STUDIES ON THE POLYPHENOLIC CONSTITUENTS OF AN INDIGENOUS VEGETABLE TANNING MATERIAL - ACACIA NILOTICA, VARIETY ADANSONII (BAGARUV7A) PODS

A THESIS PRESENTED FOR THE AWARD OF MASTER. OF SCIENCE DEGREE IN CHEMISTRY AT THE AHMADU BELLO UNIVERSITY, ZARIA

BY

R.O, ADEWOYE, B.S.(HONS.) CHEMISTRY
SCIENTIFIC OFFICER OF THE FEDERAL
LEATHER INSTITUTE, ZARIA

IN
OCTOBER, 1979

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

I am much indebted to my supervisor, Dr. J.B. Rao, whose expert advice and assistance were always freely given right from the beginning of the study. His close supervision, coupled with his invaluable suggestions, contributed in no small measure to the success of the work. I am indeed very appreciative of all his efforts.

I must express my profound gratitude also to the Head of Chemistry Department, and Deputy Vice-Chancellor, Professor A.R. Mathieson, for encouraging me to start the work in the first place and for providing me with virtually all the chemicals and pieces of apparatus used. And, as a co-supervisor, I will like to express my great indebtedness for his helpful pieces of advice and constructive suggestions. I am also very thankful for his stimulating discussions.

My grateful thanks are also due to the Project Manager of the Leather Institute, Mr. Georges Kamp, and the Director of the Federal Livestock Department, Kaduna, Dr. D.A.W. Walker, for approving of the programme, and permiting me to undertake the study virtually on full-time basis.

The assistance and helpful comments of a number of members of the academic staff, of the Chemistry Department, should not go unacknowledged. And to Mr. Smith and other members of the technical staff (Chemistry Department and Leather Institute), who have given maximum co-operation in supplying every item of apparatus and assisting in some other ways, I am most grateful.

The model compounds used to confirm the isolated compounds were supplied by the following people: ; Professor T.R. Seshadri, F.R.S., Head of the Department of Chemistry, Delhi University, Delhi, India; the Department of Organic Chemistry, Central Leather Research Institute, Madras 20 and J.B. Harbourne, Department of Botany, University of Reading, U.K., to them all I say, a very big thanks.

Finally, to my colleagues, the graduate students, I am also very thankful, for the interest they took in me and the work, and also for their hearty encouragements during many anxious moments.

F O R E W A R D

Leather industry has at its disposal a large number of organic tanning materials of which the natural tannins form the major part. The vegetable tanning process is of ancient origin in which the animal skin or hide is treated with solution of vegetable tannin extracts to give a solid compact leather. The tannins are polyphenolic substances giving colloidal solutions and having an average molecular weight in the range of 500 - 3,000.

They react with collagen through hydrogen bonding, although other mechanisms, such as dipole-dipole interaction are not ruled out. A study involving the chemistry of the most popular and potential indigenous vegetable tanning material has been taken up for a better understanding. The studies form the subject matter of the present thesis.

The thesis deals with the studies on the chemistry of vegetable tannins-polyphenolic constituents of bagaruwa pods.

C O N T E N T S

			Page
Chapter 1	-	I n t r o d u c t i o n	1
Chapter 2	-	Results and Discussions	35
Chapter 3	-	Practical Tanning Studies	51
Chapter 4	-	E x p e r i m e n t a l	54
REFERENCES			71

CHAPTER 1

STUDIES ON THE POLYPHENOLIC CONSTITUENTS OF AN INDIGENOUS VEGETABLE TANNING MATERIAL - BAGARUWA PODS - ACACIA NILOTICA VARIETY ADANSONII

INTRODUCTION

The word "Tannin", often referred to as "Tannins", relates to a particular group of substances of vegetable origin . The term "Tannin" was first introduced by Seguin[1] to indicate various plant extracts which have the capacity to convert hides and skins into leather: only substances having this property are termed "Tannins". This property was noticed by human beings as far back as 10,000 B.C.[2]

Early men used the tanned animal skins for clothing, foot wear, harness for animals, etc. Then they started equipping their armies with shields, and helmets made out of leather.[3] The twin Egyptologist, C. Schiaperelli found the garad pods, collected from jars at Gebels, to contain as much tannins as in fresh pods.[4] These pods are recorded to be the remains of an Egyptian tannery. Leather articles have also been identified from Egyptian tombs dating around 5000 B.C. One can also find the mention of tanneries in Athens by Homer, Pliny and other writers. The Greeks are said to have learned the use of Acacia pods from the Egyptians.[5] Oak bark was traditionally and extensively used by the British during olden days. This was, however, replaced gradually with imported Valonea, Myrobalan, Sumac, and later with Quebracho and Wattle. With the advent of world wars in the 20th century, there have been remarkable advances in the tanning industry, mainly in the development of tannin extracts,

and the modification of tannins to suit the desired needs.

The tannins are widely distributed in nature and occur in different parts of the plants, barks, wood, fruits, etc. A strict chemical definition of tannins has not yet been made. In general the name "tannins" implies: substances which contain numerous phenolic hydroxyl groups; are of limited solubility in water in their crystalline state, and tend to form super saturated solutions.[6] It is difficult to single out any phenolic substance and call it as the respective tannin of the concerned plant. In view of this complex nature it is generally safer to use the word "tannin extract" to include many substances which are responsible for the tanning character. The commercial tannin extracts are usually obtained by leaching the plant materials with water.

The tannins are astringent in taste and fully acidic in character; they give coloured solutions with metallic salts; they combine with albumin, gelatin and various alkaloids; they have a molecular weight in the range of 500-2500.[7,8] Though the leather industry is the main agency to utilise the tannins, their use, however, is not restricted to this industry alone. They are also used as ink ingredients, astringents, and mordants in the dye industry, in the manufacture of plastics, resins and adhesives, in the prevention of corrosion, and caustic cracking, in steam boilers; to condition the clay suspension used in oil well drilling; in the production of ceramics, and refractories, in the manufacture of

cement by the wet slurry process; in mineral floatation; as anti-virus and anti-fungal agents, and in the preservation of fish nets, etc.

Unfortunately, in Nigeria, where a number of potential sources of tannins grow in abundance, knowledge about their chemistry is sadly lacking. The leather industry which is the main agency to utilize the tannins in the country, faces many problems, one of which is the defective leather produced by many of the indigenous tanning materials. In view of the increasing importance of tannins, a fundamental knowledge about their chemistry has become necessary for understanding and rectifying the defects, and for better utilization of materials. Studies have been therefore undertaken to get a first hand knowledge of the chemistry of the commercially important indigenous tanning material - pods of Acacia nilotica variety adansonii, locally known as bagaruwa pods. This, in fact, is the only material being used by the rural as well as the mechanized tanneries for production of vegetable tanned crust leathers at the present moment.

The chemistry of tannins of this material forms the subject matter of the present thesis. On a preliminary examination, the tannins in this material have been found to be complex mixtures of both hydrolysable and condensed nature. Before proceeding further it would be worthwhile to briefly review our present knowledge about the chemistry of the vegetable tannins.

Tannins occur throughout the greater part of the vegetable

kingdom, and are more prevalent among the higher plants or angiosperms, especially in certain dicotyledenous families.[10] Important tannin bearing dicotyledenous families are: Leguminosae, (e.g. wattle, burma cutch) Anacardiaceae, (Quebracho), Rhizophoracea (mangroves), Myrtaceae (eucalyptus), Polygonaceae (conaigre) and Combrataceae (myrobalan). Monocotyledons are generally devoid of tannins. There are tannin bearing plants in both tropical and semi tropical climates. However, the commercially important materials like wattle, Quebracho, myrobalans, mangrove, etc. belong to the warmer countries, and there are twice as many species containing appreciable amounts of tannins within the tropics, as there are outside them.[7] In tanniferous plants, though tannins are distributed all over the plant, ranging from roots to leaves and fruits, they usually concetrate in certain portions of the plant. Examples would include: bark (wattle) fruits (myrobalans) pods (tara), leaves (sumac) beards and cups (valonea) and roots (canaigre).

Though it is suggested that tannins play several functions in plants, such as:

(a) protective agents (due to their astringency)

(b) toxic agents (being phenolic)

(c) resistance to frost in temperate zones, etc.,

the exact role played is not yet clearly understood.[7]

CLASSIFICATION OF TANNINS

Plants produce different types of phenolic substances, some of which may contribute to the form of tannins. In approaching a

study of the chemistry of the various tannins, it must be borne in mind, that one is not dealing with a single substance of unique character; not even a group of compounds very closely related. The tannins from different plant species may not all be the same. The procedures for the study of tannin chemistry might be said to have been laid by Emil Fischer[11] in the early years of this century. However, as Professor Haworth has aptly remarked, "The spirit of optimism produced by Fischer's work on Chinese gallotannins has since died, and this study has been considered as an untidy section of Organic Chemistry".

Earlier attempts to classify the vegetable tannins, based on their astringency, or such other properties, were unsuccessful.[7] However, Freudenberg's[6] classification of vegetable tannins into: (a) hydrolysable, and (b) condensed tannins, provides a convenient basis for a study of the chemistry of vegetable tannins. While the hydrolysable tannins undergo hydrolysis with mineral acids or enzymes, the condensed tannins, which are non-hydrolysable, would produce coloured solutions and/or precipitates known as "Phlobaphenes" with same.[7]

HYDROLYSOBLE TANNINS

These are based on esters of phenol carboxylic acids (gallic acid and/or ellagic acid), or related substances with a central carbohydrate core. Depending on the polyphenolic acids that are obtained as products of hydrolysis, these are again sub divided into: (a) gallotannins, and (b) ellagi tannins.

Gallotannins are those that give gallic acid and glucose on hydrolysis:

e.g. (a) Chinese gallotannins (galls produced by Aphis Cinensis on leaves of Rhus semialata)

(b) Turkish gallotannin eynips tinctoria (galls or twigs of Quercus infectoria)

(c) sumach gallotannin (leaves of Rhus Coriaria) and

(d) Dhawa gallotannin (leaves of Anogeissus latifolia), etc.

On the other hand ellagi tannins on hydrolysis, give ellagic acid and glucose; examples of ellagi tannins would include (a) myrobalan nuts, (terminalia chebula) (b) divi-divi pods (ceasalpinia coriarea) etc.

Studies have been made on the hydrolysable tannins paperchromatographically, using the solvent pair:[13] (a) 6% acetic acid in the first way, followed by (b) sec butanol-acetic acid-water (14:1:5 v/v) in the second way. The spots are studied under ultra violet, (galloyl and hexahydroxy diphenoyl esters absorb or fluoresce under the ultra violet light, both characteristics being enhanced by fuming with ammonia vapor), and are usually developed using the spray reagents (a) a mixture of ferric chloride-potassium ferricyanide (b) bis-diazotised benzidine,[14] (c) Gibbs reagent[15] and (d) potassium iodate solution.[13]

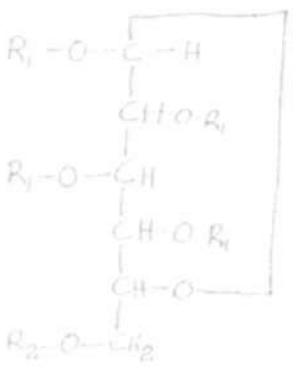
Gallotannins: Studies on the chemistry of gallotannins started with the observation of Macquer and Monnet[16] Scheele,[17] Kunsmuller,[18] Berthollet[19] and others who isolated from plant galls the materials

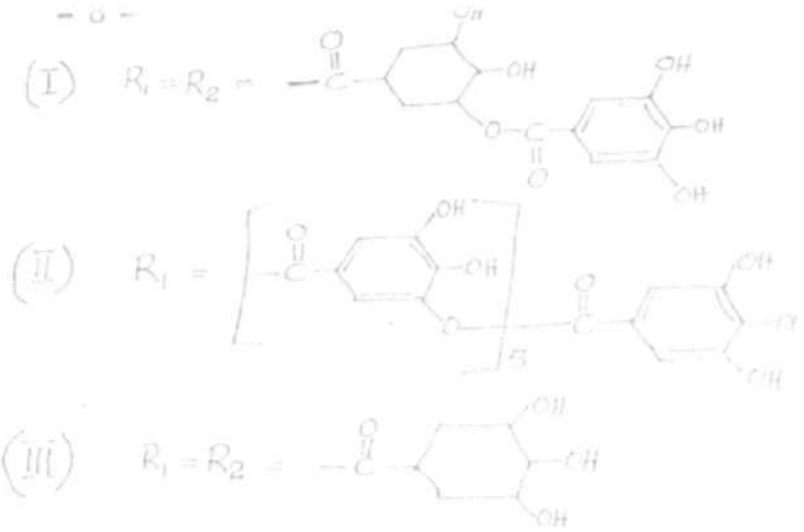
termed by Scheele "gallapfelsaure", by Proust,"tannin",[20] and by other workers, "gallotannin" and "tannic acid".

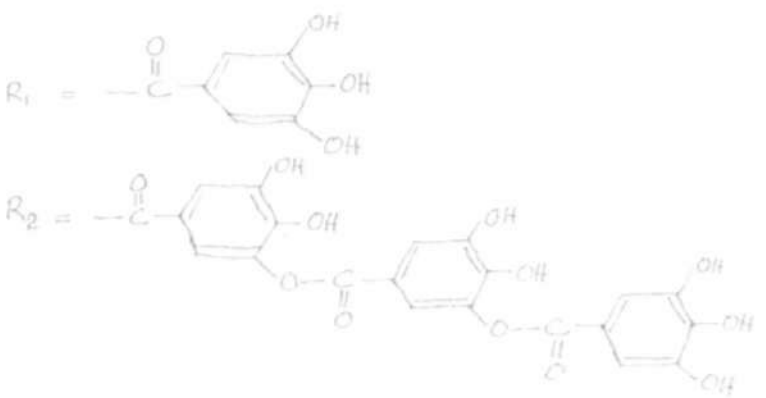
The subject matter of gallotannins formed the classical researches of Fischer,[21,22] Freudenberg,[23,24] and Karrer, et al.[25] Karrer, et al, suggested the presence of polygalloyl chains in the Chinese gallotannin, as they had found considerable difference in the optical rotation of the various fractions obtained by the gradual precipitation of the tannins with aluminum hydroxide. Based on his experimental evidence, Freudenberg[26] concluded that many arrangements, varying from the β-penta-o-m- di-galloyl glucose (1) structure to the extreme 1,2,3,4, tetra-o-galloyl 6-o-m hexagalloyl glucose (II) were possible.

