies on related compounds led him to suggest that the position of the phenolic group in the benzopyran unit is in the 5 position, and not the 7. If the group is in the 7 position, replacement of the acetyl derivative by methoxyl group should be relatively easy. If however the hydroxyl group is in the 5 position, steric hindrance would render replacement much more difficult. This was shown to be the case.

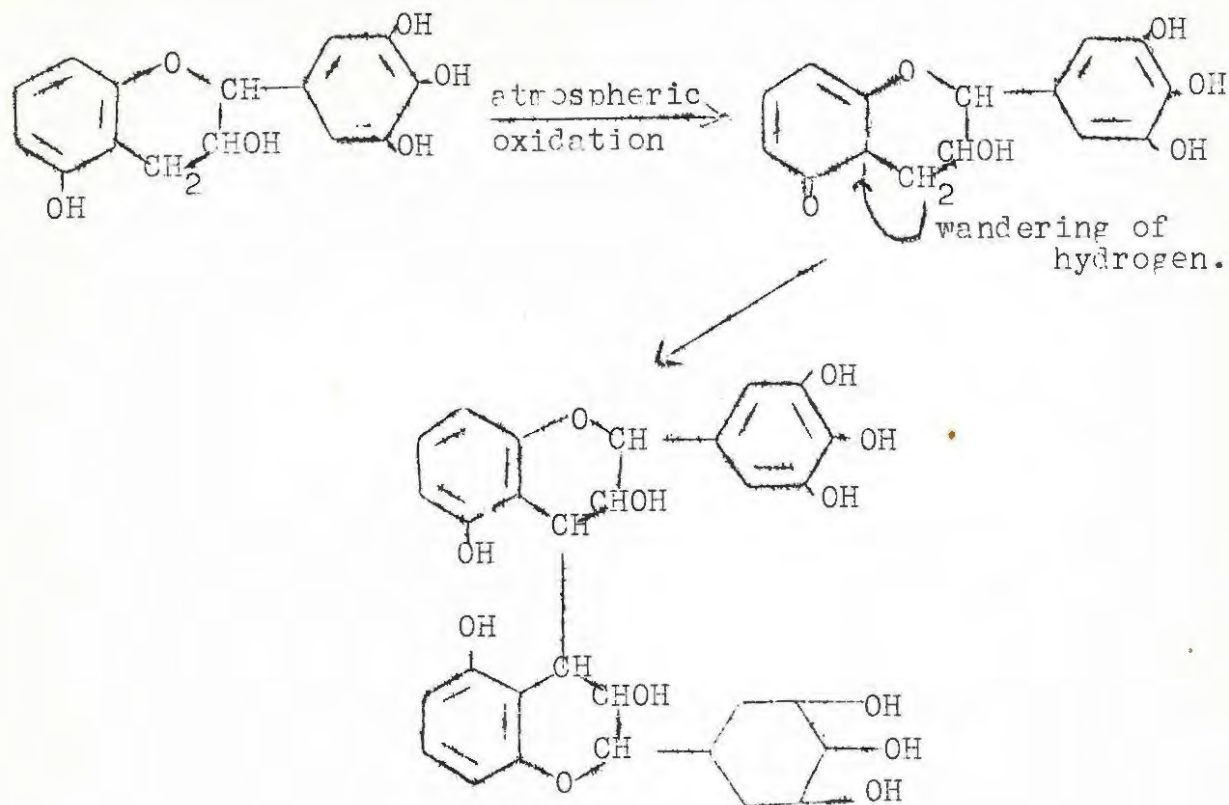
Williams (12) has tentatively proposed an alternative structure for the tannin. He points out that Stephen has shown that on bromine oxidation of tannin an anthocyanin results, and that on bromination of tannin (a process in which oxidation and

nuclear substitution occur simultancously) up to 10% of the bromine in the isolated bromide could be removed by silver nitrate treatment. This, Williams reasons, points definitely to a pyrrillium salt formation.

This oxidation cannot be explained on the Freudenberg structure, since oxidation would lead to complete degradation of this molecule into phenolic acids. It may however be explained on the Russell formula. Stephen failed to detect a glycol grouping in tannin, essential for a flavpinacol structure, but Williams pointed out that this does not exclude linkage through the 4 positions of two pyran nuclei to give a molecule of the following type:-



No direct experimental findings as yet confirm the presence of the above molecule, but he pointed out that polymerisation appears to be associated with oxidation and hence quinone formation. The origin of this molecule he explained as a primary atmospheric oxidation of the building unit, with the wandering of a hydrogen from the 4 position to leave this an active centre through which polymerisation occurs.

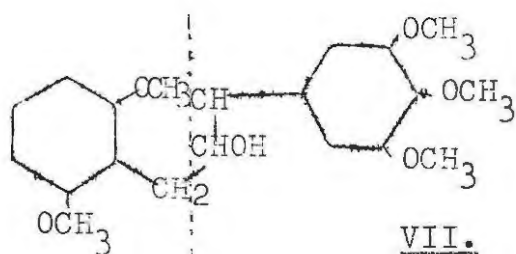


DISCUSSION.

The identity of the acid of equivalent weight 182, melting point  $178^{\circ}\text{C} - 179^{\circ}\text{C}$ , reported by Corbett and Stephen, and assumed by them to be veratric acid, was not confirmed. It must be pointed out therefore, that there is every possibility that this acid is 2.6. dimethoxybenzoic acid (dimethoxy  $\gamma$  resorcylic acid), which has exactly the same carbon and hydrogen values, equivalent weight, percentage methoxyl, and melts at  $179^{\circ}\text{C}$ .

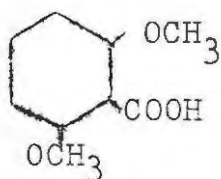
The origin of this acid can also be explained from the proposed Freudenberg structure. The methylation of tannin was effected by means of dimethyl sulphate and KOH, and the alkali is always maintained in excess. In the presence of alkalis, according to Nierenstein (13), the pyran ring opens at the oxygen

bond, and the hydroxyl group thus formed immediately becomes available for methylation. The structure for the resulting tannin would then be VII.

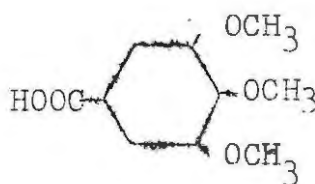


In this structure it has been assumed that the 3 hydroxyl group has not been methylated. This is a reasonable assumption to make on consideration of the low methoxyl determinations of Stephen, and the difficulty experienced by Lowitt in methylating a compound with a similar group.

An oxidation of the above molecule could yield therefore trimethylgallic acid and 2,6-dimethoxybenzoic acid by scission at the 3 position as indicated by the dotted line.



2,6-dimethoxybenzoic acid.



trimethylgallic acid.

CATECHIN.

A brief summary of the work conducted on catechin has been included, as the studies on this molecule, which is thought to be similar to the tannin molecule, provide a useful guide to the methods of attack which may be applied in the elucidation of the structure of black wattle tannin.

Perkin in 1902, reviewed the early work on the catechins (14). Since that date catechin has only received brief mention in the Annual Reports (15), but Russell (4) in 1935 reviewed these compounds in conjunction with the tannins.

Catechin was first described by Nees van Esenbeck (16) in 1832, and in 1837 Svanberg (17) assigned to it the formula  $C_{15}H_{12}O_6$ ; this combustion was performed on the air dried sample. Subsequently formulae ranging from  $C_{12}H_{12}O_5$  to  $C_{22}H_{22}O_9$  received favour (18), until the work of Karnowski and Tambor (19) indicated, however, that its formula was  $C_{15}H_{14}O_6, 4H_2O$ . Perkin and Yoshitake (14) independently arrived at the same empiric formula. Later Clauser (20) showed that catechin could form a number of hydrates. Perkin (21) confirmed this work, and formulae  $C_{15}H_{14}O_6, 4H_2O$ ;  $C_{15}H_{14}O_6, 3H_2O$ ; and  $C_{15}H_{14}O_6, 1H_2O$  have been established.

Gautier (22) was the first to show that gambier catechu contained not one but three catechins of melting points  $204^{\circ}C - 205^{\circ}C$ ;  $176^{\circ}C - 177^{\circ}C$  and  $163^{\circ}C$ . Perkin (14) confirmed the one of M.Pt.  $176^{\circ}C - 177^{\circ}C$ , but could only isolate one other catechin M.Pt.  $235^{\circ}C - 237^{\circ}C$  from gambier catechu. However he succeeded in isolating a compound M.Pt.  $204^{\circ}C - 205^{\circ}C$  from acacia catechu, which he showed to be identical with Gautier's catechin. In order to avoid confusion Perkin (21) called this acacatechin.

Specific Rotation.

It was first mentioned in 1921 (23 and 24) that catechin could exist in a number of stereoisomeric forms. The early workers in this field failed to determine the optical activity of the compounds, although Perkin reported his acacatechin as optically inactive. Freudenberg and co-workers showed that gambier catechin pentaacetate was a homogeneous dextrorotatory compound, and suggested that the pure l - form may occur in acacatechin, drastic extraction methods causing partial racemisation. Nierenstein in a subsequent paper (25) denied the optical activity of acacatechin. Later it was independently established by Fiest and Futtemenger (26) that three catechins examined by them were optically active. They also established the presence of two asymmetric carbon atoms in the molecule of catechin.

Below follows the now established list of the catechins isolated from natural sources with their respective specific rotations:-

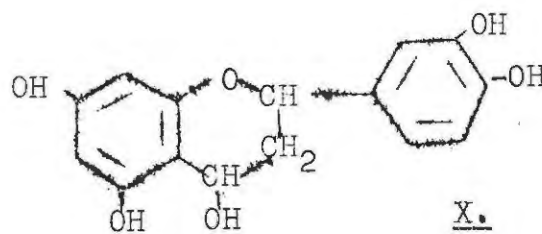
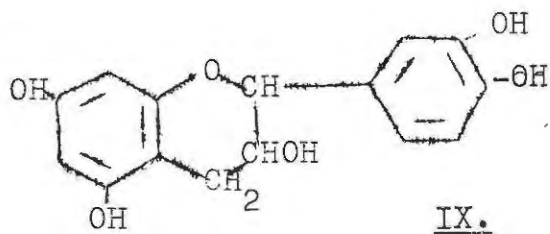
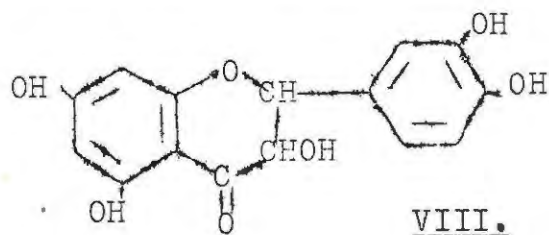
	<u>M.Pt.</u>	<u>Source.</u>	<u>Specific Rotation.</u>	<u>References.</u>
d-catechin	93°-95°C 174°-175°C (Anhydrous)	Gambier Mahogany Block- Gambier.	$[\alpha]_D^{25} +17.1^\circ$ Acetone 50% inactive alcohol 96%	14, 20, 27, 28.
l-catechin	174°-175°C	Acacia Catechu.	$[\alpha]_D^{25} -16.8^\circ$ Acetone 50% inactive alcohol 96%	30.
d-l catechin	212°-214°C	Acacia Mahogany, Catechu.	inactive.	30, 28.
d-epicatechin	245°C	Block Gambier.	$[\alpha]_D^{25} +68.9^\circ$ Alcohol 96% $[\alpha]_D^{25} +59.9^\circ$ Acetone 50%	30.
l-epicatechin	245°C	Acacia Catechu.	$[\alpha]_D^{25} -68.2^\circ$ Alcohol 96% $[\alpha]_D^{25} -59.0^\circ$ Acetone 50%	30, 14.
dl-epicatechin	224°-226°C	Pegu- Catechu.	inactive.	30, 27.

