THE CHEMICAL STUDIES OF THE TANNINS OF THE EXUDATE OF THE PLANT Eucalyptus citriodora, Myrtaceae Hook AND THE TANNING PROPERTIES.

THE CHEMICAL STUDIES OF THE TANNINS OF HIE EXUDATE OF THE PLANT Eucalyptus citriodora. Myrtaceae Hook AND THE TANNING PROPERTIES.

BY

BENEDICT BINCHAN DASHE

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY
ZARIA-NIGERIA

DECEMBER, 1999

DECLARATION

I declare that the work recorded in this thesis is my own research work and has not been submitted elsewhere for the award of degree or diploma. All sources of information are specifically acknowledged by means of reference.

_____

B.B. Dashe

_____

Date

CERTIFICATION

This thesis entitled "The Chemical Studies of the Tannins of the Exudate of the Plant Eucalyptus citriodora and their tanning properties" by Benedict Binchan Dashe meets the regulation governing the award of the degree of Doctor of Philosophy (PhD) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation

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ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisors, Prof. J.O Amupitan and Professor Musa Shok for their very deep commitment to this work, which has been a great inspiration and encouragement to me throughout. They thoroughly reviewed the manuscript and supplied the initial samples of the kinos for analysis. I am greatly indebted to them. Their encouragement is greatly appreciated.

The assistance of Mr. P. Ukoha of the Federal College of Chemical and Leather Technology (CHELTECH) Zaria in the running of the various chromatograms is greatly acknowledged. Also, the contributions of Messrs A. Ejila and H.I Echewe both of the CHELTECH are greatly appreciated. I sincerely thank the entire staff of the Research Laboratory of CHETECH where all the analyses of this work were carried out. I appreciate greatly the assistance of the staff of the (CHELTECH) tannery, particularly Messrs S. Tanko, M. Ejeh and A. Balarabe who processed the leather with the exudate. To them I am very grateful.

I appreciate with thanks the assistance of National Institute of Pharmaceutical Research and Development (NIPRD) Abuja through Dr. Nelson Ochekpe and his staff in the Department for supplying the HPLC and its use for this work.

I sincerely thank the Federal College of Chemical and Leather Technology for sponsoring me and offering its facilities for use, in this work.

My profound gratitude to Miss Mary Oyewo of Leather & Leather Products Department CHELTECH who typed the original manuscript and to my Secretary Miss Gloria Bauchi who completed the typing and correction of the manuscript. I appreciate deeply the assistance of Mr. S.O Ladeji of CHELTECH in drawing the structures in the computer. To them all I am grateful.

I wish to express my sincere thanks to the Head of Department of Chemistry, Ahmadu *Bello University, Prof. J.O* Amupitan and the staff of the Department for their support and encouragement throughout the period of this work. To them all I am very grateful.

I am grately indebted to my wife, Vehcit, for her devoted love and patience through out the period of this work. I thank our children too for their co-operation and the understanding they displayed.

DEDICATION

This work is dedicated to my late Mother, Mrs. Mbai Dashe, and my last two children, Master Domnan and Nanbyen, who were given birth to during the period of this work.

Also to the generation of young Leather Chemists and Technologists who will use this information to develop Leather Science and Technology in this country.

ABSTRACT

The exudate of Eucalyptus citriodora. (family) which was initially thought to be a gum was found to contain mainly tannins using 5% aqueous $FeCI_3$. Such tannin containing exudates that issue from the trunks of Eucalyptus and other trees are referred to as "kino". The exudate was collected from four identified trees throughout the investigation at a plantation behind the settlement. Laying Zomo, opposite the premises of the Nigerian College of Aviation Technology, Zaria

The reagents that were used to establish the nature of the "kino" included 5% Ferric Chloride, 5% gelatin solution, bromine water, ferric ammonium citrate, formalin, sodium nitrite and lime. The reagents indicated that the "kino" contained mainly hydrolysable tannins whose main constituents are gallotannins and ellagic tannins but with traces of condensed tannins otherwise known as catechol tannins or catechins. It is worthy of note that inspite of the different periods of collection of the kino, the 1R spectral properties of the various samples were identical. This further indicated that the same spectrum of tannins as stated above were present.