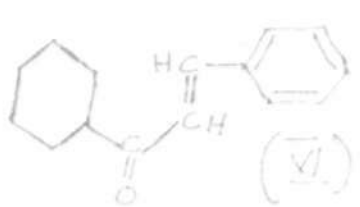
Similar studies carried on Turkish gallotannin led Fischer and Freudenberg[31] to suggest an average composition corresponding to β-penta-o-galloyl glucose, and a synthetic specimen of the latter prepared from tri ortho acetyl galloyl chloride and β-glucose, with subsequent hydrolysis, using sodium hydroxide, exhibited many variabilities with the natural tannins. However, with the identification of significant quantities of ellagic acid in Turkish gallotannin by Fischer and Freudenberg[27] and later by Karrer and his co workers,[28] the problem was again open for question.

As regards sumach gallotannin Lowe[29] suggested its identity with Chinese gallotannin, but Karrer, et al,[28] suggested a close resemblance with Turkish gallotannin.

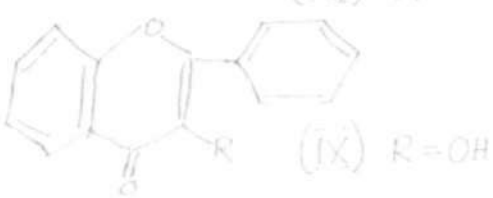












glucose is unesterified. As regards the constitution of the acidic tara gallotannin,[30,33] it was found to have the average composition of a penta-o-galloyl Quinic acid as shown in the structure IV.

In view of the conflicting ideas on the problem of gallotannins, and in view of the complexity of tannin extracts, earlier work was reinvestigated by Haworth, et al,[30,31,32,33] with the aid of recently developed techniques. These workers have not only established the carbohydrate core of the Chinese, Sicilian, and Staghorn sumach and Turkish tannins, but established their structures to a large extent.

By a study of the hydrolysis of the methylated tannins, and then analyzing the ratios of the 3,4-di-o-methyl and 3,4,5-tri-o-methyl gallic acids, liberated by hydrolysis, methanolysis of the gallotannins at neutral pH, and analyzing the products of methanolysis, both in the intermediate and final stages, and estimation of gallic acid and glucose in the tannins, together with the aid of N.M.R. and mass spectral data, these workers have established the structure of the gallotannins to a large extent, unravelling the earlier anamolous ideas. According to these workers, the structure of the tannins of Chinese, Sicilian and Staghorn sumach was based on a β-penta-o-galloyl glucose core (III) to which two gallic acid units are attached depsidically chain wise at 2-position of the glucose. The Turkish gallotannin was shown to be a hexa galloylated glucose based upon a tetra galloyl-glucose core in which the two hydroxyl groups of the glucose is unesterified.[30,32] As regards the constitution of the acidic tara gallotannin,[30,33] it was found to have the average composition of a penta-o-galloyl Quinic acid as shown in the structure IV.

Recently, Reddy, et al,[34] have isolated a gallotannin from the leaves of Dhawa (anogeissus latifolia) which is an indigenous tanning material of India. From analysis and other degradation studies this gallotannin was found to be an octa-or-nona galloylated glucose. All the hydroyl groups in the glucose are esterfied by gallic acid, having a depsidic[8] chain containing at least 3-4 galloyl groups. Other members of this group would include Hammameli and acer tannins.

ELLAGITANNINS

The ellagi tannins are different from the gallotannins in that they deposit, on standing, ellagic acid from their tannin infusions. The characteristic phenomenon of the formation of bloom on leathers is due to the deposition of ellagic acid on hydrolysis. These produce gallic acid in addition to ellagic acid.

Knowledge about ellagi tannins has been considerably advanced by the elaborate studies of Schmidt and his co workers[35] on myaobalan nuts and divi-divi pods. Chebulagic acid, chebulinic acid, corillagin, glucogallin and di-galloyl glucose were isolated and identified from the plants. Recently Schmidt[36] reported the isolation of terchebin and tri galloyl glucose from myrobalan nuts. The importance of the above compounds lies not only in that several of these compounds were isolated for the first time, but also in that it was possible to assign structures to most of them.

Schmidt and Bernauer[37] isolated from algarobilla (caesalpina brevefolia) extract, three crystalline products: brevilagin, brevifolin carboxylic acid and brevifolin. From Valonea (quercus valonea) extract Schmidt and Komarok[38] reported the isolation of a new dilactone which they name "Valonea-di-lactone". Mayer[39] obtained from chestnut leaves a dehydrodigallicacid. Jurd isolated "Juglanin", and ellagi tannin from walnut skin (Juglans regia)[40]

Minor polyphenols of hydrolysable tannin group

Mention may be made of the minor polyphenols in the vegetable tannin extracts, which are less important, so far as the tanning action is concerned; however, they are important from the biogenetic point of view. These may be present as such or they may have formed as artifacts during isolation. Examples would include β-D-glucogallin (first isolated from (Rheum Officianale)[41] 3,6-di-o-galloyl-D-Glucose, and B-1,3,6-Tri-O-galloyl-D-glucose (both isolated from Terminalia chebula)[42] derivatives of hydroxy cinnamic acids.[43]

Thus, our knowledge about hydrolysable tannins has been greatly advanced by the brilliant researches of Schmidt, Haworth and co workers.

Biogenesis of hydrolysable tannins

It may not be out of place at this stage to briefly indicate the biogenesis of the hydrolysable tannins. From the above review, it is clear that the hydrolysable tannins can be assumed to be derived from gallic acid, and or hexa hydronoxy diphenic acid,

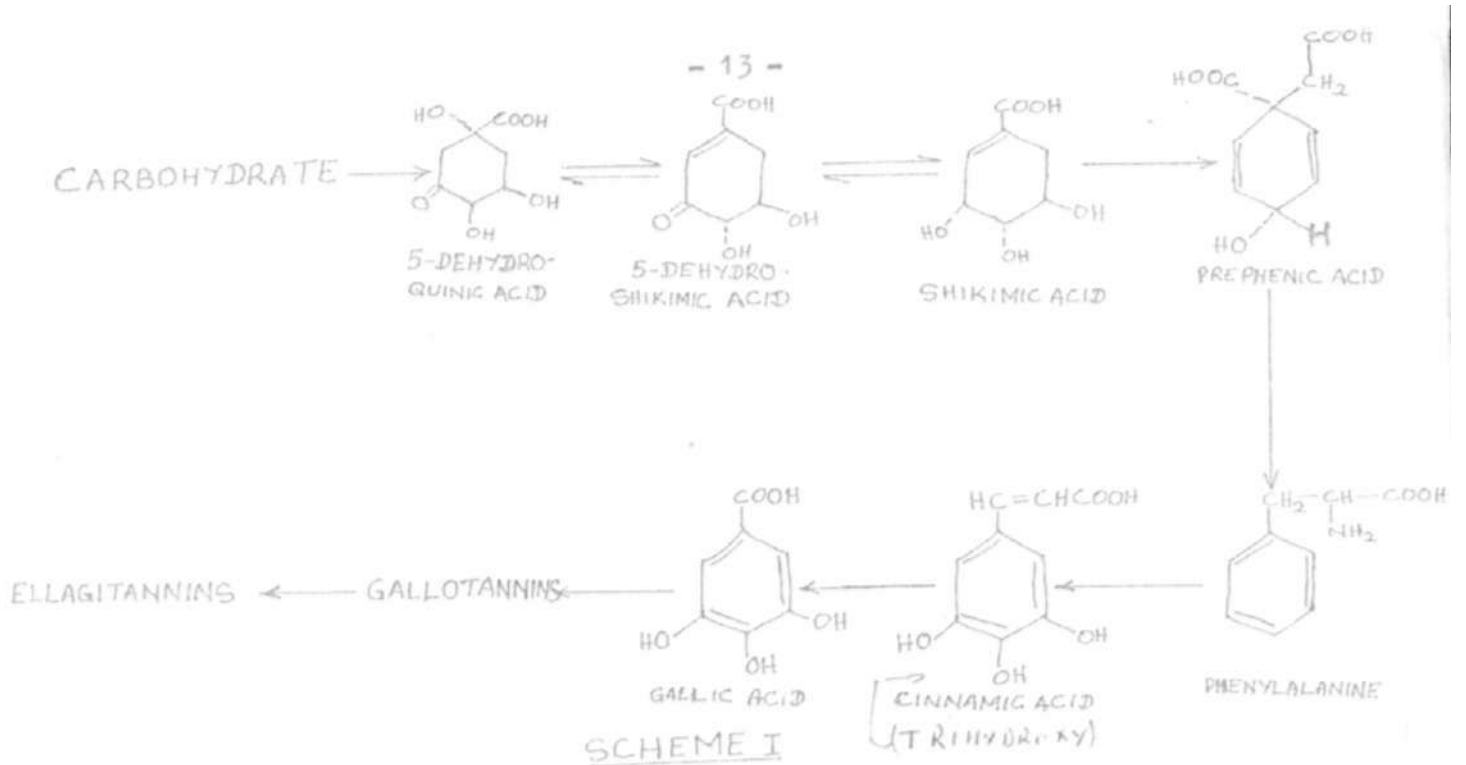
present as ester with a sugar molecule usually glucose and that a probable bio-synthetic route for the two acids is enough to understand the formation of these tannins. It is to be noted that hexa-hydroxy diphenic acid, being unstable, does not occur in the free state. It is isolated as dilactone, ellagic acid, which is more stable.

Using isotopic feeding experiments, several workers studied the bio synthesis of gallic acid in plants, and the work was reviewed by Haslam.[44] Haslam, Haworth and Knowles[45] proved that 5-dehydroshikimic acid was the immediate, and 5-dehydro quinic and shikimic acids were near precursors of both gallic and protocoatechuic acids. Haslam[44] later proposed that gallic acid could be formed from 5-dehydroshikimic acid by dehydrogenation.

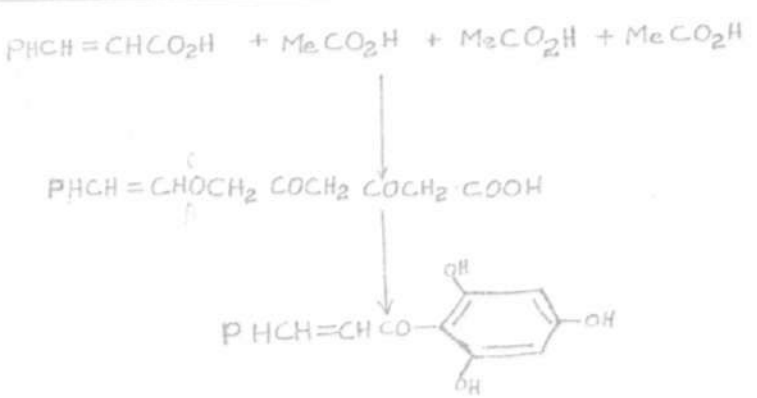
Recently, Zenk[46] concluded that gallic acid was derived from the metabolism of phenylalanine as shown in Scheme I. Thus it is seen that more than one route operates for the formation of a natural product, But the exact pathway is yet to be clearly understood.

CONDENSED TANNINS

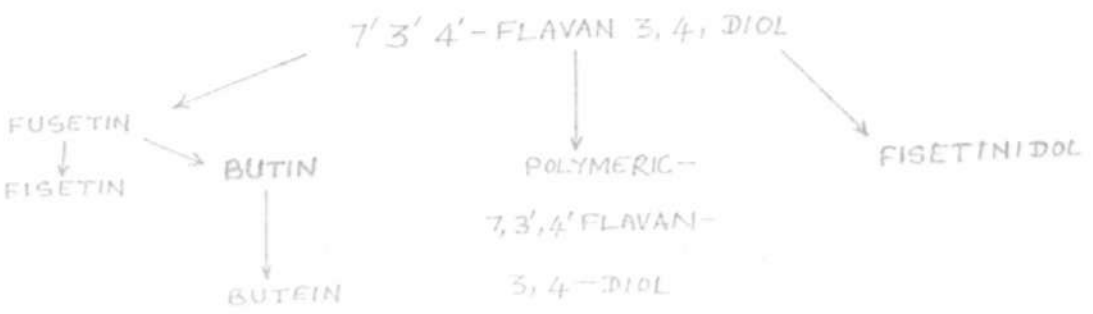
These are commercially more important than hydrolysable tannins. Tanning materials like wattle (Acacia mearnsii), quebracho (schionopsis lorentzii) gambier (uncaria gambier), cutch (Acacia catechu) Hemlock (Tsugu canadensis) spruce (picea abies)



SCHEME I



SCHEME II



SCHEME III

mangroves (rhizphorecea species) avaram (cassia auriculata), etc., belong to this group of tannins.

Structurally related to flavanoids, these tannins are widely distributed in nature and constitute a heterogeneous group, possessing the common characteristic of forming soluble "phlobaphenes" on treatment with hot, dilute mineral acid in aqueous medium.

The C-15 skeleton (V) of the flavanoid compound is made up of two distinct units: viz: A-ring (consisting of a C6 unit), and the B-ring (made up of C_6-C3 unit). Based on the configuration and the state of oxidation of the C3 unit in the molecule the flavanoids are broadly classified into six major groups, namely:

(a) Chalcones

(b) aurones

(c) flavones and flavonols

(d) flavanones and flavanonols

(e) flavanols

(f) isoflavones.

In addition, the glycosides of these flavonoids are also known.

CHALCONES

In this class two aromatic rings are linked by an aliphatic C3 unit, as represented by (VI). The C3 unit does not participate in forming a hetero ring as contrasted to other classes of flavonoids.

AURONES

The aurones (VII) are the most recently discovered group of flavanoids, and are, as their name implies, essentially golden yellow flower pigments.[47]

FLAVONES AND FLAVONOLS

Von Kostanecki and Tambor[48] were the first to suggest the name "flavone" to compounds having the structure (VIII). Flavonols are 3-hydroxy flavones (IX).

FLAVANONES AND FLAVONONOLS

Flavanones (X) differ from flavones in that the former are saturated at carbon atom 2 and 3. These are colorless, natural compounds having a single center of asymetry. The 3-hydroxy compounds are flavanonols (XI).

FLAVANOLS

These are the most important class of compounds from the point of view of vegetable tannins.[49] They possess the property of being transformed into amorphous polymeric tannins by the action of enzymes, mineral acids, or even by merely being in aqueous solution, or in the solid state.[50] There are mainly two such groups of compounds in this class, namely:

(1) flavon-3-ols (XII)

(2) flavon3,4-diols (XIII) with two asymetric centers at atoms 2 and 3.

flavonoids from the point of view of their being the precursors of condensed tannins[49] and as such their biosynthesis and vis-a-vis the biosynthesis of other flavanoids has been subject of greater importance.