Freudenberg and Purrman (29) have also shown that it is possible to convert d-catechin to d-epicatechin by heating it at 115°C for twelve hours in aqueous potassium carbonate solution. By similar method l-epicatechin has been converted to d-catechin and dl-catechin.

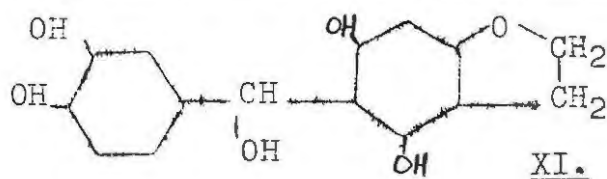
Structure of Catechin.

By dry distillation of catechin Wachenroder (30) detected catechol, while Miller (31) found acetic acid, catechol, and phenol. Etti (18) on alkali fusion identified phloroglucinol and protocatechuic acid.

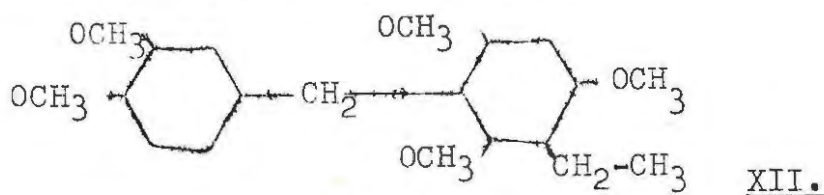
Perkin (14) confirmed the presence of phloroglucinol and protocatechuic acid, and on oxidation of catechin tetramethylether with  $\text{KMnO}_4$  (21) he isolated veratric acid. Because of the marked similarity between the catechins and quercetin VIII which accompanies them in the plant, he suggested that they may be viewed as reduction products of quercetin, and proposed the following structures for the catechin molecule IX and X.



Kostanecki and Lampe (32), because only a monobromotetramethyl catechin could be prepared from tetramethyl catechin, and because on oxidation only veratric acid could be isolated, suggested that catechin could not contain a chroman group but had a coumaran type. They proposed the following structure for catechin XI.

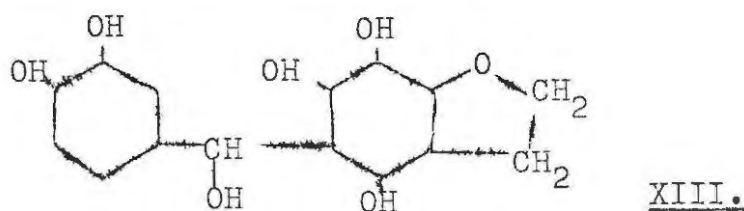


They claimed to confirm their proposed structure by isolating a compound whose combustion analysis agrees with 2.4.6.3'.4'. pentamethoxy 3 ethyldiphenylmethane XII.



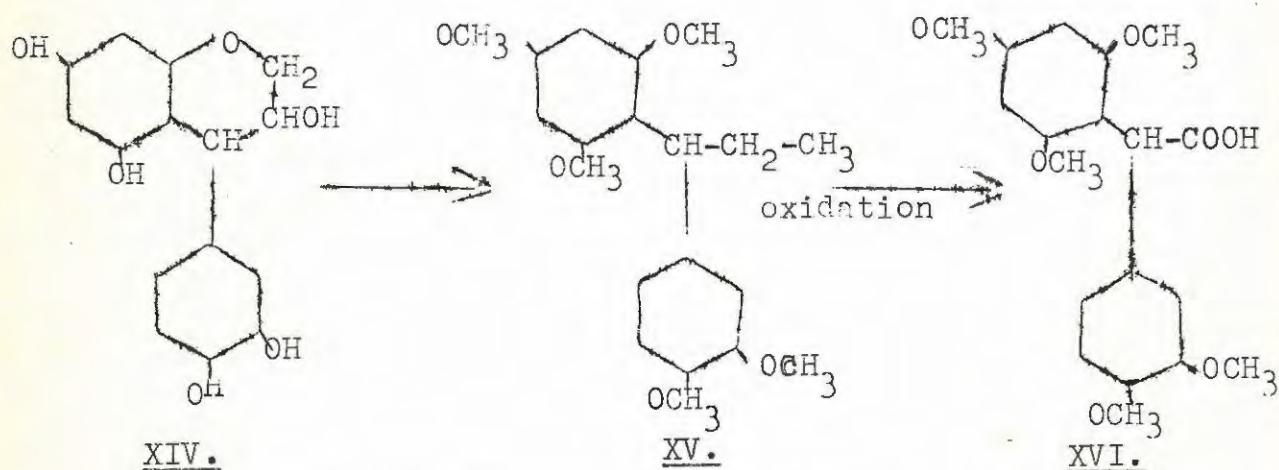
This compound was obtained by reducing catechin tetramethyl-ether with sodium in alcohol and methylating the resulting oil (33).

Nierenstein (34) obtained a compound M.Pt. 284°-285°C with decomposition, which he called hydroxycatechin XIII. This compound was obtained by the reductive acetylation of catechone with acetic anhydride and zinc dust.

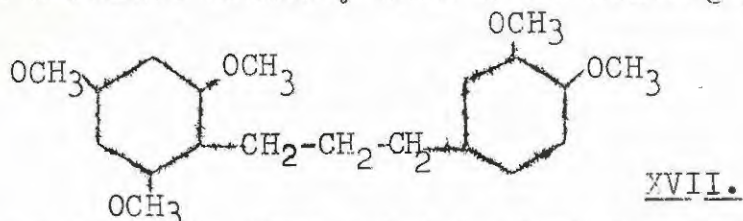


Karrer and Widmer synthesised a number of this type of compound from hydroxycoumarones and hydroxycoumarans (35). None were identical with the catechins. Because Ryan and Walsh (37) observed that by opening the pyran ring in the Perkin structure for catechin, a pentamethyl derivative could be formed,

Nierenstein (36) suggested an alternative structure for catechin XIV. This he claimed to prove by opening the pyran ring and oxidising the product to give an acid, catechin carboxylic acid XVI, which he stated was identical with an acid of the same structure synthesised by him. Later he synthesised the degraded methylated product XV, which he claimed was identical with the original product.



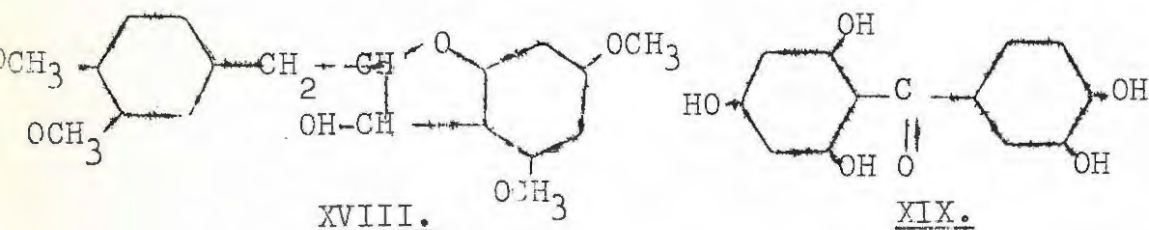
Freudenberg (38), however, claimed that 3,4,2',4',6'-penta-methoxy ~~oxy~~ diphenylpropane XVII is identical with the product obtained by Kostanecki and Lampe (33) on sodium and ethanol treatment of catechin, with subsequent methylation. Nierenstein denied this, so Freudenberg (39) synthesised this compound and established absolute identity with mixed melting points.



Nierenstein (40) still maintained that catechin carboxylic acid could be prepared according to his method, and to explain the discrepancy suggested that gambier catechin and acacatechin differ. Freudenberg and Cohn (41) however, reestablished the

identity of their reduced product.

Drumm (42) suggested that either of the two formulae originally proposed by Perkin, IX and X (14), are applicable. Freudenberg (43), however, stated that there were four possible structures for catechin, of which XVIII was the most probable.



Hazleton and Nierenstein (44) now announced that by the action of penicillium solitum on acacatechin, maclurin XIX was produced in 86% yields. This was claimed to support Nierenstein's structure.

Freudenberg and co-workers (45) synthesised compounds of the figure XVIII type and showed them to differ from catechin. Nierenstein (46), in an attempt to disprove Perkin's structures IX and X, unsuccessfully attempted to reduce quercetin to catechin. Freudenberg and Kammüller (47), however, successfully reduced quercetin pentamethylether with a platinum catalyst to d-1 epicatechin (48). This conforms with Perkin's structure IX.

While Nierenstein criticised this synthesis on the grounds that the constitution of cyanidin hydrochloride was still indefinite (49), Robinson (50) pointed out that it had been independently synthesised by two chemists, and that there was no reasonable cause for doubt as to its structure. Robinson himself later successfully oxidised d-catechin pentamethylether to cyanidin hydrochloride (51).

In 1937, however, Nierenstein agreed that, since Maitland (52) had isolated pentamethyl dl-epicatechol from the reduction products of pentamethyl quercetin, Freudenberg's structure - or more correctly that of Perkin IX - must hold (53).

The final comment on the catechin structure has been taken direct from the abstract (54):-

"The dispute over the structure of catechin is now ended as Maitland has isolated pentamethyl epicatechin from the reduction products of pentamethyl quercetin, and Nierenstein accepted this as establishing the Freudenberg formula".

In conclusion it should be noted that the so called Freudenberg formula was originally suggested in 1902 by Perkin, an author who has received little credit for his work. Perkin also observed that on boiling catechin with HCl, an amorphous powder originated with the loss of water. Freudenberg has used the same method for the synthesis of quebracho tannin, but states that no loss of water occurs. It is essential that no loss of water should take place for the Freudenberg structure of tannin to hold.

PART II.

EXPERIMENTAL.

### PREPARATION OF SAMPLES FOR ANALYSIS.

The work recorded in this thesis has been conducted on tannin obtained from two sources. At first commercial black wattle extract was used as the starting material. This is prepared in the factory by the extraction of the bark with water at 90°-100°C. After the sludge has been allowed to settle, evaporation is carried out under reduced pressure, and the viscous extract obtained sets to a brittle solid on cooling (55). It was, however, subsequently found advisable to extract the tannin directly from the fresh bark which had been preserved in paraffin, and it was on this material that the bulk of the work was conducted.

#### Extraction of Commercial Extract with Acetone.

300 grams of the commercial extract, finely ground, was added to 1,200 ml. acetone and was left for three days with intermittent shaking. The acetone was then filtered off, leaving a dark brown gummy mass. The filtrate was taken down on a waterbath to a brown syrup, and the remainder of the acetone was removed under vacuum. When free from acetone, the solid was powdered yielding 220 grams of a light brown amorphous powder. This will henceforth be referred to as "acetone extract".

#### Extraction of Fresh Bark.

During the various extractions the acetone extracted material had been exposed to heat and light. If, as is reported by many workers in this field, tannin polymerises and oxidises so very easily, then the resulting extract would almost certainly be highly polymerised. It was therefore decided to obtain a better starting material.

Wet bark was finely cut up and just covered with distilled

water in a flask. The air was displaced with hydrogen and the flask was well stoppered. After standing in the dark for six hours, the resulting dark solution was filtered off. Since methylated tannin has been used throughout in this work, it was decided to methylate the tannin as soon after extraction as possible to stabilise the molecule and render it less subject to polymerisation. The filtrate was therefore immediately methylated with dimethyl sulphate and KOH. The product, however, was highly coloured, and as this is an indication of polymerisation a better method of extraction was sought.

Wet bark was once more cut up, and just covered with methanol which had been freed from acetone. The flask was stoppered and placed in a dark cupboard for six hours, after which the light red methanol solution was filtered off. This was immediately methylated as above to yield a light flesh coloured amorphous powder. After drying thoroughly under vacuum at 60°C, a methoxyl determination was carried out according to the standard Zeisel method.