A solution of the kino in water was spotted against solutions of tannic and gallic acids on cellulose paper and chromatographed. The presence of free gallic acid in both the "kino" and tannic acid was confirmed. Free ellagic acid was, however, absent. The exudate was then hydrolysed with 2N hydrochloric acid and again chromatographed with tannic acid and gallic acid. The presence of gallic was further confirmed. There was also a strong indication of anthocyanidins as shown from the red colour of the hydrolysate. Alkaline hydrolysis (2N NaOH) of the exudate, subsequent chromatography and the use of appropriate detective methods, UV *(X* max) 366 nm before and alter exposure to NH_3, suggested that one of the probable components of catechol tannins was kaempferol

Quantitative analyses of the exudate showed that it contained an average of 50% tannins which compares well with all known commercial sources of tannins. Subsequent tanning trials and leather analyses (both physical and chemical) showed the leather to be of good resulting in the good quality leather products. These are the shrinkage temperature, 58°C, load at crack, 250NM^2, distention at crack, 6.7, waterproofness, 42.7gm, tensile strength, 212.03 NM^2, % Elongation at break average, 73NM", pH of unfiltered extract, 3.10, pH of 10 x dilution, 3.7, Difference Figure, 0.6, substance extractable by Dichloromethane, 4.57, volatile matter, 8.46 and Degree of Tannage, 39.50.

It was concluded that the exudate ("kino") contains mainly gallotannins and ellagitannins with traces of catechin tannins. It was established that the leather produced was of high quality as attested to by physical appearance and physical and chemical tests.

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CHAPTER ONE

1.0 INTRODUCTION:

Tannins are polyphenolic compounds found in plants. They have diverse structures and chemical properties depending on the monomeric phenolic molecules that make up the polymers. The molecular weights of these polymers vary from 500 - 4000 units. Tannins are soluble in water and generally miscible with solvents such as methanol, ethanol, acetone etc. Tannins have an astringent taste which may be detected either in fresh leaves, barks or in solution. Monosaccharide molecules like glucose are always found associated with the tannins in the form of glycosides. These would normally add to their easy solubility in water (Reeds 1956).

A chemical test that would easily indicate the presence of tannins is that of mixing a 5% $FeCl_3$ solution with a dilute solution of a suspected tannin. A dirty green or blue-black coloration suggests the presence of catechol and pyrogallol respectively, depending on whether the monomeric unit is catechol (catechin) or gallic/ellagic acid, respectively.

One of the most important chemical properties of tannins is their reactions with proteins, particularly the protein matrices of animal skins, to form leather. This reaction is not only important chemically but is important economically. Tannin converts putrescible or unstable raw hides and skins into leather which has adequate strength and resistance to various biological chemical, and physical agents. This is because additional cross-links are introduced into the collagen of the skin binding the active groups of the tanning agents to some functional groups of the protein matrices.

Tannins from different plants have been used, traditionally, for the production of leather. In Africa, for example, the use of vegetable infusions for the production of leather is believed to date as far back as 5000 years (Reeds, 1956).

The use of the pods of Acacia nilotica was first recorded in Upper Egypt while tannage with vegetable materials were common in the Mediterranean around 600 BC (White, 1956). Oak bark has been used in Britain and Germany since the Medieval Age, (Hathway, 1956). Oral evidence has shown that the use of Acacia nilotica pods for tanning in Northern Nigeria may be as old as in North Africa and the Mediterranean.

Whereas, elsewhere the chemical studies of tannins had been going on for a long time, the screening and assessment of the quantity and quality of tannins in Acacia nilotica and other plants did not really start in Nigeria until the early 1960s when the Leather Institute was established by the Food and Agricultural Organisation (FAO) of the United Nations Development Programme (UNDP) in conjunction with the Federal Government of Nigeria and the then Northern Nigerian Government. David Winters (1964) was the first person to screen Nigerian trees for tannins. Thereafter students of the Institute and other researchers have screened many more Nigerian trees for tannins both qualitatively and quantitatively (Adewoye, et al, 1971). However, not much of the chemistry of these tannins has been studied. Although some reasonable work has been done on the chemistry of Acacia nilotica (Adewoye et al, 1986), Parkia Clappertoniana (Adewoye and Shok and Lemus 1986), Anogeissus schimperii (Nduji and Okwute,1987), the study of the polyphenols of these vegetable materials has been very scantily done.