Robinson[60] was the first to suggest that flavonoids, which may be considered to have been derived basically from a 1,3-diaryl propane could be formed in plants by the condensation of a C6 and C6-C3 units. Also it was stated[49] that the C-6 unit (ring A) is derived by the acetate pathway and ring B (C6-C3 unit) from the shikimic acid pathway. This hypothesis was further confirmed by the formation of Quercetin (in buck wheat, Fagopyrum tataricum and F. esculentum,[61,62,63] and Cyanidin [in red cabbage and crude wheat, F. esculatum] using isotopic tracer studies. Hence the more probable route for the biosynthesis of flavonoids would be starting from a cinnamic acid (or related compound), derived by the shikimic acid pathway) with the addition of three acetic acid units. The basic C6-C3-C6 compound on this view could be the chalcone although other oxidation levels of the C3 unit are not excluded, since evidence in other series show[66] that a variety of acids can add acetic acid units, (Scheme II). Based on the concomittant occurrence of different closely related flavonoids this similar oxygenation pattern in the A and B rings, many theories have been put forward favoring the sequential formation of flavonoids from a single precursor like chalcone.[67,68]

More recently, working on wattle tannins, Reux[69,70,71] isolated a number of related compounds like fisetinidol fustin, fisetin, from the heart wood of wattle. Based on this he proposed an entirely different idea of flavonoid biosynthesis. According to him, a flavan 3,4-diol is first formed, followed by its conversion to other flavonoids by a combination of reduction and dehydrogenation. (Scheme III).

Thus, there seems to be several,[49,66] opinions regarding the biosynthesis of flavonoids. However, it appears that to understand more clearly, more experimental evidence using enzymatic tracer, techniques, etc., is needed.

FORMATION OF FLAVONOID TANNINS

Bate Smith[72] identified the presence of leucocyanidins in a number of dicotyledenous plants by applying phlobaphene test and concluded that the red color produced was due to the generation of anthocyanidins from leucoanthocyani(di)ns present in these extracts. The presence of leuco anthocyanadins in eucalyptus, mangrove, wattle, etc. was noticed by Hillis.[73] Hillis[74] noticed that synthetic monomeric flavan 3,4-diols yielded anthocyanidins under a set of conditions as they were obtained from eucalyptus wood and bark extracts and he concluded that eucalyptus leuco anthocyanidins are flavan 3,4-diols and precursors of condensed tannins. However, Hillis made no attempt to distinguish between monomeric and polymeric leuco anthocyanidins. The isolation of a novel type of flavan 3,4-diol, melacacidin from the heart wood

of wattles led King and co-workers[75] to conclude that phlobatannins, were flavan 3,4-diols. Their statements were incorrect since they ignored the polymeric nature of condensed tannins, some of which do not yield anthocyanidins upon boiling with acid.

Roux and co-workers[76] on their studies on black wattle tannins have been able to differentiate between monomeric and polymeric leuco anthocyanidins. In the low molecular weight fractions, they isolated and characterized a number of compounds like robinetinidol, leucorobinetinidin and mollibacacidini, gallocatechin, catechin, etc. They also carried out the quantitative measurement of the generated robinetinidin from the high molecular weight fraction of wattle and concluded that the structure of polymeric condensed tannin present in these extracts are based on polymers of both fisetinidin and robinetinidin. From oak bark extractives, Hathway[77], Mayer and Bauni[78] reported the isolation of leuco delphinidin and a number of catechins. Hathway[79] suggested that gambier and acacia catechu tannins may be formed by aerobic oxidation of catechin precursors.

The significant role of flavan 3,4-diol in the production of condensed tannin is recognised to a greater extent by the isolation of a number of leucoanthocyanidins in a number of tannin bearing plants, like eucalyptus,[80] quebracho,[81] babul,[82] avaram,[83] etc.

Thus, a review of all work reported up to date definitely proves that a large majority of condensed tannins are essentially

derived through the polymerisation of flavan 3-4-diols and flavan 3-ols. Freudenberg[84] pointed out that it would be more appropriate to name condensed tanninsas "flavanoid tannins" since they are mainly derived from flavanoid compounds.

Structure of condensed (flavonoid) tannins: From the above review though it is clear that condensed tannins are primarily derived through the polymerization of flavan 3-ols and flavan 3,4-diols, the structure of these polymers (i.e. the mode of linkage between the flavanoid units) is yet to be understood fully. There are three theories viz: (a) condensation by acid as suggested by the Heidelberg school; (b) enzymic condensation through quinone polymerization mechanism as proposed by Hathway;[85] (c) unspecified mechanism to give polymers with ether linkage which has been advanced in the work of King, Roux, Hergert and others.

6) TANNIN FORMATION BY ACID CONDENSATION

Freudenberg,[86] Meyer[87] and Schmidt[88] maintained that the catechin tannins are formed in nature, just as in the laboratory, by the post mortem action of acid on catechins. They proposed two mechanisms for the polymerization process. In one of these, the basic structure may react as a pair of tautomeric diphenyl propanes to form a polymer. In the second mechanism the molecule reacts bifunctionally in the presence of hydrochloric acid and condenses to form a dimer capable of further polymerization.

In the case of flavan 3,4-diols, it was found that the rate of polymerization is greater than the catechins. The greater reactivity of the 3,4-diols in the condensation reaction lead to the supposition that the 4-hydroxyl group of the leuco anthocyanadins participitates directly in the reaction. It is the hydroxyl group of a p-hydroxybenzyl alcohol and as such is extremely reactive. Obvious conjecture is that this hydroxyl group would react readily with the loss of water between the 4-position of one molecule and the 6 and 8 positions of a second. Such a probable condensation product for leucoanthocyanidin may be represented as shown in (XV) In criticism of this hypothesis, it has been pointed out[89] that the acid catalysed reaction suggested by Freudenberg requires a low pH (< 2) and high temperature ($> 50^{o}C.$). Both the in vivo and extract pH are rarely lower than 4, and elevated temperatures are not encountered in vivo.

However, the earlier findings of Freudenberg were further confirmed by Weinges[90] who suggests that the majority of flavanoid tannins that occur in tanning woods very probably originate by acid catalysed intermolecular condensation of catechins and leuco anthocyanadins without the participation of enzymes. The mechanism for the formation of flavanoid tannins consists of a primary polymerization step and a secondary condensation with the elimination of water as represented by (XVI). It cannot be decided whether the dehydration step occurs after the addition of each new molecule of catechin to the growing tannin. For this

reason the constitution of flavanoid tannins is represented by a combination of the formulae shown in (XVII).

(b) ENZYMIC CONDENSATION

Hathway and co-workers studied[85,91] the autooxidative and enzymic polymerisation of flavan-3-ols and 3,4-diols, and as a result suggested a quinone polymerization mechanism as an alternative to Freudenberg's hypothesis. They proposed the following structure (XVIII) to the catechin polymer. The presence of c-c linkage in the 2-position was subsequently confirmed[92] by the isolation of small amounts of m-hemiphinic acid by the oxidation experiments on the methylated product of the polymers.

There are certain valid criticisms about this hypothesis. Quinones show a strong carbonyl stretching band at 1650-1685 cm-1 in their infrared spectra, a band that is lacking in the infrared spectra of naturally occurring condensed tannins tested to date. Nor are the ultra violet spectra of most condensed tannins typical of quinones.[93]

(c) UNSPECIFIED MECHANISM

In the third general structural theory, the linking of flavanols or other phenols through ether linkages has been proposed. Kirby and White,[94] examined the untra violet spectra of acetate derivatives of quebracho tannin fractions and concluded that

approximately one half of the oxygen atoms were occupied in the ether linkages. In his recent work Roux[95] has suggested that the mode of condensation is such that the flavans-3,4-diol structure remain intact. Ether links between flavanoid units through the 4-position of leuco anthocyanidins to the 6 or 8 position of some other or similar flavanoid nucleii were considered to be a likely mode of linkage. It is reasonable to assume that more experimental evidence will have to be brought forward before any mechanism can be accepted as generally applicable to biosynthesis of condensed tannins.

Stilbenes

Stilbenes belong to the group of compounds which exhibit an intense blue florescence under ultra violet light. The hydroxy stilbenes of plants are gaining importance because of their fungicidal and fungistatic properties.[96] Their effect on pulping under acid conditions,[97] cause colouration in pulps, etc.[98]

Grasman and others[99] have observed that the tannins of spruce bark (picea abies) are made up of the polymerization products of piceatonol (3.4.3', 5' tetra hydroxy stilbenes) which occurs in the free state or as glycoside. Later hydrogenized stilbenes have been isolated from the tannin bearing plants like eucalyptus wandoo,[100] and cassia marginata.[101] The close metabolic relationship of hydrogenized stilbenes with the condensed tannins was first suggested by Erdtman[102]

and corroborated by later workers.[103] Further it was indicated that in both groups one aromatic ring is derived via the acetate pathway, and the other from the shikimic acid.[104]

Present Work

It is seen, from the above survey that detailed studies on the nature of polyphenolic constituents present in various tanning materials of commercial importance are available in various parts of the world. This knowledge, apart from giving insight into the formation of tannins and the tanniferous bearing plants could be useful from another angle also, viz: with regard to tannage mechanism. For it would be then possible to picture the chemical entities taking part in the interactions involved in vegetable tanning and to have a better understanding of the process of vegetable tannage; also the various post tanning changes occuring on and in leather can be properly understood.

Nigeria is rich in flora and since tannins occur in many plants, it would not be surprising if the country has resources untapped yet. The only tanning material being used by the industry at present is the pods of acacia nilotica variety adansonii, popularly known as bagaruwa pods . A recent survey of the various indigenous tanning materials available in the various parts of the country has, however, revealed that apart from bagaruwa pods which is the most potential and popularly used tanning material, other species containing sufficient tannins

(though comparatively less tannins) are available and could be exploited for use in the leather industry. No chemical studies, even on bagaruwa pods, the most potential tanning material, have been made so far. A complete knowledge of the various polyphenolic constituents present in this material is not available. This knowledge, as earlier pointed out, could be very useful for a better understanding of the properties of this material, and therefore a better utilisation of the material. Therefore, in the present work studies have been carried out on this indigenous tanning material - bagaruwa pods. A systematic approach to the study of tannin extracts could involve the following steps:

(a) Study of the occurence and distribution of tannins in the various parts of the pod.

(b) separation of the tannin extracts into various fractions by extraction with suitable solvents.

(c) isolation and identification of polyphenolic components in the various fractions, and

(d) isolation and identification of intermediate and degradation products of the tannin compound.

(e) tanning chracteristics of the various fractions extracted with solvents in comparision with aqueous extracts.

Acacia nilotica variety adansonii

Except for tan, non-tan content of this material[105] there is no other systematic work carried out with the same. A complete

examination of the material for tannin, non-tannin, acid/salts, sugar, etc., has been initially carried out.

Extracts of the pods have been prepared and analysed for their tannin contents, as well as their leather making properties. The extracts have been analysed for the different components present in them. Gallic acid, protocatechuic acid and leuco cyanidin and glucose have been isolated and/or identified from the pods.

The presence of both gallic acid and leucocyanidin in the pods is extremely interesting. That the components characteristic of both the hydrolysable and condensed are present in the pods indicating that the good leather making characteristics could be explained by the presence of both these in the bagaruwa pods. It also throws light on the probable biogenetic path of the formation of the tannins as well as the structure of the tannin present in the pods.

BOTANICAL FEATURES

(a) Classification: The biological classification[106] of the tree under study is as follows:

Division:	Spermatophyta
Sub-division:	Angiosperm
Class:	Dicotyledon
Family:	Leguminosae
Sub-family:	Mimosoideae
Genus:	Acacia
Species:	nilotica
Variety:	Adansonii

(b) Local names: In Nigeria, acacia nilotica (adansonii) has been given different names by different tribes. The following are a few of them:

Hausa:	Bagaruwa (or Gabaruwa)
Yoruba:	Bonii
Kanuri:	Kangar
Fulani	Gabdi
Arabic:	Sunt-garad.

(c) Historical:[107] Acacia nilotica is said to have furnished a valuable wood exported from ancient Egypt and used for house-beams, furniture, panelling, statues, implement handles, etc. It was regarded as impermeable and incorruptible, and was specially used in the construction of boats from times of remote antiquity.

This tree was also the original source of gum-arabic before the latter's detection in acacia senegal. The leaves, boiled, with addition of a small piece of tamarind pod, are used as a cure for a disease of the eye which causes the lashes to fall out.

The sap of the leaves and especially of the young fruits, being rich in tannin, yield an astringent extract which was long ago exported from Egypt under the name acacia vera, and had much the same reputation as Indian cutch.

Silvicultural Features of Acacia nilotica - variety adansonii:

Silviculture[108] is the art of applying the knowledge of the

requirements of different trees, in tending and regenerating existing woods or in rearing fresh wood-land crops. In this study it is the requirements, climatic or otherwise, needed for a healthy acacia nilotica (adansonii) that will be considered.

(d) Habitat:[109] This tree, like other acacias, is found in abundance in the Sudan Savannah and Northern Guinea Savannah Zones. In these zones the average annual rainfall falls between 25 and 30 inches. Bauchi area from where the pods used for this study were collected falls inside Northern Guinea Savannah, near the border with the Sudan Savannah zone. In this area, as in other areas in the two zones involved, the variation in temperature is not excessively great thoughout the year. The temperature vary from as much as a maximum of 95°C. (March-April) to a minimum of 48°F. (during the month of January).

The soils[110] in the area under study have low content of organic matter because the latter is continually destroyed during the annual bush-fire. It is in this area that we have great accumulation of salts in the upper layers of the soil leading to alkaline reaction because the rate of evapo-transpiration always exceed the amount of rainfall. The tree does well without much phosphorus or nitrogen since the soils in the savannah generally are deficient in these minerals.

The tree abounds[109] very well in areas clothed with loamy or sandy soils. The seed germinates freely in low lying country

liable to submergence by rains or floods and the tree flourishes in such situations.

The tree is very frequent around towns where it is utilised for the tanning properties of its pods. It varies largely in size, reaching to a height of about 50 feet in some cases. The average height, however, is about 30 feet. It could be up to 8 feet in girth with crown which may vary from umbrella shape to high rounded. It may be found occuring gregariously in clumps or small forests, growing densely, with the crowns meeting overhead. It flowers during the period of November to March and fruits during December to June. Thus it can be seen that flowering and fruiting spread over periods of time which overlap each other.

(e) Morphological features: With its fairly black stem and branches, bluish green foliage, and graceful, symetrical form, it is readily distinguished from other Acacia species.[107]

The BARK is dark, almost black, with long stringy ridges and narrow longitudinal fissures. The branchlets are dark reddish brown, covered with very short soft hairs. A clear yellow gum exudes from the slash which has a pale pink colour. The slash is red brown and blackish in streaks.

The THORNS are in pairs on the branchlets and twigs. They can be up to 3 inches long and projecting at a very wide angle to each other on the older wood. They are slender, tapering to a sharp point, quite short, greyish, at narrow angle and sometimes

even curved on the brachlets.