0.1093 gms. gave 0.2856 gms. AgI whence %OCH<sub>3</sub> = 34.50

0.1089 " " 0.2831 " AgI " %OCH<sub>3</sub> = 34.43

#### Method of Methylation.

Corbett (1) reported that a fully methylated derivative of tannin was obtained by methylating three times with dimethyl sulphate and KOH. The method adopted in the present investigation was as follows:-

10 grams acetone extracted tannin was dissolved in 100 ml. methyl alcohol, and 20 ml. dimethyl sulphate was added all at once. The resultant solution was kept just alkaline by the

slow addition of a concentrated solution of KOH in methyl alcohol, while stirring vigorously. The reaction was vigorous. After stirring for twenty minutes the methylation was repeated using the same quantities of reagents, and was then finally repeated for the third time. During the second and third methylations the solution heated to boiling point.

When the third methylation was completed, the solution was made slightly acid with dilute  $H_2SO_4$ , and poured into excess water, when the methylated product separated as a pink granular mass. This was filtered off and taken up in hot ethyl alcohol in which it was completely soluble. Boiled with animal charcoal and filtered hot into water, a lighter coloured product separated. This was filtered and dried to give 9 grams methylated tannin.

A methoxyl determination on the dried product gave the following figures:-

0.1122 gms. gave 0.2953 gms. AgI whence  $\%OCH_3 = 34.70$ .

0.1106 " " 0.2900 " AgI "  $\%OCH_3 = 34.65$ .

This method was adhered to for the methylation of the tannin from the extraction of the fresh bark.

OXIDATION OF METHYLATED TANNIN WITH  $\text{KMnO}_4$ .

Corbett (1) and Stephen (2) both oxidised methylated tannin successfully, and claimed to have isolated trimethylgallic acid and veratric acid, although, as has been pointed out, some doubt exists as to the identification of the latter acid. In order therefore to establish the presence of this acid, and to attempt to increase the yields of oxidation products, a number of oxidations were carried out under varying conditions. Acetone extracted material was used, as by Corbett and Stephen.

Procedure.

10 grams methylated tannin, of 32% methoxyl content, was finely crushed and suspended in 200 ml. water, the whole being kept thoroughly stirred. Finely ground  $\text{KMnO}_4$  was slowly added over a period of two hours until no further oxidation was apparent, 60 grams in all being required. The oxidation proceeded readily at room temperature but rose quickly to  $45^\circ\text{C}$ , dropping again as the oxidation neared completion. The excess permanganate was removed with sodium bisulphite. The resulting  $\text{MnO}_2$  was filtered off and washed with warm water, and the combined filtrates and washings were concentrated on the waterbath, acidified with  $\text{HCl}$  and extracted five times with ether. The ether extract was evaporated to give a small quantity of crystals in a reddish gum. These were taken up in sodium bicarbonate solution, when only a trace of amorphous material remained undissolved. The solution was filtered, acidified, and extracted once more with ether, giving on evaporation of the ether and thorough drying .6 grams of a yellow gum which crystallised on cooling.

Being soluble in hot water, these crystals were boiled with animal charcoal and filtered. On recrystallising twice from water, a small quantity of colourless crystalline acids was obtained, melting at  $142^{\circ}\text{C}$ . The examination of these crystals is reported later.

An oxidation was now carried out on the completely methylated product i.e. 34.7%  $\text{OCH}_3$ , in exactly the same manner as described above, except that 10 grams of sodium carbonate was added to the suspension before starting the oxidation. In this case the oxidation would not proceed at room temperature, and no decolourisation of the permanganate was apparent after  $1\frac{1}{2}$  hours, although very little permanganate had been added. The suspension was therefore heated up slowly to  $55^{\circ}\text{-}60^{\circ}\text{C}$  and the oxidation was carried out at this temperature. 55 grams  $\text{KMnO}_4$  were used in oxidising 11 grams of the methylated tannin.

The products were worked up exactly as before, and 3.3 grams of a reddish gum, crystallising on cooling, was obtained.

As these acids were probably not fully oxidised, they were once more taken up in 50 ml. sodium bicarbonate solution and filtered, giving a red solution. This was reoxidised on a boiling waterbath at  $80^{\circ}\text{C}$  with  $\text{KMnO}_4$ . Permanganate was reduced quickly at this temperature, and 5 grams were required to complete the oxidation. The  $\text{MnO}_2$  was filtered off, washed thoroughly with hot water, and the combined filtrates and washings concentrated on the waterbath. On acidification and cooling in the ice chest for 24 hours the filtrate yielded 0.5 grams of a crystalline acid. On twice recrystallising from water the crystals melted sharply at  $178^{\circ}\text{C}$ . This appeared

to be the one acid previously suggested to be veratric acid.

In order to do a complete analysis of this acid, 0.5 grams more was obtained by a similar oxidation, except that in this case no sodium bicarbonate was added to the suspension before oxidation. This appeared to decrease the yield of the mixed acids. M.Pt. acid 178°C.

Analyses were now carried out on the acid as follows:-

0.0654 grams neutralised 13.36 ml. of 0.02675 N NaOH

whence equivalent weight = 183.0

0.1056 grams acid gives 0.2710 grams AgI

whence % OCH<sub>3</sub> = 33.9

0.1011 grams acid gives 0.0506 grams H<sub>2</sub>O and 0.2195 grams CO<sub>2</sub>

whence % H = 5.56                      % C = 59.2

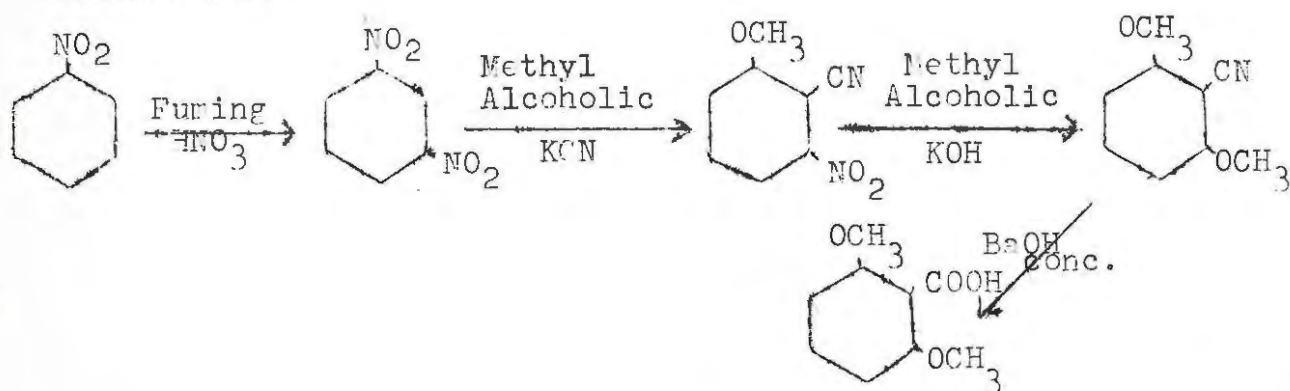
C<sub>6</sub>H<sub>3</sub>(OCH<sub>3</sub>)<sub>2</sub>COOH requires :- Equivalent Weight = 182

(veratric acid or                      % OCH<sub>3</sub> = 34.07  
2.6-dimethoxy-

benzoic acid) % C = 59.3

% H = 5.49

In order to establish the identity of this acid and to exclude the possibility of 2.6. dimethoxy - benzoic acid, both these acids were synthesised. Veratric acid was easily prepared by oxidising dimethyleugenol with KMnO<sub>4</sub>. 2.6. dimethoxybenzoic acid was prepared in four stages from nitro benzene as follows (56):-



As the original paper was unobtainable, difficulty was experienced in the hydrolysis of the 2,6-dimethoxybenzotrile, but on increasing the concentration of the alkali and lengthening the time of hydrolysis, the acid was successfully isolated. M.Pt. 181°C.

The identity of the unknown acid was now firmly established as veratric acid, since a mixed melt with veratric acid showed no depression, melting at 178°C, whereas a mixed melting point with the 2,6-dimethoxybenzoic acid gave the value 162°C.

The methyl ester of the unknown acid and that of veratric acid were prepared with diazomethane to give, in each case, a white crystalline solid. Melting points were as follows:-

Veratric acid methyl ester	56°C (Theoretical 56°-57°C).
Unknown acid methyl ester	55.5°C
Mixed melt	56°C

Having now established the presence of veratric acid, a number of further oxidations of the methylated tannin were carried out in order to obtain enough of the mixed acids to effect a separation. The conditions of each oxidation were varied in an attempt to increase the yields of these acids, and the acids in each case were boiled with charcoal and recrystallised from water. A table showing the effect of the conditions on the oxidation has been included on page 30.

The mixed acids from the various oxidations were now combined, and a separation was effected by means of the silver salt, as reported by Corbett (1). 3.5 grams of the acids was dissolved in a slight excess of ammonia and boiled until neutral. The solution was made to 300ml. and heated almost to boiling

when a hot solution of 4 grams silver nitrate in 100 ml. water was added. A blueish silver salt settled out immediately. This was allowed to cool to room temperature, and the silver salt filtered off, washed four times with water at 60°C, the washings being discarded. The silver salt and the filtrate were treated separately.

The silver salt was suspended in 100 ml. hot water and a slight excess of conc. HCl was added. The AgCl precipitate which formed was then heated almost to boiling point and filtered hot. The filtrate, when cold, was now extracted with ether, and the ether evaporated to give a white crystalline acid which, on recrystallising twice from water, gave 0.2 grams of needle-like crystals melting at 178.5°C, and showing no depression of melting point when mixed with veratric acid. M. Pt. 178.5°C.

The filtrate was cooled thoroughly in the icechest for 24 hours, when a little cream-coloured silver salt was found to have precipitated. This was filtered off and the filtrate treated with HCl to precipitate the silver, and extracted with ether to yield an acid melting at 147°C. There had evidently been an incomplete separation, so the acidic crystals were subjected to a further silver salt separation. The filtrate yielded 0.15 grams of a crystalline acid which, recrystallised from water, had a melting point of 167°C. A mixture of this acid with synthetic trimethylgallic acid (M. Pt. 167°-168°C) gave no depression of the melting point, melting sharply at 167°C.

Having now confirmed the presence of both the pyrogallol and catechol nuclei in the side chain, it appears that Stephen's suggestion that tannin is a mixture is correct. However, commercial extract has been used for all the oxidations so far

recorded, and Russell (4) points out that sometimes when the origin of the extract is not known, and if bark and wood are not extracted separately, a mixture of phlobatannins is sometimes encountered, thus confusing the issue.

In order to exclude this possibility, 30 grams methylated tannin, from fresh bark extracted in the laboratory, was oxidised at  $50^{\circ}$ - $60^{\circ}$ C as before, to give 5.1 grams mixed acids which, on boiling with charcoal and recrystallising from water, melted at  $143^{\circ}$ C. These were subjected to a silver salt separation, giving two acids as before which were shown by melting points and mixed melts to be veratric acid and trimethylgallic acid.

It should be noted here that all subsequent work was carried out on fresh bark extract.

## OXIDATION RESULTS OF METHYLATED TANNIN.

Weight Oxidised	Percentage Methoxyl	Temperature of Oxidation	Na <sub>2</sub> CO <sub>3</sub>	Wt. KMnO <sub>4</sub> used.	Yield Mixed Acids	% Yield	M. Pt. of acids.	Time of Oxidation.
10 gms.	32%	45°C	No	60 gms.	0.6 gms.	5%	142°C	2 hrs.
11 gms.	34.7%	55°-60°C	Yes	55 gms.	3.3 gms.	30%	--	3½ hrs.
12 gms.	34.7%	80°C	Yes	75 gms.	2.0 gms.	16.6%	Melted over a range 150-170°C	6 hrs.
10 gms.	34.7%	55°-60°C	No	60 gms.	2.5 gms.	25%	145°C	7 hrs.
10 gms.	34.7%	80°C	No	65 gms.	1.2 gms.	12%	164°C	8 hrs.
10 gms.	34.7%	55°C	No	60 gms.	2.3 gms.	23%	--	6½ hrs.
3 gms.	34.7%	Refluxed.	No	25 gms.	0.2 gms.	6.67%	165-175°C	9 hrs.

Conclusions on Oxidations.