The plants of the genus Eucalyptus are alien to Nigeria (Dashe and Shok, 1993) even though many species such as Eucalyptus citriodora, E. camaldulensis E. terecticonis, E. closeana

and E. toreliana are presently grown in the country. The country of origin of the Eucalyptus plants is Australia. In Australia, parts of the plants particularly, the barks, have been used for tanning (Penfold and Willis, 1961). In addition to the morphological parts, that are used, some of the plants produce an exudate called "kino" which is almost completely a tannin product. E. citriodora is not known to exudate or produce "kinos" in its natural habitat, Australia. (Penfold and Willis, 1961) but it does so in Nigeria. Probably because Eucalyptus is alien to Nigeria, no tanner has tried any part of the plant for leather production. There is only very scanty information on any work being done on any species of Eucalyptus except that by the National Research Institute for Chemical Technology, Zaria (Mustapha. 1992), and the preliminary work on the "kinos" of E. citriodora as a source of tannins (Dashe and Shok, 1993).

1.1 Objective of the Work

The objective of this work is to study the chemistry of E. citriodora exudate with a view to identifying the major components. This, when done, would give an insight into the type of tannins and therefore the kind or type of tannage it can be used for. Preliminary work on the "kino" has shown that the "kino" contains about 33.7% tannins (Dashe and Shok, 1993) which was considered to be reasonably high. If this can be properly studied and the quantity established, then the plant could be a very promising one in Nigeria and establishing plantations of the same would be a worthy economic venture. Earlier work has also shown that leather produced from these tannins has very satisfactory characteristics (Dashe and Shok, 1993) compared, and in some cases, better than the leather normally prepared using Acacia nilotica tannins. The second objective of this work, therefore, would be to study the utilisation of "kinos" in the production of

leather. Comparative studies on the types of leathers produced would be made with similar leathers from the traditional "bagaruwa" tanning pods and combination tannage with chromium sulphate. This aims to establish the advantage or otherwise of the tannins of "kinos" over "bagaruwa" in view of the high tannin content.

The chemical and the physical properties of the leathers would be carefully studied in accordance with the Official Methods of the Society of Leather Technologist and Chemist. The parameters so studied would bring out the strong and the weak points of the leathers and this also would give a guide on the economic viability of using 'kinos' for the production of these kinds of leather.

1.2 Justification of the work

The current aspiration in the manufacturing industries and, in particular as regards chemicals, is the use of and the production of environmentally friendly chemicals and products. Every nation, and particularly the developed nations, is advocating for cleaner technology. This has resulted in stricter environmental laws at Local, States and Federal Government levels. Consequently the Federal Environmental Protection Agency (FEPA) in Nigeria is taking the matter very seriously. Chrome tannage, and indeed mineral tannages are undergoing very serious investigation to develop processes that would reduce environmental pollution (Clean Technology, 1995, IULTCS, 1997). Vegetable tannage as presently practised using bagaruwa pods produces a very high proportion of solid waste which currently is considered as a serious pollutant. Parkia Clappartoniana leaves a reasonable quantity of solid waste. It is imperative for Nigeria to look at sources of tanning materials that would leave less pollutants and yet are rich in tannins, and which

would give rise to and encourage the development of Clean Technology as is being advocated internationally. The crown eucalyptus, through its members such as E. citriodora has provided the immediate necessary raw material. Its potential from the preliminary work (Dashe and Shok, 1993) is very high. The high amount of tannins contents of the exudate and the lack of solid waste or any waste at all makes the exudate very attractive. The quality leather obtained from the preliminary work has added greatly to this attraction.

The fact that Eucalyptus does not seem to require any conscious extraneous factor before exudation is very attractive. The quantity and quality of tannins in the exudate is sufficiently high to warrant investment in the project. Similarly the quality of the resultant leather from the preliminary trials, makes the exudate a very good source of tannins for the tanning industry.

CHAPTER TWO

TANNINS AND TANNING

LITERATURE REVIEW

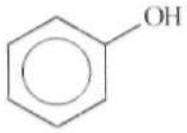
PART I

TANNINS

2.0 INTRODUCTION

2.1. Phenolic Compounds:

Tannins form a large part of the natural compounds known as phenols. Phenolic compounds, or simply phenols are derived from the simple molecule, phenol (1).