The WOOD is ■ deep red brown with almost purple bands. The sapwood is yellow. In transverse section, the colour is darkest, the rings show as dark, ill-defined bands, the pores are small, few and scattered about between the fine straight rays which are visible against the dark ground of the hard tissue. There are many double and nested pores and zones with very few pores. The contents nearly close them. In vertical section, the grain is twisted, there are dark bounds and the pores have black contents. The wood is very hard to saw and plane. The grain picks up badly in places.

The LEAVES are often densely but finely hairy; with a common stalk 1.5 to 3 inches long; 3 to 6 pairs of pinnae up to 3 inches long; usually 15 to 20 pairs of leaflets up to 0.2 inch long; narrow, with parallel margins, rounded at the apex and with a central midrib, closely crowded. They are dark bluish green with grey bloom and soft texture. Figure i

The FLOWERS are bright yellow, in axillary heads, about 0.5 inch across on the stalks, about one inch long covered with very short hairs and with a whorl of minute bracts half way up.

The FRUITS are flat, straight or sickle-shaped, 4 to 6 inches long by about 0.75 inch broad with a thick wavy margin. Fig.ii They are sometimes fairly deeply, but usually irregularly, constricted between the seeds and are densely covered with velvety,

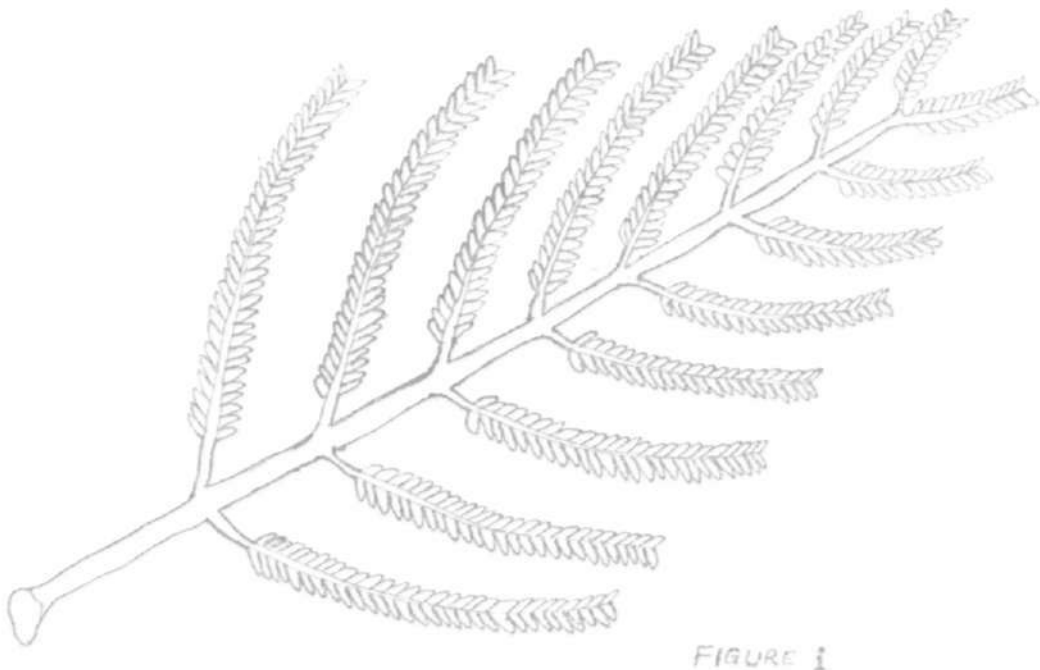


FIGURE i

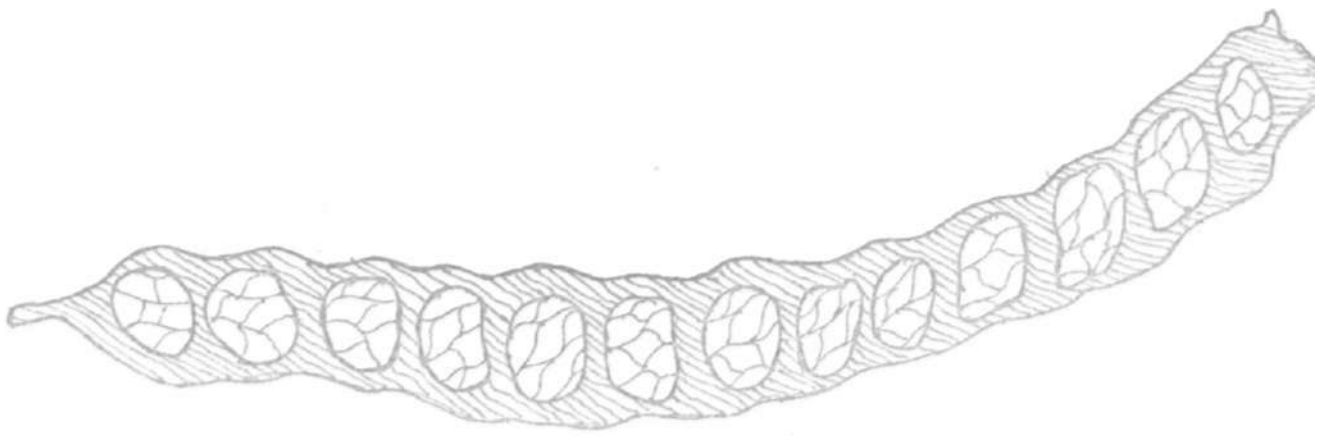


FIGURE ii

very short whitish hairs; singly or in small clusters arising from the remains of the flower-head. Each pod is with a stalk of about half-inch long; containing up to 12 smooth flattened, shiny brown seeds about 0.75 inch across.

CHAPTER 2

RESULTS AND DISCUSSIONS

In the present work, studies have been carried out with a view to understand the nature of components present in the pods.

Sample of the pods has been collected from Bauchi (North Eastern State of Nigeria) and the whole pods were analysed for their tannins, non-tans,[111] moisture, insolubles, acids, salts, weak acids and salts of weak acids. The pods, composed of grains, husk, and seeds, were then crushed and separated into the three portions.

The grains present in between the inner and outer pod coats constitute (50.0%) tannins, while the husk (42.0%), and the seeds (25.0%). The three portions have also been analysed for their tans, non-tans, etc. contents along with the whole pods.

The pods have been crushed and extracted with acetone-water mixture. The residue extracted subsequently with water and the aqueous extract was repeatedly extracted with ethyl acetate. The ethyl acetate extract was fractionated into phenolic and carboxylic fractions. All the solvents were evaporated at low temperature (in vacuo) in an inert atmosphere to minimise secondary oxidative changes to a large extent. All the extracts were examined by two dimensional chromatography in the solvent systems (a) 6% acetic acid in the first way followed by (b) Sec-butanol-acetic acid-water (14:1:5) in the second way.

The phenolic nature of the components was ascertained by spraying with (a) ferric chloride and (b) a mixture of ferric chloride-potassium ferricyanide; flavanoid nature of the polyphenolics was confirmed by spraying with (c) vanilin-ethanolic hydrochloric acid, and (d) 1% ethanolic hydrochloric acid. Preliminary colour reactions and paper chromatographic examination of the extracts indicated that bagaruwa tannins belong to the mixed (hydrolysable and condensed) group of tannins. This was confirmed by the formaldehyde-hydrochloric acid reaction, though it was not possible to determine the exact proportion of each type of tannins.

Chemical analysis of the bagaruwa pods (whole) (grains, husk, and seeds).

The whole pods have been analysed chemically for tan, non-tan, insolubles and moisture contents, following the International Society of Leather Trades' Chemists (S.L.T.C.) methods.[112]

The total acid and salt contents have been determined according to the method of Okell[113] and the amount of weak acids and their salts according to the method of Atkin and Burton.[114]

The results are given in Table I

Table I

Analysis of Bagaruwa Pods and its various Parts

Test-Material	% Tans	% Non-Tans	Moisture	% Insolubles*	Colour	PH	Total Acids **	Total Salts **	Weak Acids **	Salts of Weak Acids **
Whole Pods	29.8	18.0	12.0	50.2	Y=17.0 R= 5.0	4.5	44.0	102.0	35.0	55.0
Grains	50.0	22.6	10.8	16.6	Y=10.0 R= 2.0	4.3	40.0	110.0	38.0	60.0
Husk	18.0	17.5	12.5	52.0	Y=18.0 R= 6.0	4.5	-	-	-	-
Seeds	2.0	6.5	13.5	78.0	Y=10.0 R= 1.0	4.5	-	-	-	-

From the results, it can be seen that the tannin content of the whole pods is fairly high (30.0%) and compares favourably with other commercially important tanning materials, such as mimosa (Acacia mearnsii) which has 30 - 35% tannins, and myrobalans (Terminalia chebula) which also has 30 - 35% tannins.

It is very interesting to note that the grains present to the extent of 33% on weight of the whole pods contain a much higher percentage of tannins 50.0%, as high as some of the commercial tannin extracts. It is a unique phenomenon that a highly concentrated tannin is produced by nature and it would be interesting to study the biosynthesis of the formation of the same. This could be a subject for further investigation on this material. However, since

* mg/100 gm Total Solubles

** results expressed as mg-equivalents/100 gm Total Solubles.

the whole pods are normally used for tanning in industry, all the studies made on the polyphenolic constituents of this material have been on the extracts of the whole pods.

Another interesting point that has been observed in the chemical analysis of the pods is the quantities and nature of acids and salts present in the material. Most of the acids and salts present are in the form of weak acids and salts of weak acids and their presence in a tanning material will have a protective action against oxidative changes.[115]

The presence of salts of weak acids in tanning materials impart greater resistance to darkening of the colours of the leathers tanned with such materials, and such materials are ideally suited for production of leathers for export where they are prone to darken during transportation to overseas countries. Leathers tanned with bagaruwa are more resistant to oxidative changes due to the presence of these salts and therefore is an excellent material for tanning vegetable leathers for export. Qualitative analysis showed it to be composed of mixed tannins (hydrolysable and condensed).

A brief reference may at this stage be made to other indigenous tanning materials available in Nigeria besides bagaruwa not presently being utilised by the industry which may at a later date be exploited for use in the tanning industry. A few of such materials have been collected and analysed for their tannins and non-tan contents, and the results are given in Table II.

Table II

Comparison of its properties with those of other commercially important tanning materials

Tanning Material	Average % Tans	Average % Non-Tans	Insolubles Average %	Moisture Average %	Colour	PH
Acacia nilotica adansonii- Pods	30.0	14.6	46.8	8.6	R= 2.3 Y= 7.8 B= 0.6	4.26
Acacial nilotica adansonii- Bark	12.8	6.7	73.8	6.7	R= 2.0 Y= 8.0 B= 0.00	4.3
Annogeiosus Schimperii - Leaves	19.8	13.0	58.0	9.2	R= 3.1 Y=14.4 B= 0.6	4.58
Annogeiosus Schimperii - Bark	11.6	6.0	70.2	12.2	R= 3.0 Y=10.4 B= 0.00	4.45
Acacia sieberiana Hausa: Fara Kaya -, Bark mainly condensed	20.7	6.0	64.6	8.7	R= 2.3 Y= 8.1 B= 0.00	5.65
Prosopis Africana; Hausa:Kiriya - Bark	19.1	5.3	66.9	8.7	R= 3.5 Y= 4.0 B= -	4.05
Pterocarpus erinaceus (African rosewood) Hausa: Madobiya - Bark	18.3	30.5	59.5	11.7	R=14.0 Y=19.0	5.25
Lanuncularia racemosa (white mangrove)-Bark	13.7	9.6	61.6	15.1	-	-
Rhizaphora harrisonii-(Bark)	20.3	10.0	56.7	13.0	-	-
Acacia nilotica var.nilotica-Pods Hausa: Bagaruwa Namiji or Marje	27.2	17.4	41.4	14.0	-	-

It can be seen from the table that apart from bagaruwa pods, there are other indigenous tanning materials with sufficient amount of tannins and could be commercially exploited at a later date. The pods of Acacia nilotica variety nilotica contain a high percentage of tannin comparable with bagaruwa pods but the pods are not available in sufficient quantities for commercial exploitation in view of the fact that this species is not commonly grown in Nigeria. So, as pointed out earlier, bagaruwa is the most important commercial tanning material in the country and studies have therefore been carried out on the same.

The pods were extracted with acetone-water mixture. The residue was taken up with water and the aqueous extract was filtered to free it from chlorophyll and other gummy matter, and concentrated. The concentrated extract, free from chlorophyll and gummy matters was subsequently extracted with ethyl acetate and evaporated to dryness. The solid obtained was subjected to two dimensional paper chromatography. The chromatogram (Figure I) indicated the presence of six well defined spots together with a streak of polymeric tannins.

Three of the spots were identified as gallic acid, protocatechuic acid and leucocyanidin - from the Rf values and colour of the spots when sprayed with different reagents.

Since tannins are not the sole constituents of the vegetable tanning liquors some of the phenolic non-tans may be among the unidentified green spots (when sprayed with ferric cholide).