It has been shown that the oxidation of the incompletely methylated tannin proceeds easily, while that of the completely methylated tannin (34.7%  $\text{OCH}_3$ ) has to be more drastic. From the table on page 30 it will also be seen that the yields of mixed acids are very low in the case of the tannin of 32.0% methoxyl content, in comparison to oxidations on the fully methylated product. Both Corbett (1) and Stephen (2) report low yields of these acids, and the oxidation is reported as going at room temperature. It would seem, therefore, that their work was conducted on incompletely methylated tannin.

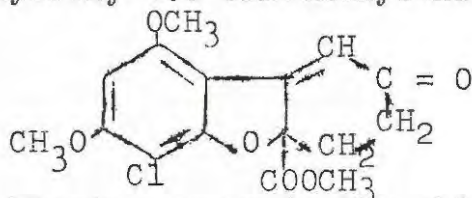
From the table of comparison it can be seen that satisfactory conditions for a maximum yield of oxidation products would be a temperature of  $55^\circ\text{--}60^\circ\text{C}$ , with the addition of sodium carbonate before oxidation. A 30% yield was obtained. A higher temperature decreases the yield of mixed acids and prolongs the oxidation, as some of the acids formed are evidently further oxidised. If the oxidation is carried out without sodium carbonate the same effect is observed, while an oxidation at  $80^\circ\text{C}$  without sodium carbonate gave only a 12% yield in eight hours.

The temperature also appears to affect the relative yields of the acids, as is shown by the rise in the melting points of the unseparated mixture. The more drastic the oxidation the less trimethylgallic acid appears to be present. (M.Pt. trimethylgallic acid  $167^\circ\text{C}$ , M.Pt. veratric acid  $180^\circ\text{C}$ ). On refluxing with  $\text{KMnO}_4$ , the mixed acids softened at  $165^\circ\text{C}$  and melted slowly over a range, collapsing finally at  $175^\circ\text{C}$ . It seems, therefore, that the trimethylgallic acid is itself undergoing oxidation under drastic conditions. On reoxidation of the mixed acids at  $80^\circ\text{C}$  only veratric acid is isolated.

NITRATION OF METHYLATED TANNIN.

Since only veratric and trimethylgallic acids could be isolated from the oxidation of methylated tannin, it would seem that the other half of the molecule is disrupted completely. This is due possibly to the formation of a phenolic group by the scission of the pyran ring, thus rendering the resorcinol unit less stable.

Oxford (57) oxidised griseofulvin and successfully isolated 3 chloro 2 hydroxy 4,6 dimethoxybenzoic acid



This has a chlorine group in the phloroglucinol nucleus which would to some extent stabilise this nucleus. It was thought that if a stabilising group such as a nitro group could be introduced into the resorcinol nucleus of the methylated tannin, this might have the required effect, enabling this unit to be isolated on subsequent oxidation. Accordingly various methods of nitration were attempted.

By Means of Ethyl Nitrate.

One gram of methylated tannin was taken up in 10 ml. acetic acid, and 5 grams ethyl nitrate was added. 5 ml. phosphoric acid was added dropwise with stirring over a period of  $\frac{1}{2}$  hour, and then 3 ml. conc.  $H_2SO_4$  was added. The brown solution darkened considerably and warmed up on the addition of the  $H_2SO_4$ .

The reaction mixture was now poured into distilled water, when a very dark red amorphous product separated. This was

filtered, washed well with water, dissolved in acetic acid, boiled with animal charcoal, filtered and precipitated in water to give a dark red amorphous product. A qualitative nitrogen test on the dried product was negative.

A repeat attempt at nitration was made by dissolving 1 gram of the methylated tannin in 5 ml. ethyl nitrate, and adding 5 ml. conc.  $H_2SO_4$  dropwise with shaking. The solution once more darkened considerably and warmed up. After shaking for  $\frac{1}{2}$  hour the mixture was poured into water, when a red-brown amorphous precipitate settled out. This was filtered off, washed, and purified as before to give a very fine red suspension on precipitation in water. This was centrifuged and dried. A nitrogen test on the dried product was negative.

By Means of  $HNO_3$  in the Cold.

1 gram of methylated tannin was dissolved in 10 ml. acetic acid, to which 5 ml. conc.  $HNO_3$  was added dropwise in the cold with constant shaking. The solution darkened, but no other signs of reaction were apparent. This was shaken for  $1\frac{1}{2}$  hours, after which it was precipitated in water to give a light brown amorphous product. This was centrifuged, washed, and once more reprecipitated from acetic acid to free it from any  $HNO_3$ . The precipitate on centrifuging and drying, gave once more a negative test for nitrogen.

By Means of  $HNO_3$  at  $60^\circ C$ .

Since the nitration did not take place in the cold, it was repeated as follows on the waterbath at  $60^\circ C$ :-

1 gram methylated tannin was dissolved in 10 ml. acetic acid and 2 grams urea added to remove  $HNO_2$ . This was warmed to  $60^\circ C$  on the waterbath and 5 ml. conc.  $HNO_3$  (previously boiled with

urea to remove  $\text{HNO}_2$ ) was added dropwise with stirring. The reaction was vigorous with effervescence, but no  $\text{NO}_2$  fumes were evolved. The brown solution darkened as the  $\text{HNO}_3$  was added. When the acid had been added, the mixture was heated on the waterbath for one hour, and so concentrated. On the addition of water an amorphous brown precipitate settled out, which was filtered, washed well with cold water, taken up in hot acetic acid and reprecipitated in water. The precipitate was then filtered and washed well. A nitrogen test on the amorphous brown precipitate was positive. Yield 0.8 grams.

After thorough drying a methoxyl and a nitrogen determination was carried out as follows:- (The method of nitrogen determination is described later).

0.0840 grams gave 0.0864 grams AgI whence %  $\text{OCH}_3$  = 13.6

0.1056 " " 0.1093 " AgI " %  $\text{OCH}_3$  = 13.7

0.0510 grams gave  $\text{NH}_3$  equivalent to 0.3555 ml. N acid  
whence % N = 9.7.

The methoxyl content of 13.6% appeared to be too low, and it was thought that a partial demethylation of the methylated tannin had occurred. The compound was therefore taken up in ethyl alcohol and remethylated with 5 ml. dimethyl sulphate and KOH. Addition of water after acidification with  $\text{H}_2\text{SO}_4$  produced a red-brown amorphous precipitate which was filtered and washed well with water. This was dried thoroughly under vacuum at  $60^\circ\text{C}$ , and methoxyl determinations were conducted.

0.1004 grams gave 0.1580 grams AgI whence %  $\text{OCH}_3$  = 20.8

0.1010 " " 0.1597 " AgI " %  $\text{OCH}_3$  = 20.89

This compound is soluble in dilute alkali, being reprecipitated on acidification. It is very soluble in ethyl acetate and acetone, being reprecipitated from the latter on the addition of water. It is insoluble in ether.

As it was suspected from the above that free phenolic groups were still present, it was remethylated in methyl alcohol three times with dimethyl sulphate and KOH. After precipitating the product, washing and drying, analyses were carried out.

0.1037 grams gave 0.1790 grams AgI whence % OCH<sub>3</sub> = 22.80

0.1010 " " 0.1745 " AgI " % OCH<sub>3</sub> = 22.82

0.1046 grams gave 0.0413 grams H<sub>2</sub>O and 0.2000 grams CO<sub>2</sub>

whence % H = 4.39 % C = 52.12

0.1050 grams gave 0.0421 grams H<sub>2</sub>O and 0.2010 grams CO<sub>2</sub>

whence % H = 4.45 % C = 52.25

0.0524 grams gave NH<sub>3</sub> equivalent to 0.2473 ml. N acid

whence % N = 6.61

0.0563 grams gave NH<sub>3</sub> equivalent to 0.2626 ml. N acid

whence % N = 6.53

This product, methylated as highly as possible, showed only very slight solubility in alkali.

A comparison of these figures with those of a theoretical dinitrated methylated tannin is given overleaf.

	Analytical Results.	Theoretical for Dinitrated Tannin with 3'4' Dimethoxybenzene side group.	Theoretical for Dinitrated Tannin with 3'4'5' Trimethoxybenzene side group.
% OCH <sub>3</sub>	22.8	22.9	28.4
% C	52.2	53.21	52.2
% H	4.42	4.43	4.58
% N	6.61	6.90	6.42

It will be seen that, except for the methoxyl value, the analysis agrees well with dinitrated methylated tannin, assuming a mixture of the two units according to Stephen's suggestion. No higher methoxyl value could be obtained.

METHOD OF NITROGEN ESTIMATION.

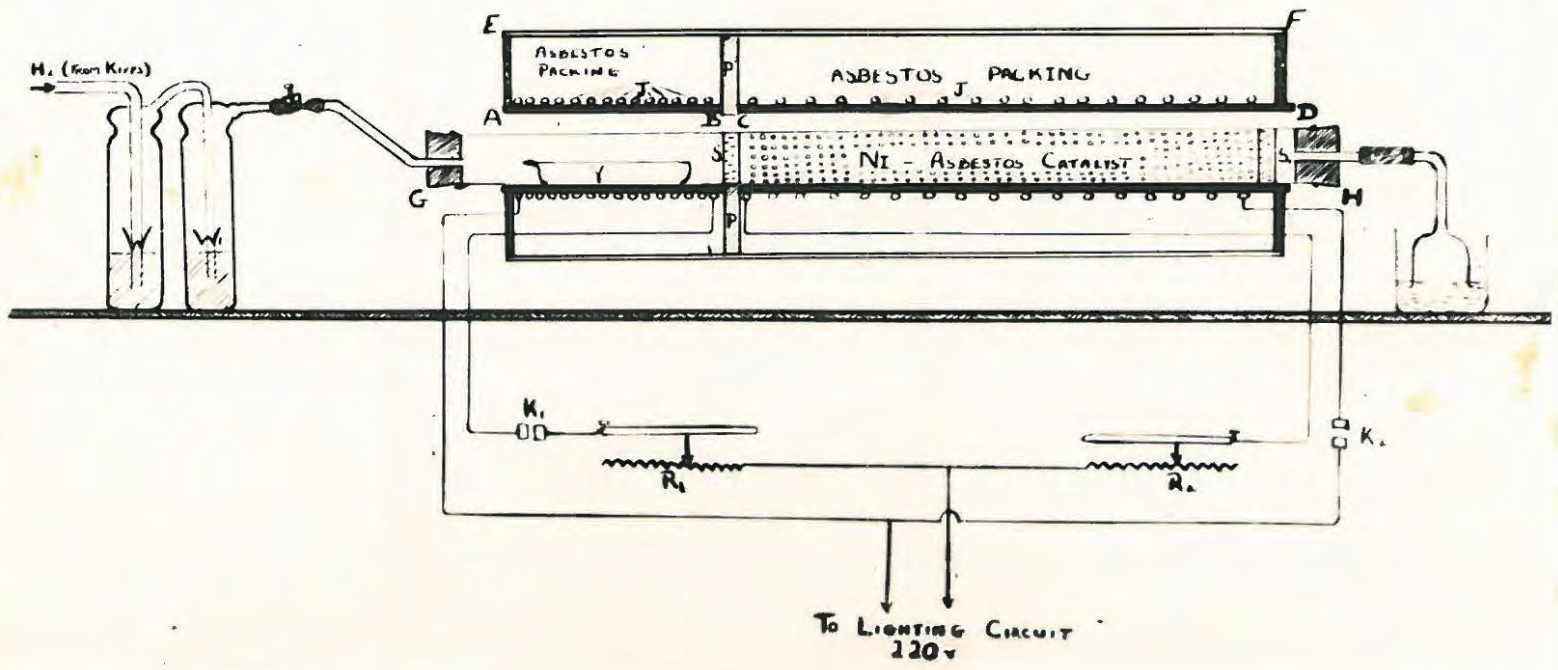
The method of nitrogen estimation adopted, known as the "Hydrogenation Method", has been described fully by Shuttleworth (58). The principle of the procedure is to combust the compound in a stream of hydrogen over a suitable catalyst, when the nitrogen is converted to  $\text{NH}_3$  which is then absorbed in a standard acid. The amount of  $\text{NH}_3$  evolved may thus be determined by titration of the acid, and hence the nitrogen value is obtained. As the method is not widely employed, a brief description is included.