(1)

R - OH

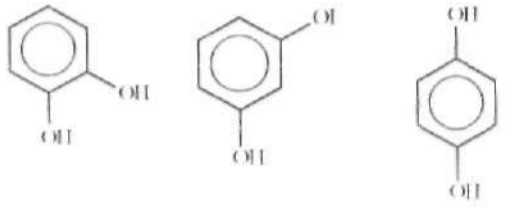
R = aliphatic group

R= CH_3CH_2- = Ethanol

(2)

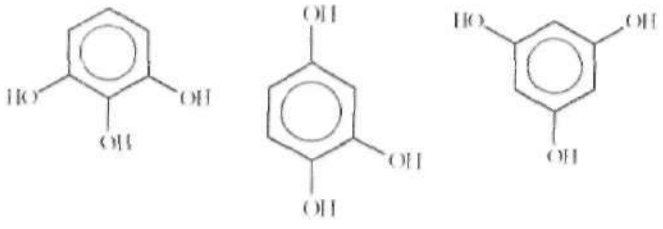
Phenols are classified as monohydric, dihydric, trihydric phenols, etc. according as they contain one, two, three, etc. hydroxyl groups (3-6). Although theoretically, the benzene ring may carry as many as six phenolic groups, in practice, the most common phenols are the monohydric, dihydric and trihydric phenols. The dihydric and trihydric phenols have possibilities of existing in isomeric forms.

Diphenols:



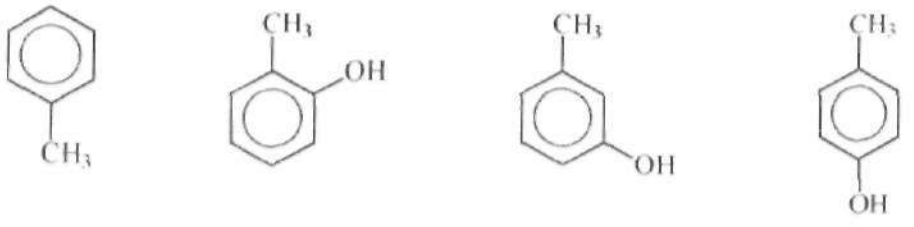
(3) (4) (5)

Triphenols



(6) (7) (8)

There also exist homologues of phenol which are called cresols (3 isomers). Cresols (10-12) are hydroxytoluenes.



(9) (10) (11) (12)

Higher homologues of phenol also exist e.g. thymol (3-hydroxy-4-isopropyltoluene) (13) and carvacrol (2-hydroxy-4-isopropyl toluene) (14) which are isomers.



(13) (14)

The higher homologues of phenol constitute a very large number and variety of compounds which will be dealt with in detail subsequently. In addition to these there are some important phenolic compounds that are derived from polynuclear hydrocarbons such as the naphthols and α- sorigenin glycoside from naphthalene and the anthraquinones from anthracene.

Phenols probably constitute the largest group of plant constituents referred to as secondary metabolites. They are widespread in nature and are to be found in most classes of natural compounds having aromatic moieties. As has already been shown, they range from simple structures with one aromatic ring to highly complex polymeric constituents such as tannins and lignins.

Phenols and similar compounds which have the grouping $\overset{|}{\underset{OH}{C}}=C$, as in the enols, have characteristic colour reactions with ferric chloride (5% solution):

Phenol + $FeCl_3$ →	Violet
Catechol + $FeCl_3$ →	Green
Resorcinol + $FeCl_3$ →	Blue-violet
Phloroglucinol + $FeCl_3$ →	Blue
Pyrogallol + $FeCl_3$ →	Dull-yellow
o – Cresol + $FeCl_3$ →	Violet Blue
p – Cresol + $FeCl_3$ –	Blue
α – *Naphthol* + $FeCl_3$ →	White ppt.
β – *Naphthol* + $FeCl_3$	White turbidity
Gallic acid + $FeCl_3$ →	Blue Black
Ellagic acid + $FeCl_3$ →	Blue Black

Salicylic acid, however, does not give any colour with $FeCl_3$

2.2. The Classification of Plant Phenolic Compounds.

Harborne and Simmonds (1964) have attempted to provide a fairly comprehensive classification of phenols based on the basic carbon skeleton; as shown below:

C_6	Simple Phenols
$C_6 - C_1$:	Phenolic Acids and Related Compounds.
$C_6 - C_2$:	Acetophenones and Phenylacetic Acids
$C_6 - C_3$:	Cinnamic Acids and Related Compounds