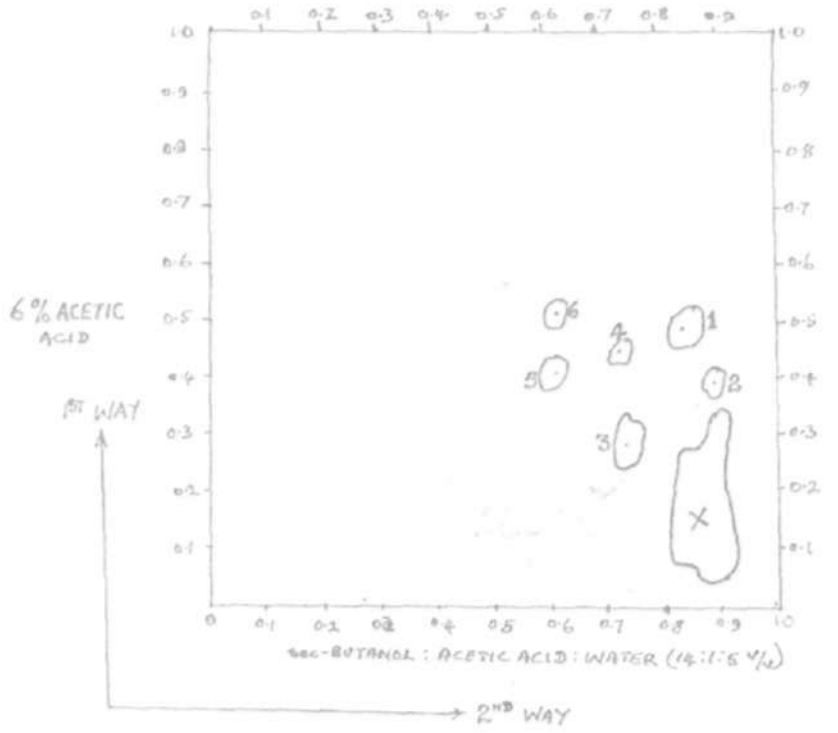


FIGURE I

1. PROTOCATECHUIC ACID.
2. UNKNOWN.
3. UNKNOWN.
4. GALLIC ACID.
5. LEUCOCYANIDIN.
6. UNKNOWN.
X. POLYMERIC TANNINS.

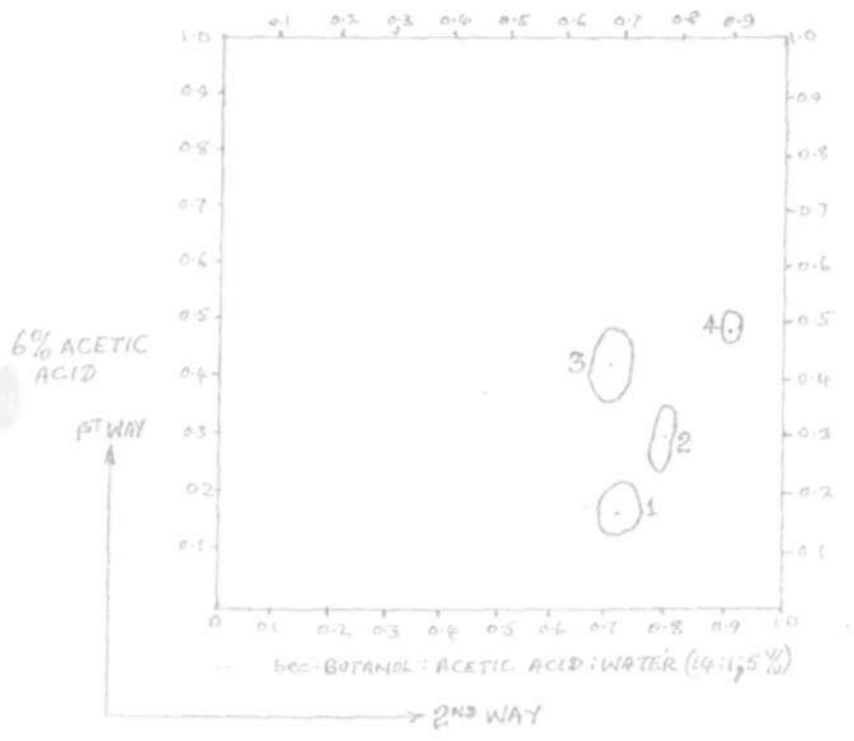


FIGURE II

1. UNKNOWN
2. UNKNOWN
3. GALLIC ACID.
4. PROTOCATECHUIC ACID.

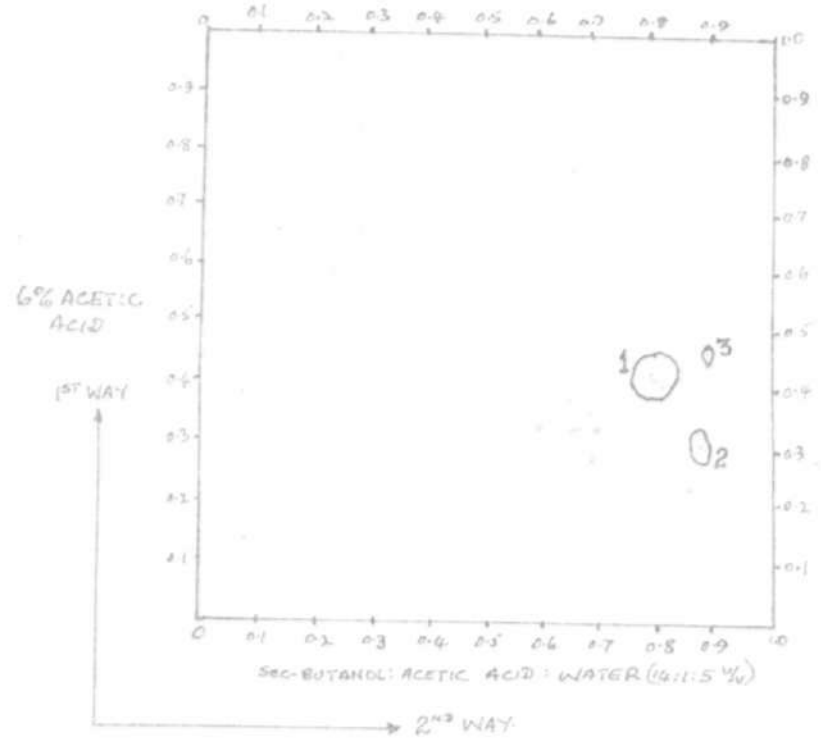


FIGURE III

1. GALLIC ACID.
2. PROTOCATECHUIC ACID.
3. PYROGALLOL.

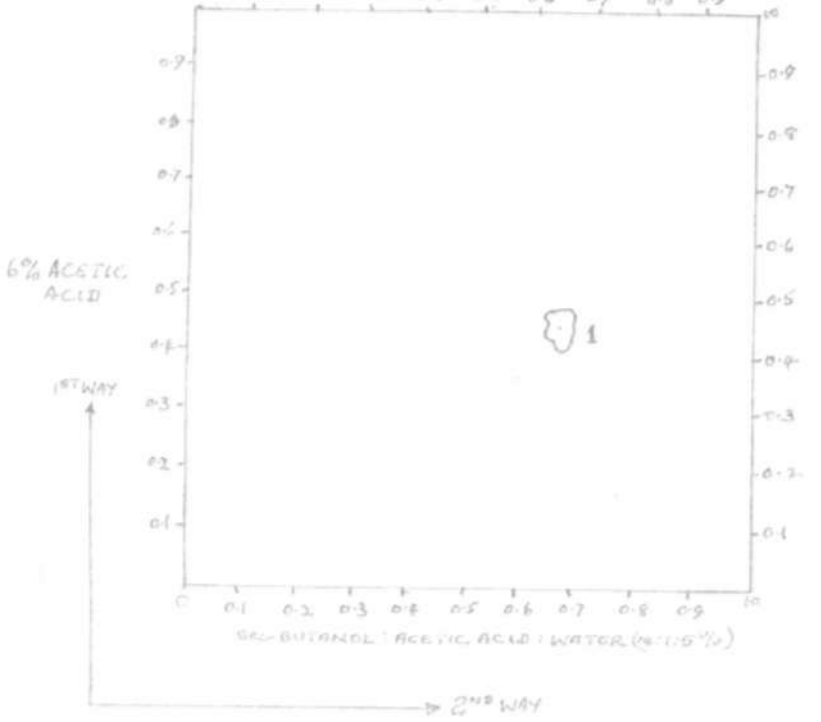


FIGURE IV

1 PROTOCATECHUIC ACID.

The test of the fraction with gelatin salt reagent gave a brownish precipitate indicating the presence of tannins, but it was not as copious as was obtained for the phenolic fraction under similar conditon of test, indicating that the phenolic fraction contains higher proportion of tannins.

The test with ferric chloride gave a greenish yellow solution, with a tinge of violet. This, being a characteristic test for phenols, show that most of the acids present in the fraction may be phenolic. Gallic and protocatechuic acids are, in actual fact, aromatic polyhydroxy acids.

Further separation of gallic acid and protocatechuic acid from the ether extract was effected taking the advantage of the limited solubility of gallic acid in icecold and cold water as compared to protocatechuic acid. The ether extract was washed in ice cold water followed by cold water. The ice water soluble portion was concentrated and subjected to two dimensional chromatography, Figure IV.

The chromatogram on spraying with dilute ferric chloride gave one distinct spot, green[116] and feeble black spot. Colour reactions and Rf values indicated them to correspond to protocatechuic acid (green spot) and gallic acid (black spot).

Attempts made to crystallize protocatechuic acid from the aqueous solution have failed as it is perhaps present in small quantities. However, the confirmation of protocatechuic acid was made by co-chromatography as detailed in the experimental procedures.

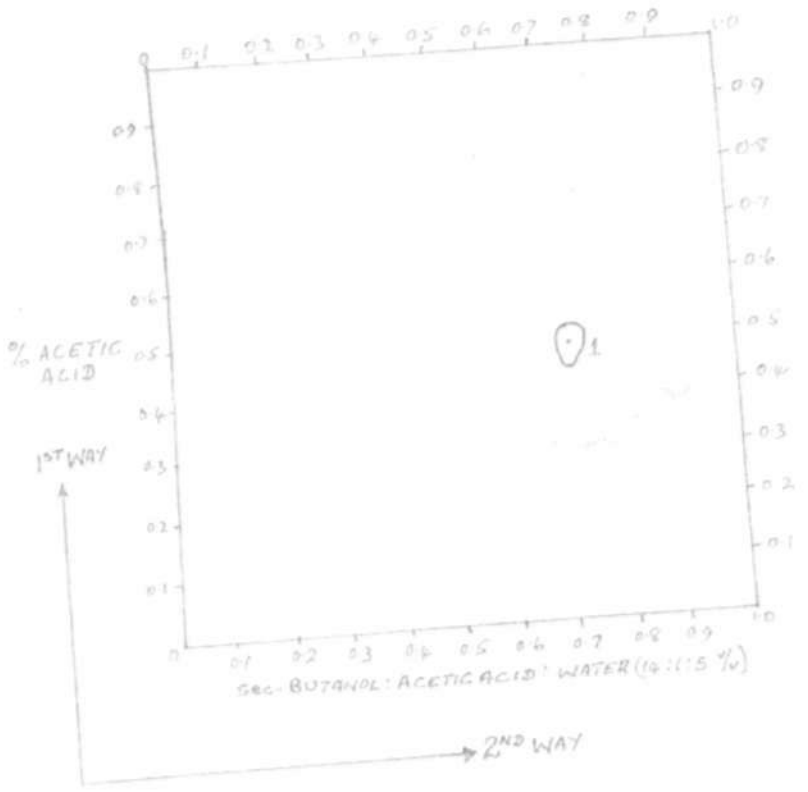


FIGURE V

1. GALLIC ACID

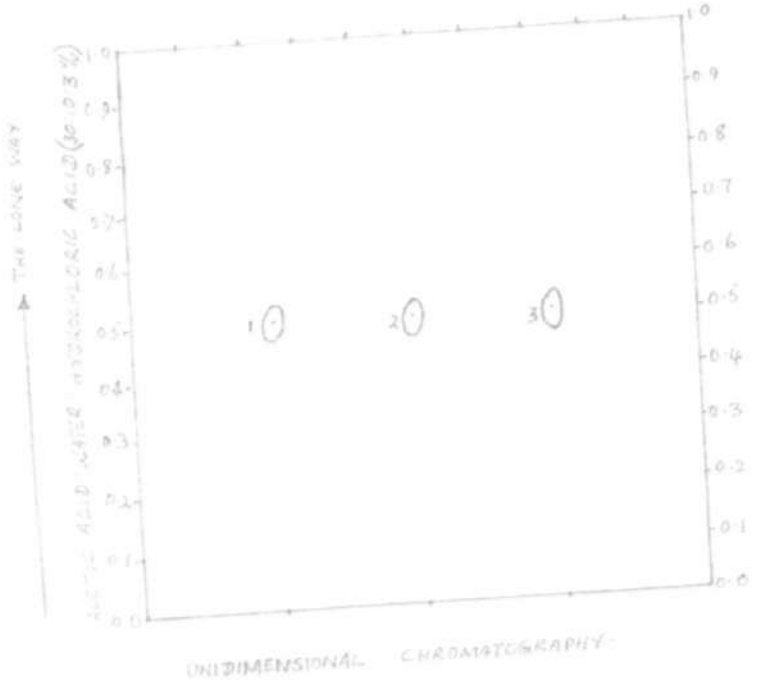


FIGURE VI

1. AUTHENTIC CYANIDIN CHLORIDE.

2. CYANIDIN CHLORIDE FROM TESTMATERIAL

3. MIXTURE OF 1 + 2 ABOVE

Isolation of gallic acid[116,117] The ether extract left after washing it with cold water was crystallized from hot water at 70°C; (charcoal) to give gallic acid needles, m.p. and mixed m.p. 250°C. (decomposition).

Analysis found - C:49.7; H:3.6

$C_7H_6O_5$ requires C:49.5, H:3.5

When a solution of the crystals was subjected to two dimensional chromatography, a clear single spot was noticed. Figure V.

Methylation - The acid in methanol was treated with diazomethane and the methyl derivative - methyl-tri-o-methyl gallate was obtained as fine needles m.p. and mixed m.p. 82.5°C. confirming that the compound isolated was gallic acid.

Since hydrolysable group of tannins normally contain ellagic acid associated with gallic acid, work has therefore been carried out to confirm conclusively the absence of this constituent. Hydrolysable group of tannins often deposit ellagic acid along with gallic acid. Though the chromatograms of the various fractions have not indicated the present of this acid, further confirmatory work with the phenolic and carboxylic fractions, by hydrolysis and chromatographic studies, have been carried out and have conclusively proved the absence of ellagic acid in the various fractions.

Studies on the phenolic fraction - The phenolic fraction was subjected to two dimensional chromatography in solvent system

6% acetic acid in the first way followed by sec-butanol-acetic acid-water (14:1:5) in the second way. The chromatograms were sprayed with (a) ferric chloride (b) mixture of ferric chloride and potassium ferricyanide, (c) vanilin/ethanolic hydrochloric acid. Four spots were noticed - Figure II.

This fraction, from the paper chromatographic studies indicated that it is made up of one leucocyanidin together with other minor components. Catechin, etc., were absent.

Identification of Leucocyanidin[118] - The leuco anthocyanidin was found to be leucocyanidin. The identification of the compound was made by converting it into corresponding anthocyanidin chloride by treatment with butanolic hydrochloric acid following the method of Swain and Hillis[119] with subsequent unidimensional paper chormatography in the solvent system (a) acetic acid-water-hydrochloric acid (30:10:3 v/v) and (b) 90% formic acid-3N hydrochloric acid (1:1v/v). The Rf values 0.5 in (a) and 0.35 in (b) correspond with those of authentic cyanidin chloride run under identical conditions. Figure VI. Further confirmation of the compound was obtained by taking the λmax values (λmax 545 mu) in the visible region of the cyanidin chloride. The anthocyanidin present must therefore be leucocyanidin. The sign of rotation of the leucocyanidin could not be determined due to its presence in small quantity.