Description of Apparatus.

A diagram of the apparatus constructed is given on page 38. AB and CD are two lengths of brass tube,  $1\frac{1}{2}$ " diameter, and 6" and 18" in length, respectively, which are wrapped in mica for insulation. They are wound with separate heating coils J, which are connected to the lighting circuit through two rheostats,  $R_1$  and  $R_2$  as shown. AB and CD are insulated from each other by an asbestos plug P, and are packed firmly with asbestos packing into a circular asbestos cylinder EF, which is mounted on a wooden stand.

GH is a silica combustion tube, 2'3" long, packed loosely with a nickel-asbestos catalyst which is kept in position by two porous asbestos plugs S and  $S_1$ .  $W_1$  and  $W_2$  are two wash bottles containing, respectively, an acidic solution of  $\text{KMnO}_4$  and an alkaline solution of  $\text{KMnO}_4$  to purify the hydrogen which is generated in a Kipps apparatus. Y is the boat containing the nitrogen containing compound mixed with the catalyst.

**— ELECTRIC FURNACE FOR  
NITROGEN ESTIMATION —**



### Catalyst.

The nickel-asbestos catalyst is prepared by mixing well 1 part by weight of small asbestos fibres with 2 parts of nickel oxide powder ( $\text{Ni}_2\text{O}_3$ ), or with 4 parts of nickel formate. This is then packed loosely into the tube as shown, leaving the portion of the tube lying in AB free. The tube is now placed in the furnace and the hydrogen train is connected.

A stream of hydrogen is passed rapidly through and, when all the air is displaced, key  $K_2$  is plugged in, the rheostat  $R_2$  being adjusted to maintain the temperature of CD at  $250^\circ\text{--}300^\circ\text{C}$ . The catalyst takes about 2 hours to reduce, and should always be kept in hydrogen to prevent reoxidation.

### Reagents Required.

- 1)  $\frac{N}{20}$   $\text{H}_2\text{SO}_4$  and  $\frac{N}{20}$  NaOH solutions free from  $\text{CO}_2$ .
- 2) 50% KOH solution.
- 3) Nickel formate.
- 4) Reduced nickel, made by reducing nickel oxide in the furnace.
- 5) Methyl red indicator.

### Procedure.

The temperature of CD is adjusted by means of  $R_2$  to remain steady at  $250^\circ\text{--}300^\circ\text{C}$ . A position on the rheostat which will bring the temperature up to  $250^\circ\text{C}$  in 10 minutes is marked, and the exact time taken is noted. When it has reached  $250^\circ\text{C}$  the rheostat is shifted to position 2 which is such that it will maintain the furnace at this temperature.

Weigh out  $\pm 0.05$  grams of the nitrogen containing compound in a porcelain boat Y, and mix it with  $\pm 0.2$  grams nickel formate and  $\pm 0.5$  grams reduced nickel. Cover this with a few ml. 50%

KOH solution, and allow to stand for a few minutes before sliding the boat into the position shown in the diagram.

The outlet of the furnace is connected, in the meanwhile, to a thistle funnel inverted and dipping into a 100 ml. beaker as shown. The beaker contains 10 ml. standardised  $\frac{N}{20}$  acid and 50 ml. water. Pass hydrogen through at the rate of 1-2 bubbles per second, and allow a few minutes for the displacement of any air in the tube. Now connect  $K_1$ , leaving rheostat  $R_1$  in a position, previously determined, which will allow AB to heat up to a bright red heat in 45 minutes. The furnace is maintained at this temperature for a further  $\frac{1}{2}$  hour, after which the beaker is removed and the thistle funnel washed well into the solution. The excess acid is now titrated with  $\frac{N}{20}$  NaOH using methyl red as indicator.

It was found that using a weight of compound greater than 0.05 grams low results were obtained, probably due to incomplete reduction of the nitrogen along the catalyst. The rate of bubbling of the hydrogen had to be reduced from 2 to 1 bubble per second, as the faster rate recommended by Shuttleworth led to low values. The method was standardised using m-nitrophenol.

OXIDATION OF NITRATED METHYLATED TANNIN.

6 grams nitrated methylated tannin was finely crushed and suspended in 150 ml. water. 5 grams sodium carbonate was added, the whole being kept thoroughly mixed. 20 grams finely ground  $KMnO_4$  was now added over a period of 5 hours, when no further reduction of the permanganate was discernable. The oxidation proceeded readily at room temperature, the suspension warming up of its own accord.

At the end of the 5 hour period the  $MnO_2$  was filtered off and washed thoroughly with warm water, and the combined filtrates and washings were concentrated on the waterbath. The solution was now extracted with ether to yield on evaporation a trace of oil. The solution was acidified with dilute  $H_2SO_4$ , when a solid precipitated, and the whole extracted with ether. The solid was not completely soluble in ether, but a subsequent ethyl acetate extraction removed it all. Both solvents were evaporated, the ether extract yielding 0.2 grams of a yellow gum which showed a tendency to crystallise (Fraction I). The ethyl acetate fraction yielded 0.15 grams of an oily dark yellow gum which failed to crystallise (Fraction II). A nitrogen test on each fraction was positive.

In attempting to recrystallise fraction I from water, droplets of a highly viscous oil separated. These were filtered off from the yellow solution, which was then concentrated slightly. On cooling, more oil separated. This was again filtered off and the solution placed in the icechest overnight. A yellowish needle-like crystalline precipitate separated.

Fraction II was insoluble in hot water, toluene, benzene, Xylene, chloroform, carbon tetrachloride, and very soluble in

ethyl alcohol, methyl alcohol, ethyl acetate, and acetone. All attempts to crystallise this compound from the above solvents and mixtures of them proved fruitless. In an attempt to purify the gum further, it was taken up in sodium bicarbonate solution in which it was completely soluble. This solution was extracted with ethyl acetate, and a trace of a red gum was removed. Acidified with dilute  $H_2SO_4$  and once again extracted with ethyl acetate, on evaporation it again yielded a yellow gum.

To obtain enough of these fractions for analysis, 30 grams of the nitrated methylated tannin was oxidised as before with 15 grams sodium carbonate and 90 grams  $KMnO_4$  over a 6 hour period. The oxidation products were worked up as above, yielding 2.2 grams of ether soluble acidic material (Fraction I) and 1.7 grams ethyl acetate soluble material (Fraction II).

#### Examination of Ether Extract (Fraction I).

This was taken up in hot water, when a quantity of dark brown gum remained undissolved. The golden brown solution was filtered off and allowed to cool to room temperature when more gum separated. This was filtered off and the filtrate placed in the icechest. After a few days a light yellow crystalline needle-like solid separated, the needles forming agglomerates and giving the whole an amorphous appearance. After a week the crystals were filtered, washed with a few ml. cold water, when they lost their yellow colour yielding 0.05 grams of colourless crystals. Starting at  $174^{\circ}C$  these melted finally at  $178^{\circ}C$ . An equivalent weight determination was carried out after thorough drying.

0.0326 grams acid neutralised 6.60 ml. 0.02606 N alkali

Whence equivalent weight = 189.5

The acid was recovered by acidification and extraction with ether, and on drying melted at  $179^{\circ}\text{C}$ . A mixed melt with veratric acid showed no depression. M.Pt  $179.5^{\circ}\text{C}$ .

The mother liquors from the above extraction of the gum with water were added to the gum separated previously and heated on the water bath. On filtering and cooling as before, a further crop of crystals was obtained on seeding the solution with a crystal of the acid. These were filtered off and washed well with a little cold water to yield 0.04 grams of acid on thorough drying. These were combined with those previously recovered, and a redetermination of the equivalent weight was made.

0.0346 grams acid neutralised 7.24 ml. 0.02606 N alkali  
whence equivalent weight = 183.4

A qualitative nitrogen test was negative. This acid is therefore established as veratric acid, which has M.Pt.  $180^{\circ}\text{C}$  and eqt. wt. 182.0

The dark brown acidic gum remaining after the extraction of the veratric acid with water was thoroughly washed with hot water to remove any traces of veratric acid. All attempts to crystallise it failed. It was then dried thoroughly and left in a vacuum desiccator for a week, when it set almost solid. A nitrogen test was positive and analyses were performed.

0.1048 grams gave 0.0450 grams  $\text{H}_2\text{O}$  and 0.1861 grams  $\text{CO}_2$   
whence % C = 48.43      % H = 4.77

0.1046 grams gave 0.0445 grams  $\text{H}_2\text{O}$  and 0.1858 grams  $\text{CO}_2$   
whence % C = 48.45      % H = 4.73

0.1068 grams gave 0.2185 grams AgI whence %  $\text{OCH}_3$  = 27.03

0.1020 " " 0.2093 " AgI " %  $\text{OCH}_3$  = 27.11

0.0512 grams gave  $\text{NH}_3$  equivalent to 0.2134 ml. N acid

whence % N = 5.88

0.0567 grams gave  $\text{NH}_3$  equivalent to 0.2414 ml. N acid

whence % N = 5.96

0.0429 grams neutralised 8.08 ml. 0.02566 N alkali

whence equivalent weight = 206.5

0.0585 grams neutralised 11.0 ml. 0.02566 N alkali

whence equivalent weight = 207.2

The results indicated a benzoic acid with two methoxyl groups and one nitro group. A comparison with such an acid follows.

	Analytical Results.	Theoretical for a dimethoxy nitrobenzoic acid.
%OCH <sub>3</sub>	27.1	27.3
%C	48.4	47.6
%H	4.7	3.97
%N	5.9	6.17
Eqt.Wt.	207	227

Examination of Ethyl Acetate Fraction (Fraction II).

As has been stated before, all attempts to crystallise this gum failed. However, on evaporation of the ethyl acetate after extraction, fine white crystals were deposited around the edge of the beaker,  $\pm$  0.01 grams in quantity. These were brushed into a watch glass and were found to be strongly acidic towards litmus and very easily soluble in water, melting at 89°C.

On evaporation of the water solution long flat needles crystallised, melting at 95°C. These were shown to be oxalic acid crystals by the normal qualitative tests.

The gum was washed thoroughly with hot water to remove any oxalic acid, and dried well in a vacuum desiccator for a week.

Analyses were as follows:-

0.1045 grams gave 0.0438 grams H<sub>2</sub>O and 0.1700 grams CO<sub>2</sub>

whence % C = 44.36      % H = 4.66

0.1061 grams gave 0.0450 grams H<sub>2</sub>O and 0.1728 grams CO<sub>2</sub>

whence % C = 44.41      % H = 4.72

0.0529 grams gave NH<sub>3</sub> equivalent to 0.2606 ml. N acid

whence % N = 6.9

0.0491 grams gave NH<sub>3</sub> equivalent to 0.2393 ml. N acid

whence % N = 6.82

0.1100 grams gave 0.1153 grams AgI whence % OCH<sub>3</sub> = 13.85

0.1018 grams gave 0.1060 grams AgI whence % OCH<sub>3</sub> = 13.76

0.0598 grams neutralised 13.13 ml. 0.02566 N NaOH

whence equivalent weight = 177.5

0.0469 grams neutralised 10.26 ml. 0.02566 N NaOH

whence equivalent weight = 178.2

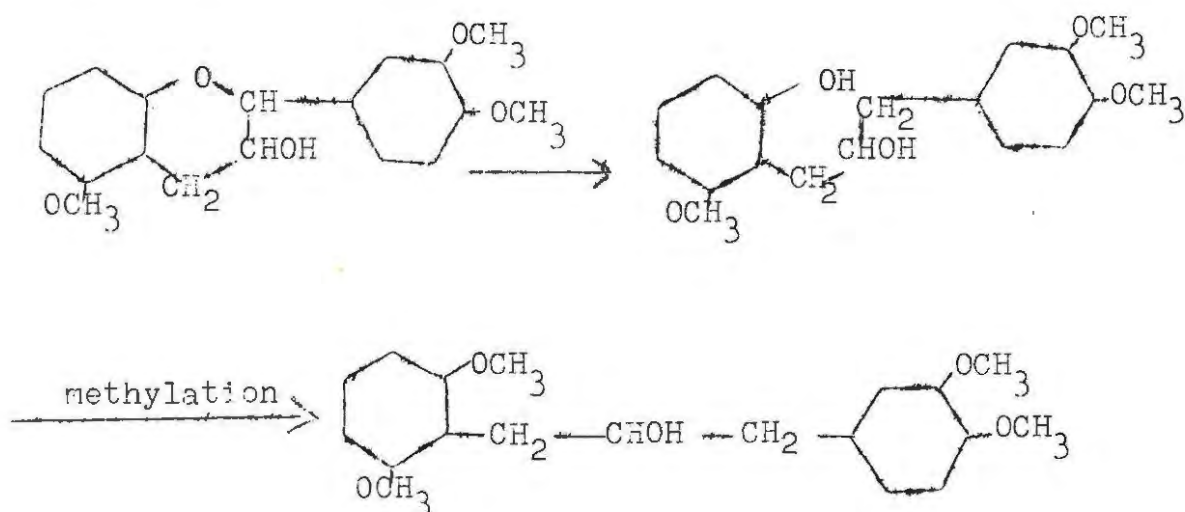
These figures are inconclusive.

It must be emphasised that as both the acidic gums from this oxidation refused to crystallise from the numerous solvents used, it is probable that the analyses were conducted on impure substances. They were freed from oxalic acid as thoroughly as possible, but it is still likely that a small quantity was tenaciously held by the gums. This would account for the low equivalent weights obtained in each case.

ATTEMPTS AT OPENING OF PYRAN RING.

I - Alkali Treatment of Methylated Tannin.

Having failed to isolate the resorcinol nucleus from the oxidation products of the previous oxidation, attempts were now made to open the pyran ring before oxidation. If the pyran ring could be split as shown at the oxygen link, and the resulting phenolic group liberated, methylated, then by subsequent oxidation of the chain still linking the two benzene nuclei, the other half of the molecule might be obtained as an acid, as the resorcinol nucleus would now be stabilised.



Karrer (59) showed that methylated anthocyanins were satisfactorily degraded by boiling with dilute alkalis, and Stephen (2) reports small yields of acids by the same treatment of methylated tannin. This method of attack was therefore attempted.

1 gram samples of the methylated tannin were dissolved in 20 ml. methyl alcohol, and refluxed for 5 to 6 hours with increasing concentration of KOH. The tannin during this treatment was preserved from atmospheric oxidation by a floating

layer of benzine, with which methyl alcohol is immiscible. The solution in each case darkened slightly on refluxing.

At the end of the 5 hour period the benzine layer was separated and evaporated on the waterbath. The methyl alcohol solution was remethylated with 3 ml. dimethyl sulphate and KOH, acidified and poured into water, when, in each case, a light precipitate very similar to methylated tannin separated. After cooling in the icechest this precipitate was filtered and well washed. It was then dried thoroughly in a vacuum desiccator, and the methoxyl contents determined. The filtrates from the above were extracted with ether to remove any water soluble material, and the ether was evaporated.

A table of comparison is given below:

Samples.	% KOH by weight.	Methoxyl Content after treatment.	Benzine Soluble material.	Ether Soluble material.
A	5	34.7		
B	10	34.8	NO	INCREASING
C	25	34.4		TRACES
D	50	35.0	BENZINE	
E	100	35.0		OF A
F	200	34.8	SOLUBLE	
G	300	34.5		REDDISH
H	400	35.0	MATERIAL.	OIL.
I	500	34.7		
J	600	34.5		
K	700	35.0		
L	800	34.9		

The methoxyl content of the original tannin was 34.8%, so that no change had taken place. Should a phenolic group have been liberated, an increase of methoxyl content of at least 9% would be anticipated. The material after treatment was identical in appearance with the original methylated tannin.

It was found impossible to increase the concentration of the alkali further, since the methanol solution at 800% by weight was already saturated, a small quantity of KOH remaining undissolved.

## II - Reduction of Methylated Tannin with Sodium.

Having failed to open the ring by boiling with dilute alkali, a more drastic reduction was attempted. Kostanocki and Lampe (33), by boiling tetramethyl catechin with sodium in alcohol, were able to split the pyran ring at the oxygen link to give an oil which was remethylated to give the pentamethyl reduced catechin. An investigation of a satisfactory method of obtaining a reduction of the methylated tannin was therefore now conducted.

### (i) Boiling with the Sodium Salt of the Alcohol.

The sodium salts of ethanol, n-butanol, and pentanol were prepared by the addition of 2 grams sodium, in flakes, to 45 ml. of the respective alcohol, which was kept boiling until all the sodium was dissolved. A solution of 1 gram methylated tannin in a minimum of the respective alcohol was added to each, and the solutions were boiled for 5 hours, when they were observed to have lightened in colour.

After cooling these solutions, 50 ml. water was added to break the sodium salt, and in the case of ethanol the solution

was acidified and extracted with ether. Water being immiscible in butanol and pentanol, two layers separated, the water layer being dark brown in colour. These layers were separated and the water layer was acidified and extracted with ether. The alcohol was steam distilled from the other layer, leaving in each case a brown semi-solid which was then acidified and extracted with ether, the extract being combined with that of the water.

In each case a small amount of brown highly viscous oil resulted, which set to a glassy solid after being kept in a vacuum desiccator for a week.

Molecular weight determinations, according to the method of Rast, were performed on each of the solids to ascertain whether they were still of low molecular weight, or whether polymerisation had taken place under the conditions used. Combustion analyses were also conducted.

Sodium Ethanoate Fraction (Fraction I).

0.0211 grams with 0.1455 grams camphor gave a depression of  $13.5^{\circ}\text{C}$  (Molecular Depression Constant for camphor = 395).

whence molecular weight = 424

0.1023 grams gave 0.0627 grams  $\text{H}_2\text{O}$  and 0.2483 grams  $\text{CO}_2$

whence % C = 66.2      % H = 6.80

Fraction from Sodium Salt of Butanol (Fraction II).

0.0345 grams with 0.1588 grams camphor gave a depression of  $20.0^{\circ}\text{C}$  whence molecular weight = 420

0.1021 grams gave 0.0628 grams  $\text{H}_2\text{O}$  and 0.2498 grams  $\text{CO}_2$

whence % C = 66.7      % H = 6.83

Fraction from Sodium Salt of Pentanol (Fraction III).

0.0324 grams with 0.1608 grams camphor gave a depression of 19.5°C whence molecular weight = 410

0.1044 grams gave 0.0660 grams H<sub>2</sub>O and 0.2566 grams CO<sub>2</sub>  
whence % C = 66.5      % H = 7.02

A methoxyl determination was made on the combined fractions, after which they were remethylated with dimethyl sulphate and KOH. The remethylated product was dried well and a redetermination of methoxyl content was made.

Methoxyl on combined fractions.

0.0998 grams gave 0.1912 grams AgI whence % OCH<sub>3</sub> = 25.3

Methoxyl on remethylated fractions.

0.1027 grams gave 0.2021 grams AgI whence % OCH<sub>3</sub> = 26.0

We have therefore:-

	Mol. Wt.	Yield.	%C	%H	%OCH <sub>3</sub>	%OCH <sub>3</sub> after remethylation.
Fraction I	424	.2gms.	66.2	6.8	} 25.3	} 26.0
Fraction II	420	.4 "	66.7	6.8		
Fraction III	410	.5 "	66.5	7.0		

From the molecular weight determinations it can be seen that we are still dealing with the low molecular weight unit, and polymerisation does not seem to have taken place. A certain amount of demethylation probably occurred, the methoxyl content having dropped to 25.3%. However, remethylation did not raise this appreciably. A certain amount of reduction appeared to have taken place with the complete elimination of methoxyl groups, but it cannot be said that the ring had opened.

A more drastic reduction was now adopted.

(ii) Reduction of Methylated Tannin with Sodium in Alcohol.

1 gram methylated tannin was dissolved in 30 ml. ethyl alcohol and 3 grams sodium was slowly added, in small flakes, to the boiling solution. When all the sodium had been used up, the solution was boiled for 1 hour, cooled, and remethylated with 3 ml. dimethyl sulphate and KOH. On the addition of water, an amorphous product, identical in appearance with the original methylated tannin, separated. This was filtered and dried thoroughly after washing. The methoxyl content was determined.

0.1170 grams gave 0.3046 grams AgI whence %  $\text{OCH}_3$  = 34.4

No reduction or opening of the ring had occurred. As a higher temperature might facilitate the degradation, the above was repeated using n-butanol and pentanol respectively.

When the reaction was completed after one hour, it was observed that the solutions had lightened in colour considerably. They were cooled, and water was added to break the sodium salt. The dark brown water layer in each case appeared to have dissolved some of the reaction products. This layer was separated and acidified with  $\text{H}_2\text{SO}_4$ , when an amorphous precipitate separated. It was extracted with ether. The alcohol was steam distilled from the other layer, when a brown solid was left floating on the water. This was acidified and extracted with ether, a little resinous black gum remaining undissolved. The ether extracts from the water layer and the alcoholic layer were combined, and after drying over anhydrous sodium sulphate and evaporation yielded, in the case of butanol, 0.45 grams of a dark brown gum (fraction I). That from the reduction in pentanol yielded 0.75 grams of a similar gum (fraction II).



On drying these gums for a week in a vacuum desiccator they both set solid. Combustions and molecular weight determinations were conducted:-

Fraction I.

0.0291 grams with 0.1835 grams camphor gave a depression of 15.7°C whence molecular weight = 400

0.1013 grams gave 0.0642 grams H<sub>2</sub>O and 0.2546 grams CO<sub>2</sub>  
whence % C = 68.5 % H = 7.03

0.1041 grams gave 0.2058 grams AgI whence % OCH<sub>3</sub> = 26.1

Fraction II.

0.0200 grams with 0.1381 grams camphor gave a depression of 15.5°C whence molecular weight = 370

0.1028 grams gave 0.0646 grams H<sub>2</sub>O and 0.2604 grams CO<sub>2</sub>  
whence % C = 69.1 % H = 6.99

0.1022 grams gave 0.0656 grams H<sub>2</sub>O and 0.2592 grams CO<sub>2</sub>  
whence % C = 69.16 % H = 7.08

0.1020 grams gave 0.2025 grams AgI whence % OCH<sub>3</sub> = 26.3

The combined fractions were remethylated as before, and a redetermination of the brown solid formed after thorough drying gave the following result.

0.1031 grams gave 0.2340 grams AgI whence % OCH<sub>3</sub> = 30.5

A comparative table of these results is given below:

	Mol. Wt.	% C	% H	% OCH <sub>3</sub>	% OCH <sub>3</sub> after remethylation.	Yield.
Fraction I (Butanol)	400	68.5	7.03	26.1)	30.5	.45gms.
Fraction II (Pentanol)	370	69.1	6.99	26.3)		.75 "

It will be seen from the table that once more demethylation has taken place. However, in this case on remethylation an increase in methoxyl content is observed. Whether this is due to the opening of the pyran ring with the liberation of a phenolic group, which is subsequently methylated, or whether it is due only to a partial demethylation of the methoxyl groups already present, is impossible to say.

On comparison with the carbon and hydrogen values obtained for methylated tannin of 34.4% methoxyl content, which are % C 63.4 and % H 6.25, it would appear that a certain amount of hydrogenation is accompanied with the demethylation. It appears also, from the methoxyl value of 30.5% which is 4% lower than that for methylated tannin, that apart from demethylation, a certain amount of complete elimination of methoxyl groups has taken place.

Since the highest yields of reduced methylated tannin were obtained by the reduction in pentanol, this method was used for the preparation of subsequent samples for oxidation.