Though the phenolic fraction did not indicate chromatographically the presence of catechin or quercetin,[120] further confirmatory work of their absence in the material was carried out as follows:

The pods were successively extracted with petroleum ether and acetone. The acetone extract was further fractionated into ether and ethyl acetate. The ether solubles were examined for quercetin by (a) reaction with magnesium/hydrochloric acid (b) chromatography and co-chromatography with authentic quercetin.

Both the above tests indicated that there is no quercetin confirming the original finding.

Isolation of glucose - The aqueous solution after the removal of the gallic acid in the phenolic fraction was found to contain sugars by colour tests. It was subjected to unidimensional chromatography along with model compounds and it was identified to be glucose, with the Rf value of 0.23 in solvent system sec-butanol-acetic acid-water (14:1:5 v/v). ~~See Figure VI~~.

Attempts to isolate and prepare derivatives were not successful as the quantity of glucose present was in very small quantity.

From various components isolated and/or identified in the bagaruwa tannins, gallic acid, protocatechuic, leucocyanidin and glucose, the earlier observation based on colour reaction, that it is a mixed type (condensed and hydrolysable) is thus confirmed.

Gallic acid is a typical component present in the hydrolysable

class of tannins and leucocyanidin, a typical component usually present in condensed tannins. The presence of both these in the material conclusively proves that the tannins are of mixed type.

The absence of ellagic acid further indicates that the tannins are not ellagi tannins. However, the presence of glucose and gallic acid indicates that the tannins may be present as gallotannins - usually present in hydrolysable group of tannins. The presence of leucocyanidin indicates the possibility that part of the tannins may be present as leucocyanidin gallates as well. The leucocyanidin may perhaps be the product of degradation of leucocyanidin gallate upon heating.

The presence of both types of tannins in bagaruwa perhaps explain its excellent leather making properties and the light colour of the leathers produced. Gallic acid present in tanning materials generally has a lightening effect on the colour of the extracts and ultimately on the colour of the leathers produced using such an extract.

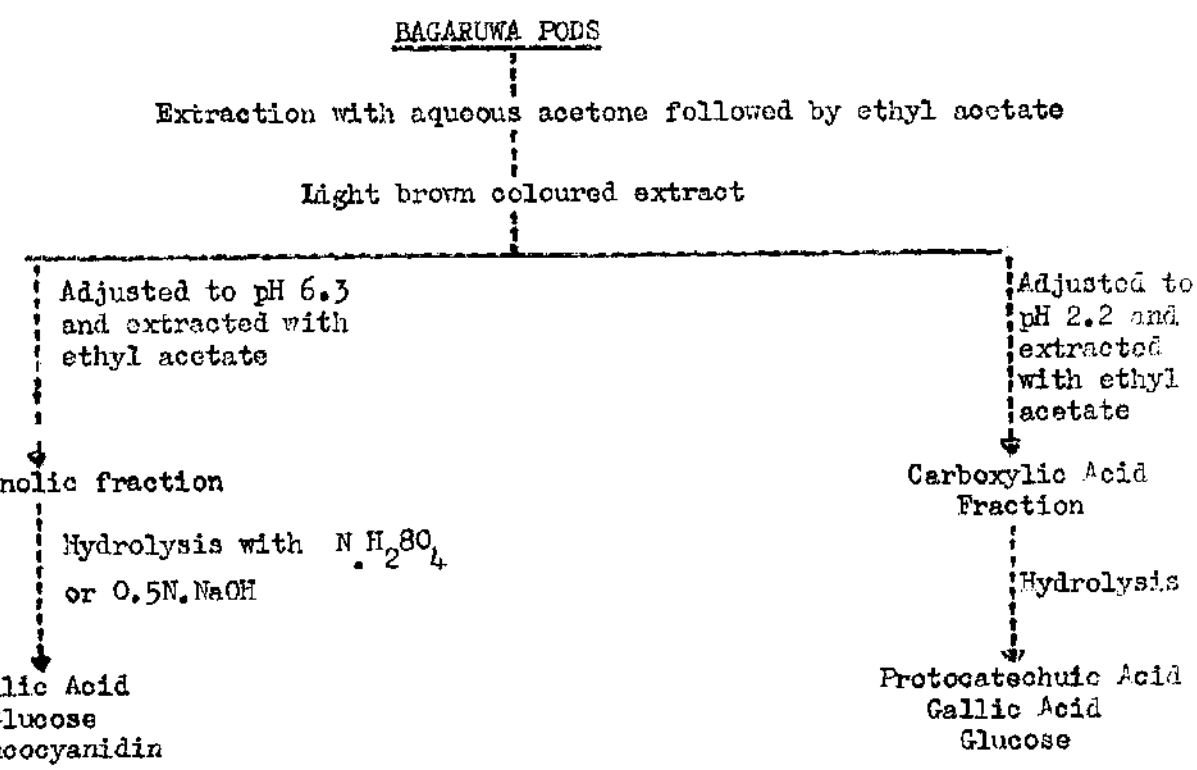
The studies on the isolation/identification of the phenolic components of the bagaruwa pods and the components identified also throw light on the possible biogenesis of the formation of tannins in the pods. The presence of gallic and protocatechuic acids along with glucose indicates as earlier pointed out that part of the tannins belong to the hydrolysable class and these can be assumed as derived from gallic acid present as esters with glucose. It

can also be assumed that gallic acid might have been formed from 5-dehydro-shikimic acid by dehydrogenation as proposed by Haslam or from phenylalanine proposed by Zenk as shown in Scheme I in the introduction.

The presence of leucocyanidin as pointed out earlier indicates the presence of condensed group of tannins as well along with the hydrolysable tannins. These may have been derived through the polymerization of flavan 3,4-diols and flavan 3-ols.

Diagram I

Constituents of Bagaruwa Pods



CHAPTER 3

PRACTICAL TANNING STUDIES ON BAGARUWA EXTRACTS

It is reported[121] that extracts of tanning materials prepared out of organic solvents are generally lighter in colour and higher in tannin content as compared to aqueous extracts. It is also reported[121] that these extracts have better tanning characteristics as compared to aqueous extracts. Studies have therefore been carried out with various solvent extracts prepared from bagaruwa pods to analyse them for their tannin and non tan contents as well as study their tanning properties with a view to see if a similar trend is observed with this material.

Extracts with the pods using acetone, methyl alcohol, ethyl acetate and water have been prepared and analysed for their tannin and non tan contents and the results are given in Table III.

Table III

Analysis of Bagaruwa Extracts prepared from various solvents

Sample	Tans	Non tans	Moisture	Insolubles	Colour	pH
1. Ethyl acetate extract	82.0	10.0	7.5	0.5	Y= 8.0 R= 1.2	4.2
2. Acetone extract	72.0	20.0	7.0	1.0	Y=10.0 R= 2.0	4.0
3. Methyl Alcohol extract	68.0	22.0	7.5	2.5	Y=10.5 R= 2.5	4.1
4. Aqueous extract	65.0	25.0	7.0	3.0	Y=12.0 R= 4.0	4.4

From the results it is observed that the ethyl acetate extract has the highest tannin content, 82% as compared to aqueous extract, next in order being acetone (72%) and methyl alcohol (68%). This confirms the earlier findings on another tanning material, myrobalans.[12]

Practical tanning tests have been carried out as follows:

An airdried goatskin, after the usual liming, unhairing, fleshing and deliming, was taken and cut into small pieces. These pieces were tanned with the various extracts prepared, using 16% of tannins on pelt weight and 300% water as float. The extract solution was added in to instalments. After complete penetration was obtained, the pieces were washed well, sammed, set, oiled with groundnut oil and dried. The dried leathers were examined for their physical properties like feel, fullness, etc., and are tabulated below:

Physical Characteristics

Extract	Leathery character	Colour
Aqueous	Leathery but some what tiny	Yellow with brown casts
Methyl Alcohol	Leathery and full	Cream yellow
Acetone	Leathery and full	Bright cream
Ethyl Acetate	Leathery and fuller	Creamish white

The better leather tanning properties of the solvent extract may perhaps be explained as follows: During the extraction of the tannins with water from the bagaruwa pods, hydrolytic and oxidation changes are likely to take place resulting in the breakdown of some of the

tannin molecules, whereas extraction with solvents, especially the non-hydroxylic ones, hydrolytic changes are not likely to take place and the tannin molecules remain intact without breakdown during the process of extraction. It may therefore be reasonable to assume that the leathers produced using these solvent extracts with their tannin molecules intact will produce fullter and better quality leathers as compared to aqueous extracts where the tannin molecules are likely to be broken down during extractions.

Summary

Studies on the chemistry-polyphenolic constituents, isolation/identification have revealed that the pods contain both hydrolysable and condensed tannins. The following components, gallic acid, protocatechuic acid and leucocyanidin, have, for the first time, been isolated/identified in this material.

These studies explain the special tanning characteristics of the pods. They also throw light on the possible biogenetic path of the formation of the tannins which may have to be confirmed by further detailed studies.

The tanning studies with the solvent extracts revealed that extracts prepared from solvents generally produce leathers of better quality. This may be due to the non break down of the tannin molecules during leaching with solvents in contrast to aqueous extracts where hydrolytic changes are likely to take place during leaching and conversion into solid or spray dried extracts.

CHAPTER 4

EXPERIMENTAL

Preparation of samples for analysis

Bagaruwa pods which has long been used for tanning purposes throughout the sudan zone of North Africa were quantitatively analysed in some details in order to determine their tannin and non tan contents.

Very numerous quantitative methods have been suggested but only the gravimetric method,[122] based on the absorption of tannins by hide powder, is of serious practical importance. The hide powder method, however, is of the nature of a laboratory tanning experiment, and is necessarily largely empirical, requiring the strictest adherence to every detail of the regulation in order to obtain concordant results. It has nevertheless, been brought to a high degree of perfection, and gives quantitative information of the utmost use.

The main crop of pods were collected during the months of December and February (the mature trees yield from 80 - 100 lbs of these pods per tree). Since the adansonii variety is not as heavily constricted as some other varieties (e.g. Nilotica), the pods are usually intact when collected and as such, much can be easily collected.

For this study, ripe fruits from Bauchi in the North Eastern State of Nigeria, were collected (green fruits are usually collected

in some other cases, but overripe fruits are known to have tannin content which is significantly lower than in the unripe ones) and each pod was thoroughly cleaned by the use of brush to get rid of extraneous matters. They were then crushed, using Apex grinder.

A known quantity of the resulting fine powder that would give, as nearly as possible, 4gm per litre of tanning matter absorbable by hide powder was always taken for extraction using Procter extractor. Two litres of the extract of the required analytical strength, were normally collected per sample.

The material was first soaked, in cold distilled water overlying a layer of sea-sand in the extractor, overnight (for condensed tannins) before commencing the extraction. In the case of hydrolysable tannins, immediate extraction was carried out at such a uniform speed that 2 litres of the extract were obtained in 4 hours.

After collecting the first 150ml at room temperature, the temperature was raised to 50°C. and the next 750ml was collected. The temperature was again raised, as rapidly as possible, to boiling point, when the remainder (to make 2 litres) was extracted. A clear solution of the extract could be ensured because apart from its passing through a fine layer of sea sand, the delivery tube inserted into the sand-bed always had a wide funnel-shaped end round which was tied a piece of finely woven filter cloth.

By the time the two litres of the required tannin extract would have been obtained, the sample must have been wholly leached of its tannins. The last few drops of the extract must show no

positive tannins test with gelatin salt (this was the major reason why the extraction was spread over 4 hours). The extract used to cool to room temperature under running tap, and then made up to the mark with distilled water.

Moisture determination - 2 Gm of the finely ground material was dried to constant weight at 98.5 - 100°C. in a vacuum oven. (The constant weight was recorded when the last two weighings differ only by less than 2 mg.).

If say, x gm. of moisture has been driven off, then the percentage moisture content is calculated by $\frac{x}{2} \times 100$.

Determination of total solids - 50 Ml. of the unfiltered tannin extract was evaporated to dryness on a water-bath and dried to constant weight in a vacuum oven at 98.5 - 100°C.

y = the amount of solids in 50cc of the extract

z = the original weight of test material (crushed) extracted

then % total solids = $\frac{2000 \times y \times 100}{50 \times z}$.

Determination of total solubles - 50 Ml. of the filtered tannin extract was evaporated to dryness over a water bath and dried to constant weight in a vacuum oven at 98.5 - 100°C.

If a = the amount of total solubles found to be present in 50cc of the filtered extract, then % total solubles = $\frac{2000 \times a \times 100}{50 \times z}$.

The Insolubles - This is the difference between the percentages for total solids and the total solubles. Alternatively, it is the difference between percentage total solubles plus percentage moisture and 100.

Determination of non-tans[111, 123] In this determination it is necessary to first prepare chrome tanned hide powder. 6.25 gm. of the dry hide powder was diluted ten times its weight with distilled water and shaken for about one hour. 1 Ml. of stock chrome alum solution per gramme of the dry hide powder taken was then stirred into it and this continued for several hours before leaving it to stand overnight.

The powder was transferred to a filter cloth, allowed to drain, and was then squeezed. A quantity of water, 15 times the weight of dry powder taken, was poured on to the powder in the filter cloth, and the mixture thoroughly stirred for 15 minutes. The cloth containing the powder was then lifted out, allowed to drain, and then squeezed to about 75% moisture content. To the powder was added water, and again stirred as above. This process was repeated three times. At the end, the powder was squeezed again to about 75% moisture content and it was then broken up, mixed uniformly, before it was weighed.

A quantity of wet chromed powder, containing 6.25 gm. of dry hide powder was added to a suitable shake bottle containing 100 ml. of the unfiltered tannin extract. The bottle was then

violently shaken by hand for 15 seconds before it was transferred to a mechanical rotatory shaker at an average speed of 60 r.p.m. for 10 minutes, after which the content of the bottle was wholly transferred on to a filter cloth supported by a funnel. When the powder had drained, it was squeezed with hand, and to the filtrate 1 gm. of kaolin was added.

After allowing the kaolin to mix thoroughly, the mixture was filtered, the filtrate was returned for refiltering, until the solution was clear. At this stage it was tested with gelatin-salt to confirm whether or not the whole of the tannins had been absorbed by the hide powder.

50 ml. of this filtrate was then evaporated to dryness and kept in the vacuum oven at 98.5 - 100°C. until a constant weight was obtained.

When the calculation for the non-tan was carried out, the residue weight was multiplied by a factor, 1.2, because of the dilution of 20 ml. of water introduced into the 100 ml. of tannin solution by the wet hide powder.