OXIDATION OF REMETHYLATED REDUCED METHYL TANNIN.

This product was found to be insoluble in water and alkali but very soluble in acetone.

8.5 grams of this compound in 200 ml. anhydrous acetone was cooled in a freezing mixture, being kept thoroughly stirred. A little  $\text{KMnO}_4$  was added, but no reduction was observed after 3 hours, so the solution was allowed to warm up slowly to room temperature ( $23^\circ\text{C}$ ) when the oxidation proceeded rapidly. 18 grams  $\text{KMnO}_4$  was added over a period of 24 hours, the solution warming to  $30^\circ\text{C}$ , but finally cooling to  $20^\circ\text{-}25^\circ\text{C}$  over the last 12 hours.

When the oxidation was complete, the acetone was sucked off and the  $\text{MnO}_2$  was washed well with anhydrous acetone to free it from any unoxidised material. 1.5 grams unoxidised material was recovered from the filtrate and washings.

The  $\text{MnO}_2$  was extracted with hot water to dissolve the potassium salts of the acids, and was filtered off and washed well with hot water. The combined filtrate and washings were concentrated on the waterbath and extracted with ether. This extraction yielded a trace of brown gummy material.

The solution was now acidified with dilute  $\text{H}_2\text{SO}_4$  when a brown, apparently amorphous solid, precipitated. This was extracted with ether 5 times giving on evaporation 2.4 grams of crystals set in a brown gum. A distinct odour of propionic acid was observed.

The acids were taken up in saturated sodium bicarbonate solution, in which they were completely soluble with the liberation of  $\text{CO}_2$ . The solution was filtered and extracted

once more with ether, when a trace of brown gum was removed. On reacidification and extraction with ether the crystalline acids set in a brown gum were once more obtained.

On heating on a waterbath with hot water the crystals were extracted, the gum remaining insoluble. The solution was filtered off and the gum was reextracted with hot water. The combined filtrates were placed in the icechest, and on cooling a crystalline precipitate settled (Fraction I). The examination of these is reported later.

Examination of Acidic Gum (Fraction II).

This gum was washed thoroughly with hot water to remove any water soluble acids, and then taken up in dilute NaOH solution. It was cooled to 0°C and slowly neutralised with 1 N HCl in an attempt to crystallise it. An oily yellow product which failed to crystallise was obtained. Further attempts at crystallisation from ethyl alcohol, methyl alcohol, and diluted ethyl and methyl alcohols also failed.

The gum, which was strongly acidic dissolving in sodium bicarbonate solution with the liberation of CO<sub>2</sub>, was then dried thoroughly in a vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. 1 gram was obtained, and analyses were as follows:-

0.1054 grams gave 0.2164 grams AgI whence % OCH<sub>3</sub> = 27.10

0.1020 grams gave 0.2099 grams AgI whence % OCH<sub>3</sub> = 27.18

0.1046 grams gave 0.0590 grams H<sub>2</sub>O and 0.2317 grams CO<sub>2</sub>

whence % C = 60.4      % H = 6.25

0.1023 grams gave 0.0568 grams H<sub>2</sub>O and 0.2262 grams CO<sub>2</sub>

whence % C = 60.3      % H = 6.17

0.0521 grams neutralised 9.13 ml. 0.02566 N NaOH

whence equivalent weight = 222.4

Three more oxidations were carried out as previously described, and the oxidation products were worked up in a similar manner to give enough of fractions I and II to continue investigations.

A silver salt method of equivalent weight determination was attempted on the gum (fraction II), and a black silver salt which appeared to be slightly gummy, was obtained. It was dried as thoroughly as possible over  $P_2O_5$ , and a subsequent determination gave the value of 242.2. However, this was considered to be unreliable, as the silver salt did not appear to be pure.

The figures obtained above indicate the compound  $C_6H_3(OCH_3)_2 \cdot CH_2 \cdot CHOH \cdot COOH$ .

A table of comparison is given.

	Analytical Figures.	Theoretical for $C_6H_3(OCH_3)_2 \cdot CH_2 \cdot CHOH \cdot COOH$ .
Eq. Wt.	222.4	226
%OCH <sub>3</sub>	27.14	27.4
% C	60.35	58.5
% H	6.2	6.19

If the compound contains a free hydroxyl group in the side chain, it should be able to be acetylated. This was attempted.

Various  $\alpha$  hydroxy acids were acetylated in order to determine optimum conditions. Acetylations using acetic anhydride with anhydrous sodium acetate as a catalyst, and refluxing for 1 to 5 hours failed, and no crystalline acetyl

derivatives were isolated.

From the literature it appeared that drastic conditions were necessary, due probably to the fact that the aliphatic OH is strongly hydrogen-bonded onto the acidic carboxyl group. Accordingly mandelic acid was treated with a slight excess of freshly distilled acetyl chloride in a small round bottomed flask fitted with a condenser. Immediate evolution of HCl occurred, and the reaction was completed by gentle heating on the waterbath. When the evolution of HCl had ceased, the mixture was poured into ice water with vigorous stirring. An oil separated and crystallised on cooling. This was recrystallised from water to give a melting point  $96^{\circ}\text{C}$ . Theoretical M. Pt.  $96.5^{\circ} - 98^{\circ}\text{C}$ .

The exact procedure outlined above was followed with the isolated oxidation product. No evolution of HCl was observed and the reaction mixture darkened considerably on heating. On pouring into water a dark oily product separated. It was evident that decomposition had taken place, and this substance, which had lost all acidic character, could not be induced to crystallise.

The less drastic acetic anhydride — sodium acetate method was now reverted to with similar results.

#### OXIDATION OF THE GUM.

In order to obtain a crystalline acid which might be identified more easily, the gum itself was oxidised as follows:

2.5 grams of the acidic gum was taken up in 100 ml. sodium bicarbonate solution and finely ground  $\text{KMnO}_4$  was added with shaking. The oxidation proceeded easily in the cold

but was eventually carried out at 80°C on the waterbath. 12 grams  $K_2Cr_2O_7$  were needed to complete the oxidation. The  $MnO_2$  was filtered and washed with hot water and the combined filtrate and washings concentrated on the waterbath. On cooling, this was extracted 4 times with ether when a small amount of yellow gum was removed. It was then acidified and extracted once more with ether, the extract being dried over anhydrous sodium sulphate and evaporated to give 0.09 grams of a slightly yellow crystalline acid, softening at 146°C and melting finally at 153°C. It was obviously impure, and was consequently taken up in 5 ml. hot water to give a yellow solution which was boiled with animal charcoal. This was filtered and washed with 1 ml. portions of hot water to give a colourless filtrate, which, on standing in the icechest for 24 hours, yielded 0.02 grams of a colourless crystalline acid. This was filtered, washed with a little cold water and dried thoroughly to give a melting point of 177°C. A mixture of this acid with veratric acid melted at 178°C.

The gum therefore appears to be a veratric nucleus with a side chain of 3 carbon atoms, which may be oxidised to veratric acid fairly easily.

#### Analysis of Fraction I.

The water extracts of the previous gum (fraction II) in each case were placed in the icechest for 10 days. Crystalline acids separated from the yellow mother liquor, and these were filtered off and combined, giving 1.5 grams of light yellow crystals. These were taken up in hot water, boiled with animal charcoal, and filtered and washed with a little hot water.

The colourless filtrate yielded, on cooling in the icechest for two days, 1.2 grams of crystalline acids which, on washing and drying, melted at 145°C.

It was suspected that these were once more a mixture of veratric and trimethylgallic acids, and accordingly a silver salt separation was effected exactly as has been described previously. From the insoluble silver salt fraction 0.15 grams of an acid was obtained. On twice recrystallising from water this melted at 178°C. Mixed with veratric acid it melted at 177°C.

The water soluble silver salt yielded 0.05 grams of a colourless crystalline acid which was recrystallised from water to give a melting point of 164°C. On mixing with trimethylgallic acid of melting point 167°C, it melted at 164°-165°C.

The oxidation of the reduced and remethylated methyl tannin yields, therefore, an acid which appears to be a veratric nucleus with an aliphatic side chain, and a mixture of veratric and trimethylgallic acids.

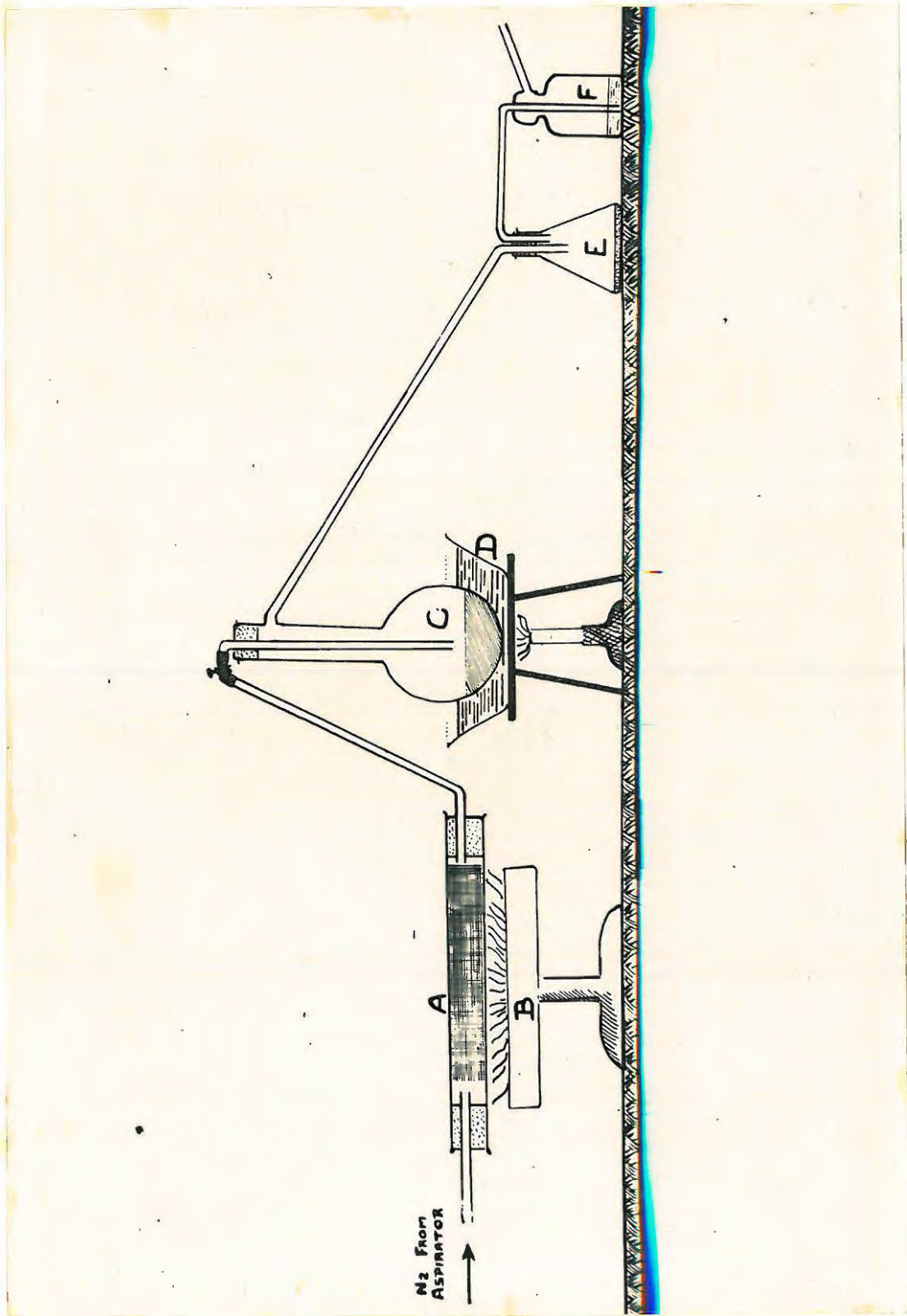
### ALKALI FUSIONS.