If c = the amount of non-tan in 50cc of the soluble fraction of the extract then % non-tan = $\frac{2000 \times c \times 100 \times 1.2}{50 \times z}$

The tannin matter absorbed by the hide powder was the difference between the percentages of the total solubles and non-tans. The percentage tannin was calculated as follows:

$$\frac{2000 \times 100}{50 \times z} (a - c).$$

Colour measurement - This was carried out using a freshly filtered tannin extract. Lovibond tintometer was used, applying artificial light source. The test solution was taken in 1 cm. cell, and the result was expressed in terms of red and yellow.

pH determination - As in colour measurement, the filtered tannin solution was employed, and the pH determined was determined by means of the glass electrode using a pH meter, after it had been standardised, using a buffer solution.

Acids and salts determinations - One litre of distilled water was boiled, to make it CO_2 free, and immediately corked up.

Acid determination - 100 ml. of the filtered test solution was diluted to 200 ml., using the CO_2 free water.

This solution was titrated with $\frac{N}{20}$ NaOH until a pH of 5.8 is reached. This was determined by the use of a pH meter. (N.B. It is necessary to stop the titration at a pH of 5.8 because it has been established that at this pH all the acids in the solution must have got fully dissociated). The volume of alkali used was at this stage recorded.

Salt determination - The cation exchanger was thoroughly washed with 100 ml. CO_2 free distilled water. This was done by allowing the water to run through the exchanger for about 10 minutes.

Another 100 ml. of the filtered tannin solution was taken and diluted to 200 ml. with CO_2 free water. 100 ml. of this solution was also run through the exchanger for another 10 minutes, followed by 100 ml. of CO_2 free water, also running through for 10 minutes, to wash down all free H+ that might still be hanging on to the resin. The combined solution collected was then titrated with $\frac{N}{20}$ NaOH, till the pH of 5.8 was reached.

(The volume of the alkali required for this titration is always much greater than the one for acid determination, because the test-solution has all its cations replaced by H+ on the resin as the solution passes through the column, thereby increasing the H+ concentration in the solution).

The amount of salts was determined by subtracting the titration figure obtained for acid deterination from that obtained for salt determination and using the figure for calculation.

Determination of sugars - To some few grammes of the phenolic fraction of the ethyl acetate extract of the test-material, say 2 gm. some 30cc of NaOH were added and heated at 70°C. for 2 hours. The lot was allowed to cool with the help of blocks of ice, and then acidified with dilute H_2SO_4. No noticeable precipitate was formed. However, the solution was filtered. The filtrate was then repeatedly extracted with ether and the residue was again filtered, neutralized with solid barium chloride, and concentrated. At this point, the test for sugars was carried out (Molisch Test) and was found to be positive and

further test confirmed it to be a reducing sugar, i.e. one that contains a (potential) carbonyl group (e.g. fructose, glucose, lactose, etc.). On running a chromatogram (unidimensional) the Rf value was 0.23 in sec-butanol-acetic acid-water (14:1:5 v/v) solvent system (c.f. literature of 0.24).

Since the presence of other sugars could not be ruled out they were quantitatively determined in terms of glucose.[122] This was done by boiling with Fehlings solution and precipitating copper oxide which can be weighed. The tannins were removed by precipitation using lead acetate solution, the excess lead salt was removed by precipitation with solid potassium oxalate and the non-reducing sugars "inverted" with HCl acid. The sugar content was determined from the weight of copper oxide by means of Munsen and Walker's Tables.

Preparation of extracts - 3 kg. of the clean fresh bagaruwa pods (crushed) were taken up in 500 ml. of water. This was allowed to stand for one hour at room temperature. 6 litres of acetone were added to the mixture and kept at room temperature for 48 hours. After decanting the supernant, which was distilled off completely ($\angle 40^{\circ}$, N_2 atmosphere), and the residue was again treated with 6 litres of water and kept at $0^{\circ}C$. for 2 hours. The solution was then filtered off from the resulting gummy matter and chlorophylls. The filtrate was concentrated in vacuum to 200cc

($<40^{\circ}$, N_2 atmosphere). It was then repeatedly extracted with ethyl acetate (acetic acid free). The ethyl acetate was then distilled off in vacuo ($<40^{\circ}$, N_2 atmosphere). The residual extract dark brown in colour, was dried in a vacuum oven kept at a temperature below 40°C. The final product (light brown granular solids) was then kept in a vacuum desiccator.

The solution of the extract in water was separately tested with gelatin and iron salts when precipitates and colour changes were observed. Another portion of the extract in ethyl acetate was subjected to two dimensional chromatography on Whatman No.1 paper using the following solvent systems:

II (c) 6% Acetic Acid containing 2% Formic Acid in the first direction, followed by (d) Benzyl Alcohol - Tertiary Butanol - Isopropanol-Water (3:1:1:1 v/v) containing 2% Formic Acid in the second way; I (a) 6% Acetic Acid in the first way, followed by (b) sec-butanol-acetic acid-water (14:1:5 v/v) in the second way.

6 spots were noted when the chromatograms were sprayed ferric chloride-ferricyanide reagent.

Characterization of the constituents of the phenolic fraction - 25 gm. of the ethyl acetate extract were taken in water (180cc) and the pH adjusted to 6.3 by the addition of solid sodium carbonate and the whole mixture was then repeatedly extracted with ethyl acetate until the acetate layer became almost colourless. The combined extracts were dried over Na_2SO_4 and the solvent

evaporated to dryness in vacuo ($< 40^{\circ}$, N_2 atmosphere) to yield the phenolic fraction, which on paper chromatographic analysis, revealed the presence of leucocyanidin, gallic acid and two unidentified spots.

Colour of the solid extract = Bright light brown
Weight (yield) = 16.40 gm.

Characterization of the constituents of the carboxylic fraction - The pH of aqueous solution left after the removal of the phenolic fraction was brought down to 2.2 by the addition of aqueous ortho-phosphoric acid (50%). The mixture was then extracted repeatedly with ethyl acetate and the extracts were combined and the ethyl acetate distilled off in vacuo ($< 40^{\circ}$, N_2 atmosphere) and the resultant solid matter was the carboxylic fraction.

Colour of solid extract - Greyish brown

Weight (yield) = 3.65 gm.

Solubility of the extract in acetone and alcohol was determined and found to be considerable. Some solids of the extract was taken in water and tested with gelatin salt. A heavy pale yellow precipitate was obtained. When ferric chloride solution was added to another solution of the extract, a greenish yellow colour with a tinge of violet developed.

A solution of the extract was subjected to two dimensional chromatography and spots corresponding to gallic acid and protocatechuic acid were located. These were later isolated and characterized.

(ii) Isolation and identification of individual constituents in the phenolic fraction

(a) Testing for ellagic acid - 20 Gm. of the phenolic fraction were dissolved in 20cc of N.H_2SO_4 and heated over water-bath for 60 hours (hydrolysis). The dark brown solid obtained was separated from the ~~color~~ clear solution by filtration. The solid was taken in pyridine, warmed to dissolve any available ellagic acid and filtered (using hot-water funnel). The resultant solution was reduced in volume and allowed to cool to room temperature. No crystals were formed. The volume was further reduced and cooled under blocks of ice for many days, yet no crystals were formed. The solution was run on two-way chromatography (6% in the first way and sec-butanol-acetic acid-water (14:1:5 v/v) in the second way). No spot could be located on spraying with ferric chloride, further confirming the absence of ellagic acid.

(b) Isolation of gallic acid - The aqueous solution left, after the removal of the brown solid in the above experiment, was repeatedly extracted with ether, and the extract dried over anhydrous NA_2SO_4. The ether was removed by distillation and the greyish white residue, left after recrystallisationfrom water, gave gallic acid (m.p. and mixed m.p. 250^d). When the crystals were subjected to two-way chromatography, the Rf values were 0.47 in solvent system A and 0.8 in B.

Its acetyl derivative, crystaliised from a mixture of alcohol and water (2:1) gave a m.p. of 167^oC. (c.f. literature value of 170^oC.). With all the above results there seemed to be no doubt

that gallic acid had been isolated.

(o) Identification of glucose - The aqueous solution after the removal of the gallic acid above, was neutralised with barium carbonate and filtered. The filtrate was shaken with Al_2O_3 for one hour and then filtered. The filtrate was concentrated in vacuo, and the tests for sugars carried out.

Molisch Test was carried out by taking a few cc's of the filtrate and adding 20% β-naphthol to it in drops. When concluded H_2SO_4 (about 1cc) was carefully poured down the side of the inclined tube, a layer of red ring, changing to violet at the interface of the two liquid layers formed, indicating the presence of a carbohydrate or a related compound. The mixture was shaken up and kept for two minutes before emptying the tube into water. A dull violet precipitate emerged, thus confirming the presence of a carbohydrate in the test-material.

The ozazone derivative was attempted but no solid crystallised out. The acetyl derivativewas also tried, and yet no crystals.

Then some of the original filtrate was further evaporated in vacuo to a syrupy consistency. The concentrated matter was subjected to unidimensional paper chromatography using authentic samples, as markers, in the solvent system (b). The chromatograms wre developed with 3% p-anisidine hydrochloride in butanol saturated with water, followed by heating at 105°C. for 5 minutes. It was discovered

that the test-material moved approximately the same distance as glucose (Rf. 0.23 as compared to 0.24).

(iii) Isolation and identification of individual constituents in the carboxylic fraction.

(a) Isolation of gallic acid - Some solids of this fraction were dissolved in minimum of water and heated at a temperature of between 70° and 80°C. for half-hour. Any solid left was filtered off while hot, and the filtrate allowed to cool to room temperature. The concentrated aqueous solution was then repeatedly extracted with ether, and the combined extracts were dried and evaporated. The solid residue, after recrystallisation from water, gave gallic acid crystals (Rf 0.48 in solvent system and 0.8 in B). Its m.p. and mixed m.p. was at 250°d, and from this was prepared the acetyl derivative which melted at 167°C.

(b) Isolation of protocatechuic acid - The solid ether extract of the carboxylic fraction was dissolved in a minimum quantity of water at 70°C. It was filtered and kept at room temperature overnight. Some solids separated and were identified as gallic acid. The solution was decanted off and slightly concentrated under vacuum ($< 70^{\circ}C$). The concentrated solution was kept at room temperature overnight during which the second portion of gallic acid crystallised out. These were filtered off and the solution tested for protocatechuic by running three two-way chromatograms. On the first the test-solution alone was spotted.

Each chromatogram gave a single clear spot, dark green on spraying, but pale purple after sometime had elapsed (Rf 0.48 in A and 0.85 in B). The first and third chromatograms each gave an additional feeble spot corresponding to gallic acid due to imperfect separation (co-chromatogrphy).

Hydrolysis of the phenolic fraction with $^{N}/_{2}$ NaOH

2 gm. of the fraction were taken and 30cc of $^{N}/_{2}$ NaOH added. The mixture was heated to 70°C. (N_2 atmosphere) for 2 hours. The solution was cooled with ice and then acidified with dilute H_2SO_4. No solid was precipitated at this point, and after filtration to remove the possibility of any solid contamination, the filtrate was repeatedly extracted with ether. The ether was evaporated off and the residue examined and found to contain, principally: gallic acid which was isolated as in the previous cases.

The aqueous solution (the filtrate in the above, was neutralised with solid barium carbonate, filtered and the filtrate concentrated. The concentrated solution gave a positive Molisch Test for sugars. The quantity of the sugar was however, found to be so small that further meaningful characterisation, such as isolation and preparation of derivatives, was prevented.

Another portion of the phenolic fraction (4 gm.) was taken in 40cc of water and repeatedly extracted with ether. The aqueous solution was fractionally precipitated with 20% lead acetate. At first, 5cc of the acetate were added and the precipitate obtained

was rejected. Then 30cc of the acetate were added to the filtrate and resultant precipitate was filtered off and the filtrate again treated with 20cc of the acetate, but no precipitate came out.

The precipitate obtained by the additon of 30cc of acetate solution was suspended in 50cc of water and then treated with DOWEX 50Wx8(H_+) Resin. The mixture was maintained at a temperature of 40°C., sunjecting to rigorous stirring for half-hour and then filtered. The filtrate was concentrated in vacuo (N_2 atmosphere) and the residue left was subjected to two-dimensional paper chromatography, using (e) 6% acetic acid containing 0.2% formic acid in the first way and (f) Benzyl alcohol-t-butanol-iso-propanol-water (3:1:1:1 v/v) containing 1.8% formic acid.

Two blue spots came out very clearly when sprayed with $FeCl_3$ - $K_3Fe(cN)_6$.

Rf values: 1st spot (0.422 in (e) and 0.514 in (f))

2nd spot (0.373 in (e) and 0.242 in (f))

These values could not be verified because there was no table of such Rf values in literature to compare.

Isolation of leucocyanidin

2Kg. of cleaned bagaruwa pods were crushed and light petroleum (b.p.40-60°, 2x3 litres) added and the mixture kept at room temperature of 2 days with daily renewal of solvent. At the end of the period, the petroleum was decanted, 90% acetone (3x3 litres) added, and the whole contents were allowed to stand for

7 days at room temperature,with renewal of acetone every two days, except the last 3 litres which was allowed to stay on for three days. After decanting the acetone, methyl alcohol (3x2 litres) was added and left at room temperature for 7 days with renewals as in the case of acetone.

The acetone extract was evaporated in vacuo ($< 40^{\circ}$, N_2 atmosphere) so as to reduce the volume to about 200cc. Although no precipitate was observed during the concentration process, the solution was still filtered, to make sure it is particles-free.

Further evaporation was resumed until all the solvent was gone. The solid left behind was successively extracted with ether and ethyl acetate. The extracts were each evaporated to dryness and labelled fraction I and fraction II respectively.

Separation and characterization of polyphenols-quercetin - 5 gm. of the ether fraction (fraction I) were dissolved in 70% ethenol (17 ml.) and kept at 5°C. for 5 days, during which some yellow needles were observed to be present in the solution. They were however, found to be very unstable and this prevented further meaningful characterisation especially to confirm whether or not they were quercetin needles.

Leucocyanidin - 5 gm. of the ethyl acetate solubles (fraction II) were re-dissolved in ethyl acetate (25cc) and dried over Na_2SO_4. Petroleum ether (m.p. 40°-60°C.) was added to the filtered solution

in small quantities and the sticky, coloured precipitate (impurities) deposited was rejected by filtration. Further addition of the ether, dropwise, to the filtrate until it was in excess and then the whole lot cooled, gave some light, pinkish coloured solids which were separated from the supernant. These were purified from a mixture of ethyl acetate and light petroleum ether and an almost white power was obtained. Its m.p. was found to be 230°C. and Rf values were 0.385 in A and 0.789 in B. The colour of the spot on the chromatogram when sprayed with ferric chloride was pale purple or pink.

R E F E R E N C E S

1. Seguin, A. — Ann. de Chemie, 20, 15 (1796).
2. Von Stokar, W. — Prehistoric leather finds and leather uses, Collegium, Haltingen, 796, 433 (1936).
3. Shuttleworth, S.G. — The story of wattle, Sc.Bull., Department of Agric. S. Africa, No.168.
4. Gannser, A. — The early history of tanning, Ciba Review 2938-2962, No.81 (1950)
5. Faller, G.A. — Greek and Roman tanners, Ciba Rev. 303-306, No.9 (1938).
6. Freudenberg, K. — "Die Chemie der natur-bi Shen Gerbstoffe" Springer verbag Berlin, pp.2-22 (1920).
7. Sastry, K.N. & Naydamma, Y. — Journal and proc.inst.chem., 38, 181, (1960).
8. Roux, D.G. — Nature, 181, 1454 (1958).
9. Berk, A.A. — Chem. and ind. London, 360 (1953)
10. Metcaye, C.R. — "Anatomy of Dicotyledons" (Oxford, 1950).
11. Fischer, E. — "Untersuchungen Uber Depside ind. Gerbestoffes" (Berlin, 1920).
12. Haworth, R.D. — J.S.L.T.C. 40, 115, (1956).
13. Haslam, E. — "The Chemistry of Vegetable Tannins", (Academic Press, Lond, p.94, 1968).
14. Linstdedt, G. — Acta Chem.Scand, 4, 448 (1950)
15. Gibbs, H.D. — J. Biol.Chem. 72, 649, (1927)
16. Macquer & Monnet — "Elements de Chymie", iii, 403, Difon (1798).
17. Scheele, C.W. — Crell's Chem. Ann., i, 3, (1787).
18. Kunsmuller, C.L. — Crell's Chem. Ann., ii, 413 (1787).

19. Bertholet, A.B. — Crell's Bey Trage, 4, 240 (17)

20. Proust, M. — Ann. Chem. 25, 225 (1799).

21. Fischer, E. — Ber. 52, 809 (1919).

22. Fischer, E. — Ber 46, 3253 (1913).

23. Freudenberg, K. — "Naturbishe Gerbestoffe"m Verlog Chemic, (Berlin, pp.101, 1920).

25. Karrer, Solomon, & Peyer — Helv-Chim, Acta, 6 , 17 (1923).

26. Freudenberg, K. — "Die chemie der Naturbisshe Gerbstoffe" Verlog chemic (Berlin, 107, 1920).

27. Fischer, E. & Freudeberg, K. — Ber, 45, 915 (1912); 45, 2709 (1912); 47, 2485 (1919).

28. Karrer, Widmor, & Staub — Ann., 433, 288 (1923).

29. Lowe, J. — Anal. Chem., 12, 128 (1873).

30. Armitage, R. et al — J.Chem.soc., 1842 (1961).

31. Haslam, E., Haworth R.D., Jones, K., & Rogers, H.J. — J. Chem. Soc., 1829 (1961)

32. Armitage, R., Haslam, E, Haworth, R.D. & Searle, T. — J.Chem.Soc., 3814 (1962).

33. Haslam, E., Haworth, R.D. & Keen, P.C. — J.Chem.Soc., 3814 (1962)

34. Redds, K.K., Rajadurai, S. Sastry, K.N.S. & Nayudamma, Y. — Aust.J.Chem, 17, 238 (1964).

35. (a) Schmidt, O.T. & Meyer, W. — Ann. 571, 1 (1951).

	(b) Schmidt, O.T. Lademan, R. & Himmele	Chem. Ber., 85, 408 (1952).
	(c) Schmidt, O.T. & Lademan, R.	Ann., 569, 149 (1950), 571, 232,
	(d) Schmidt, O.T. & Schmidt	Ann., 578, 31 (1952).
	(e) Schmidt, O.T.& Nieswandt, W.	Ann., 568, 165 (1950).
	(f) Schmidt, O.T.	"The chemistry of vegetable tannins, S.L.T.C., Symposium, Croydis 68 (1956).
	(g) Schmidt, O.T.	Cortschrete. der chemie der orgain schen, Naturstoffee VIII, 78 (1956).
36.	Schmidt, O.T.	"Seminar on veg. tannins" - 1962 (ed. Rajadurai, S. and Kotyudai Bhanu) A seminar held at C.L.R.I., Madras, P.I.
37.	Schmidt, O.T. & Bernaver	Ann., 588, 211 (1954).
38.	Schmidt, O.T. & Komerek, E.	Ann., 591, 156 (1955)
39.	Meyer, W.	Ann., 578, 34 (1952).
40.	Jurd, L.	J. Amer.Chem.Soc.80, 2249 (1958).
41.	Gilson, E.	C.R. hebd seano, Acad.Sci., Paris 136., 385 (1903).
42.	Schmidt, O.T.	Leder, 14, 40 (1963).
43.	Harborne, J.B. & Conner, J.J.	Bioch. J., 8, 242 (1961)
44.	Haslam, E.	"The chemistry of vegetable tannins" Academic Press, London, p.162 (1966).
45.	Haslam, E. Haworth & Knowles, P.F.	J. Chem. Soc., 1854 (1961)

46. Zenk, M.H. — Z. Naturforsch, 19, 1383.

47. Harborne, J.B. — "The comparative biochemistry of the flavonoids", Academic Press, London, p.33, (1963).

48. Von Kostanecki, S, & Tambor, J. — Ber, 128, 2302 (1895).

49. Haslam, E. — "Chemistry of veg. tannins", Academic Press, London, p.158 (1966).

50. Freudenberg, K. & Weinges, K. — "Seminar on vegetable tannins" 1962 (ed. Rajadurai, S. and Kotudai Bhanu, held at C.L.R.I., Madras, p.43.

51. Chaddha, J.S. — Seminar on veg. tannins 1962 (ed. Rajadurai, S. and Kotiudai, Bhanu) held at C.L.R.I., Madras.

52. Seikel, M.K. — "Biosynthesis of phenolic compounds (ed. Harborne, J.B.) Academic Press, London, p.38, (1964).

53. Ollis, W.D. — "The chemistry of flavonoid compounds" (ed. Geissman, T.A.) Pergamon Press, London, p.353 (1962).

54. Harborne, J.B. — J. Chromatography, 2, 381 (1950).

55. Kirby, K.S. Knowles, E. & White, T. — J.S.L.T.C., 37, 283 (1953).

56. Roux, D.G. — Nature, 180, 973 (1951).

57. Roux, D.G. & Evelyn, S.R. — Biochem. J. 69, 530 (1958).

58. Meyer, W. & Bauni, G. — Das Leder. 7, 33, (1956).

59. Seikel, M.K. — "The chemistry of flavonoid compounds" (ed. Geissman, T.A.) Pergamon Press, London, p.51 (1962).

60. Robinson, R. — Nature, London, 137, 172 (1936).

61. Neish, A.C., Underhill, E.W. & Watkin, J.E. — Can.J.Biochem.Physiol., 38, 219 (1957).

62. Neish, A.C. & Watkin, J.E. — Can .J.Biochem.Physiol., 38, 559 (1960)

63. Grisebach, H. & Bopp, M. — Z. Naturforsch. 14B, 485 (1959).

64. Grisebatch, H. & Patschke, L. — Chem.Ber 93, 2326 (1960).

65. Bopp, M. & Mathis, B. — Z. Naturforsch, 17B, 811 (1962).

66. Birch, A.J. — "The chemistry of flavonoid compounds" (ed. Geissman, T.A.) Pergamon Press, Oxford, p.620 (1962).

67. Seshadr, T.R. — Tetrahedron, 6, 1691 (1959).

68. Ollis, W.D. — Experientia, 17, 4 (1961).

69. Roux, D.G. & Paulus, E. — Biochem.J. 77, 315 (1960); 78, 120 785, (1961); 80, 62 (1961).

70. Roux, D.G. Mahis, A.E. & Paulus, E. — Biochem.J.78, 834 (1961).

71. Roux, D.G. — "Seminar on vegetable tannins" (ed. Rajadurai, S., and Kotyudai Bhanu) A symposium held at C.L.R.I. Madras.

72. (a) Batesmith, E.C. — J. Expt. Botany, 4, 1 (1953).

(b) Batesmith, E.C. & Lerner, N.H. — Biochem. J., 58, 126 (1954).

73. Hillis, W.E. — J.S.L.T.C. 38, 91 (1954).

74 (a) Hillis, W.E. — Nature, 175, 597 (1955); 182, 1371 (1958).

(b) Hillis, W.E. & Urbach, G. — Nature, 182., 657 (1958); Aust.J.Biol.Soc., 2, 263 (1956).

75 (a) King, F.E. & Bottomley, W. Chem. and ind., 1368 (1953), J.Chem.Soc., 1399 (1954).

(b) King, F.E.& Clark-Lewis, J.W. J.Chem.Soc., 3384 (1955)

76 (a) Roux, D.G. Nature, 180, 973 (1957), 181, 1454 (1958) J.Am.Leath.Chem.Assoc.54., 614 (1959).

(b) Roux, D.G. & Bill, M.C. Nature, 183, 42 (1959).

(c) Roux, D.G.& Evelyn, S.R. Biochem.J. 69, 530 (1958); 70, 344 (1958).

(d) Roux, D.G.& Maihs, A.E. Nature, 182, 1798 (1958).

(e) Roux, D.G.& Paulus, E. Biochem.J., 77, 315 (1960, 78, 120, 785 (1961); 80, 62 (1961).

77. Hathway, D.E. & Seakins, J.W.T. Biochem.J.,6, 239 (1957).

78. Meyer, W. & Bauni, G. Das Leder, 7, 33 (1956), Ann.611, 264 (1958)

79. Hathway, D.E. & Seakins, J.W.T. BiochemJ. 6, 239 (1957); J.Sci.Ind.Res., 17B, 168 (1958).

80. Gangoly, A.K. & Seshadari, T.T. Tetrahedron, 6, 21 (1959).

81. Freudenberg, K. & Weinges, K. Ann.613, 61 (1958).

82. Reddly, K.K. Ph.D. Thesis submited to the University of Madras (1963).

83. Bhanu, K.U. Ph.D. Thesis submitted to the University of Madras (1963).

84. Freudenberg, K. "The chemistry of flavonoids compounds" (ed. Geissman, T.A.) Pergman Press, London, p.197 (1962).

85. Hathway, D.E. J.Chem.Soc., 520 (1958); Biochem.J., 70, 34, (1958)

86. (a) Freudenberg, K. Sci.Proc.Royal Dub.Soc. 27, 153, (1956); J.Pol.Sci.,29, 433 (1958).

(b) Freudenberg, K. & AlansoDelama, J.M. Ann.,612, 78 (1958).

(c) Freudenberg, K. & Maitland, P. Collegium, 776, 656 (1954); Ann. 510, 193, (1934).

(d) Freudenbrg, K. Stocker, J.H. & Porter, J. Ber., 901, 957 (1957)

(e) Freudenberg, K. & Weinges, K. Progress in chem.org.Nat.Prod. 16, 1 (1958).

87 (a) Meyer, W. & Merger, F. Chem. and Ind.London, 485 (1959).

(b) Meyer, W.& Bauni, G. Das Leder, I, 33 (1956).

88. Schmidt, O.T. & Meyer, W. Angew chemi, 68, 103 (1950).

89. Hathway, D.E.& Seakins, J.W.T. J.Chem.Soc., 1562 (1957).

90. Weinges, K. J. Polymer Sci., 16(c) 3 625 (1968).

91. Hathway, D.E.& Seakins, J.W.T. J.Chem.Soc., 1562 (1956); Biochem.J.,67, 239 (1957).

92. Hathway, D.E. J.Chem.Soc., 520 (1958).

93. Teuber, H.J. & Staiger, G. Ber.88., 802 (1955).

94. Kirby, K.S.& White, T. J.S.L.T.C. 38, 215 (1954).

95. Roux, D.G. J.Ann.Leath.Chem.Assoc., 54, 614 (1959).

96. Lyn, H. Enzymologia, 23, 231 (1961).

97. Erdtman, H. Sevensk papperstid, 42, 344 (1939)

98. Hillis, W.E.; Nelson, P. & Zadow, G. — Appite, 19, III (1966).

99. Grassman, W.E. & Endros, H. — Leder, 10, 237 (1959).

100. Hathway, D.E. & Seakins, J.W.T. — Biochem.J.72 369 (1959).

101. Sundera Rao, V.S. & Rajadurai, S. — Aust.J.Chem., 21, 1921 (1968).

102. Erdtman, H. — "Progress in Organic Chemistry - I" (ed. Cook, J.W.) Butterworth, London, p.27 (1952).

103. Linsdtedt, G.& Misiorney, A. — Acta.ChemScand. 5, 121 (1951).

104. Birch, A.J. — Fortsch. Chem.Org.Natstoffe, 14, 189 (1957).

105. Winters, D. — Informal Technical Report, F.A.O. of the United Nations (1968).

106. Lowson, J.M. — "Text Book of Bot." 352-6 (1962).

107. Dalziel, J.M. — "Useful plants of West Trop.Africa", p.206 (1937).

108. Broun, A.F. — "Silviculture in Tropics" , p.xvii (1912).

109. Keay, R.W.J., Onochie, C.F.A., & Stanfield, D.P. — "Nigerian Trees, vol.II", p.88 (1964).

110. Lawson, G.W. — "Plant Life in West Africa, pp.39 & 72, pp. 5-10 (1966).

111. Ghosh, D., Vijayalakshmi, K. & Nayudamma, Y. — Leather Sci.14, 154 (1967).

112. Official Method of Analysis - Society of Leather Trades Chemists, Herts (1965).

113. Okell, R.I. — J.S.L.T.C. 35, 225 (1951)

114. Atkin, W.R. & Burton, D. — J.S.L.T.C. 33, 52 (1949).

115. Edwards, W.V. — "The vegetable tanning materials of India" Part III Babul (Acacia arabica) Forest Research Institute, Dehadon.

116. Elsissi, H.L., Elsissi, A.A. Ishak, M.S. & Sherbieny, A.A. — Das Leder, 18, (2), 32-5 (1967 (Gen.)

117. Santhanam, P.S. Ghosh, & Nayudamma, Y. — Leather Sci., 14, 179 (1967).

118. Bhanu, K.U.; Rajadurai, S. & Nayudamma, Y. — Aust.J.Chem. 17 (7) 803-9 (1964).

119. Swain, T. & Hillis, W.E. — J.Sci.Food Agri. 10, 63 (1969)

120. Bhanu, K.U. Rajadurai, S. Sastry, K.N.S.& Nayudamma, Y. — Bull.Central Leather Res.Inst., Madras (India) 8, 380-1 (1962).

121. Rao, J.B. — Ph.D. Thesis submitted to Madras University (1961).

122. Atkin, W.R. & Thompson, F.C. — "Leath. Chemists' Pocket Book" pp.256, 146, 136 (1912).

123. Reed, R. — "Sci. for students of Leath.Tech"., pp.218-229 (1966).