Having failed to isolate the resorcinol nucleus from oxidative degradations of the methylated tannin, alkali fusions, which Stephen (2) found to be effective on tannin itself, were attempted. These were carried out in an atmosphere of nitrogen to prevent any oxidation by the air taking place, and the apparatus employed is shown in the figure on page 61.

Nitrogen from an aspirator is passed over a copper spiral which has been reduced in methanol, and which is contained in a silica combustion tube A, to remove any traces of oxygen that might be present. B is a long burner to heat the spiral. C is a 100 ml. thick pyrex distilling flask, which is fitted with a 3' long side arm acting as an air condenser. E is a collecting vessel which is connected as shown to a bubbler F to control the rate of the passage of the nitrogen. Flask C is heated uniformly by means of a fusible metal bath D, containing Wood's metal.

### FUSION I.

5 grams methylated tannin was thoroughly mixed with 25 grams of a mixture of 60% KOH and 40% NaOH (M.Pt.  $168^{\circ}\text{C}$ ) which had been ground up thoroughly in a mortar. This was placed in the distilling flask and the apparatus was connected as shown in the diagram. Nitrogen was now passed to displace all the air from the flask, and then the passage of the nitrogen was adjusted to 2 bubbles per second. The fusible metal bath was now slowly heated, and at  $170^{\circ}$  - to  $180^{\circ}\text{C}$  the mixture started darkening at the edges, melting finally at  $240^{\circ}\text{C}$ . The temperature was taken to  $270^{\circ}$  -  $275^{\circ}\text{C}$  in 1 hour, and was



maintained there for the remainder of the fusion. At this temperature, water containing droplets of an oil distilled and was collected in E, while the melt bubbled and blackened. The distillation continued for  $1\frac{1}{2}$  hours, when the melt ceased frothing and no more distillate was obtained.

About 5 ml. of liquid distilled over, and the oil, which was slightly denser than water, was yellow in colour having the distinctive odour of resorcinol monomethyl ether. It was extracted with ether, and the ether dried over sodium sulphate to yield, on evaporation, 0.09 grams of a light yellow oil with the strong odour of resorcinol monomethyl ether.

By means of two further fusions performed as above enough oil was obtained for methoxyl and carbon and hydrogen analyses. The oil was first dried thoroughly over  $P_2O_5$  for 2 days.

0.0939 grams gave 0.1769 grams AgI whence  $\%OCH_3 = 24.89$

0.1106 grams gave 0.0651 grams  $H_2O$  and 0.2742 grams  $CO_2$   
whence  $\% H = 6.54$        $\% C = 67.60$

On the basis of the following comparison, this oil is shown to be resorcinol monomethyl ether.

	Oil	Resorcinol monomethyl ether.
Colour.	Yellow	Yellow
Density.	Slightly denser than water.	1.070
$\% OCH_3$	24.89	25.0
$\% C$	67.60	67.75
$\% H$	6.54	6.40

The odour of the two is identical.

Examination of the Melts.

The melts of the above fusions were dissolved in water, and the dark brown solution extracted with ether. Nothing was removed. The solution was now acidified with conc. HCl and ether extracted again to give a dark brown ethereal solution. This was extracted with sodium bicarbonate solution to separate the phenolic and acidic fractions, when CO<sub>2</sub> was liberated and most of the colouring matter was extracted by the bicarbonate.

The phenolic fraction was evaporated to yield 3.6 grams of a dark brown syrup. The bicarbonate solution was acidified and extracted with ether to yield, on evaporation, 3.5 grams of a dark brown almost black acidic syrup. Nothing crystallised from either of the above fractions.

To obtain a better separation, both of the above fractions were taken up in a 5% NaOH solution and then saturated with pure CO<sub>2</sub>. A sludge separated from the phenolic fraction, but nothing appeared to separate from the acid solution.

The phenolic fraction was extracted with ether, and the ether dried over anhydrous sodium sulphate yielding 1.5 grams of a dark brown gum on evaporation (Fraction I). The remainder of the solution was acidified and extracted with ether which was dried and evaporated as before to yield 1.5 grams of a similar gum (Fraction II).

CO<sub>2</sub> saturation failed to give a separation of the acidic fraction, so it was reacidified and extracted with ether, which was dried as above and evaporated to yield 2.5 grams of a brown acidic gum (Fraction III). None of the three fractions could be induced to crystallise.

The phenolic fractions were insoluble in benzene, which extracted only a trace of a gum. While soluble in alcohol, the fractions could not be crystallised from it. The acidic fraction was easily soluble in benzene and water, but would not crystallise from either.

After drying for 2 days in a vacuum over  $P_2O_5$  the following analytical results were obtained:

Fraction I.

0.1043 grams gave 0.0962 grams AgI whence %  $OCH_3$  = 12.2

0.1065 grams gave 0.0684 grams  $H_2O$  and 0.2658 grams  $CO_2$

whence % C = 68.0 % H = 7.14

With ferric chloride it gave a dark brown colour immediately. There was no observable reaction with bromine water.

Fraction II.

0.0996 grams gave 0.0542 grams AgI whence %  $OCH_3$  = 7.19

0.1162 grams gave 0.0614 grams  $H_2O$  and 0.2704 grams  $CO_2$

whence % C = 63.45 % H = 5.87

With ferric chloride it gave a dark brown colour immediately. There was no apparent reaction with bromine water.

Fraction III.

0.1039 grams gave 0.0589 grams AgI whence %  $OCH_3$  = 7.49

0.1020 grams gave 0.0518 grams  $H_2O$  and 0.2080 grams  $CO_2$

whence % C = 55.61 % H = 5.64

0.0512 grams neutralised 9.5 ml. 0.02566 N NaOH

whence equivalent weight = 210

From the low methoxyl values obtained it seemed certain that demethylation had taken place. Each of the above fractions

was therefore re-methylated in methanol with three portions of 3 ml. dimethyl sulphate and KOH. The products in each case, after evaporating the methanol, acidification and extraction with ether, were very similar to the original samples, refusing to crystallise. They were thoroughly dried and re-determinations were made.

Fraction I.

0.1034 grams gave 0.2451 grams AgI whence % OCH<sub>3</sub> = 31.32

0.1029 grams gave 0.0720 grams H<sub>2</sub>O and 0.2648 grams CO<sub>2</sub>  
whence % C = 70.2    % H = 7.77

Fraction II.

0.0957 grams gave 0.1477 grams AgI whence % OCH<sub>3</sub> = 20.39

0.1009 grams gave 0.0667 grams H<sub>2</sub>O and 0.2431 grams CO<sub>2</sub>  
whence % C = 65.7    % H = 7.34

Fraction III.

0.1033 grams gave 0.1974 grams AgI whence % OCH<sub>3</sub> = 25.25

0.1023 grams gave 0.0637 grams H<sub>2</sub>O and 0.2160 grams CO<sub>2</sub>  
whence % C = 57.59    % H = 6.92

0.0532 grams neutralised 8.40 ml. 0.02566 N NaOH  
whence equivalent weight = 246.6

0.0541 grams neutralised 8.50 ml. 0.02566 N NaOH  
whence equivalent weight = 248.3

No possible structures could be found to fit any of these figures. The increase in methoxyl contents shows, however, that demethylation has occurred.

ALKALI FUSION II.

6 grams methylated tannin was mixed with 30 grams of a mixture of 50% KOH, 40% NaOH, and 10% sodamide, and a fusion was carried out exactly as in the previous case, except that a drying tube of  $\text{CaCl}_2$  was inserted in the nitrogen train to avoid the possibility of an explosion of the sodamide. The fusion appeared to proceed in exactly the same way as reported previously, but on frothing up, the melt blocked the neck of the flask and, while the resorcinol monomethyl ether odour was very strong, nothing distilled.

After maintaining the temperature at  $270^\circ\text{C}$  for 1 hour the melt was allowed to cool, and alcohol was added to destroy any sodamide. It was then dissolved, and the solution was treated exactly as before to yield, once again, 3 fractions on separation of the phenolic fraction by the  $\text{CO}_2$  saturation of an NaOH solution.

Two phenolic fractions (Fraction IV and Fraction V), very similar to the phenolic fractions I and II, respectively, of the previous fusion, were isolated. The acidic fraction (Fraction VI) failed to give a separation on  $\text{CO}_2$  saturation of an NaOH solution. The yields were as follows.

Fraction IV	-	0.09 grams
Fraction V	-	1.0 "
Fraction VI	-	2.1 "

These fractions were remethylated three times as before with dimethyl sulphate and KOH, and on isolation they proved to be gums of the same nature as remethylated fractions I, II, and III, failing to crystallise from any solvents.

After drying these fractions thoroughly over  $P_2O_5$ , analyses were conducted.

Fraction IV.

0.1012 grams gave 0.1428 grams AgI whence %  $OCH_3$  = 18.63

0.1020 grams gave 0.0751 grams  $H_2O$  and 0.2584 grams  $CO_2$

whence % C = 69.08 % H = 8.19

Fraction V.

0.1009 grams gave 0.1191 grams AgI whence %  $OCH_3$  = 15.6

0.1031 grams gave 0.0711 grams  $H_2O$  and 0.2435 grams  $CO_2$

whence % C = 64.40 % H = 7.70

Fraction VI.

0.1059 grams gave 0.1973 grams AgI whence %  $OCH_3$  = 24.62

0.1027 grams gave 0.0621 grams  $H_2O$  and 0.2193 grams  $CO_2$

whence % C = 58.22 % H = 6.71

0.0531 grams neutralised 9.00 ml. 0.02566 N NaOH

whence equivalent weight = 230

Again no structure could be found to fit these figures.

Oxidation of Acidic Gum.

In an attempt to isolate a crystalline acid from acidic fraction VI, 0.7 grams was taken up in 20 ml. sodium bicarbonate solution and oxidised with  $KMnO_4$ . The flask containing the solution was clamped dipping into a boiling waterbath, and the oxidation was carried out at this temperature. 2 grams  $KMnO_4$  was used in 3 hours, after which the  $MnO_2$  was filtered and washed with hot water. The filtrate and washings were extracted with ether to remove any ether soluble non-acidic material. It was then acidified and reextracted with ether to give 0.015 grams of a crystalline, pale yellow acid.

This was boiled with charcoal in water, and on filtering and cooling a colourless acid crystallised. This was collected and recrystallised from water to give, on drying, a melting point of  $175.5^{\circ}\text{C}$ . A mixed melt with veratric acid of melting point  $179^{\circ}\text{C}$  showed no depression, melting at  $176^{\circ}\text{C}$ .

This was taken as sufficient evidence of veratric acid.

SUMMARY AND DISCUSSION.

The identity of veratric acid and trimethylgallic acid in the oxidation products of methylated tannin, from commercial extract and from fresh bark extract, was established, while yields of the mixed acids were increased to 30%. This would seem to support Stephen's (2) suggestion that this tannin is a mixture of two molecules.

Methylated tannin was also successfully nitrated, and it was shown that two nitro groups were introduced, although the position of these in the molecule could not be established. The isolation of veratric acid only from the oxidation of this product would seem to indicate that the nitration had occurred in the resorcinol nucleus, but the isolation of an acid which, from analysis, appears to conform to a dimethoxy nitrobenzoic acid, casts doubt on this.

Attempts at opening the pyran ring were by no means conclusive, although a reduced product was obtained. Evidently demethylation on a relatively large scale occurred, with the complete elimination of some methoxyl groups from the nucleus, and only partial demethylation of others. A general reduction in the methoxyl content was observed, with a rise in the carbon and hydrogen values. It is probable that a certain amount of hydrogenation occurred. Oxidation of this product yielded a mixture of trimethylgallic acid and veratric acid, and an acid which would seem to be a veratric nucleus with a 3 carbon aliphatic side chain.

The alkali fusion of methylated tannin yielded resorcinol monomethyl ether.



This fact, coupled with the isolation of an acid with an aliphatic side chain from both the alkali fusion and the oxidation of reduced methylated tannin, is strong evidence of the presence of a pyran ring in the tannin molecule.

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