$C_6 - C_3$:	Coumarins, Isocoumarins and Chromones
C_{15}:	Flavones
C_{15}:	Flavanones
C_{15}:	Isoflavones and Isoflavanoids
C_{15}:	Flavonols, Dihydroflavonols and Related Compounds
C_{15}:	Anthocyanidins
C_{15}:	Chalcones, Aurones and Dihydrochalcones
C_{30}:	Biflavonyls
C_6-C_1-C_6, C_6--C_2--C_6	Benzophenones, Xanthones and Stilbenes
C_6, C_{10} and C_{14}:	Quinones
C_{18}:	Betacyanins

However, even though this comprehensive classification is informative and useful, a simpler classification would serve the purpose of the present study adequately. In this simpler classification there are five groups;

a. The simple phenols
b. The phenolic acids (Benzoic acids, Cinnamic acids and Coumarins)
c. Flavonoids and Related Compounds
d. Other C_6-C_3-C_6 Phenols (Non-Flavonoid)
e. Miscellaneous Phenolic Compounds.

2.2.1. The Simple Phenols (C_6)

The simplest phenol (1), phenol itself, catechol (3) hydroquinone, (quninol (5)), phloroglucinol (8), have on occasion (except for resorcinol (4)) been reported as occuring in nature, but they are not frequently encountered.

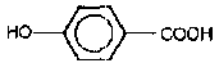
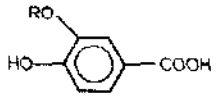
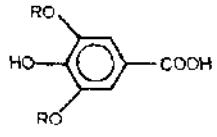
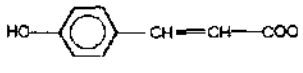
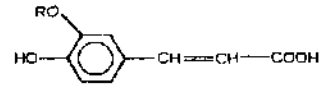
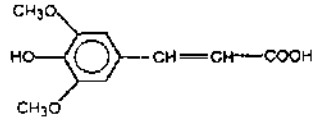
However, catechol (3), phloroglucinol (8) and, occasionally, resorcinol (4) residues occur in the more complicated molecules of the flavonoids (Ribereau-Gayon, 1972). These simple

phenolic constituents are not usually found in the free state but in the form of esters or, more generally, as glycosides.

2.2.2. The Phenolic Acids and the Coumarins (C_6-C_1, C_6-C_3)

The term phenolic acid includes the benzoic acids C_6-C_1 and the cinnamic acids, C_6-C_3. The benzoic acids include salicyclic acid (orthohydroxy benzoic acid) (15) p-hydroxy benzoic acid (16) protocatechuic acid (17), vanillic acid (18), gallic acid (19), syringic acid (20), and gentisic acid (31). The principal cinnamic acids are four in number, as shown in Table I.

Table I

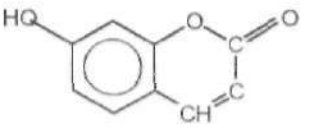
	Monohydroxy	Dihydroxy	Trihydroxy
	salicylic acid (15)	gentisic acid (19)	
Benzoic acids			
	(16) p-hydroxybenzoic acid	(17) R = H, protocatechuic acid (18) R = CH_3, vanillic acid	(20) R = H, gallic acid (21) R = CH_3, syringic acid
Cinnamic acids			
	(22) p-caumaric acid	(23) R = H, caffeic acid (24) R = CH_3, ferulic acid	(25) sinapic acid

Practically all plant tissues contain at least one of these phenolic acids. p-Coumaric acid (22) and caffeic acid (23) are among the commonest of all the phenolic constituents. Ferulic (24) and sinapic (25) acids are also widely distributed (Table I). All the phenolic acids occur naturally in combination, usually in the form of esters. These combinations are well known in the case of

the cinnamic acids (combinations with quinic acid, sugars, tartaric acid), the ester of caffeic-acid (23) with quninic acid, chlorogenic acid, is the classic example.

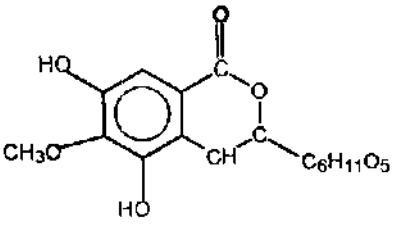
The coumarins are considered along with the cinnamic acids, since they also possess the C_6-C_3 configuration, the C_3 chain in their case being in the form of an oxygen heterocycle in the form of what is called internal ester (lactone).

Umbelliferone (26) is an example of a coumarin.

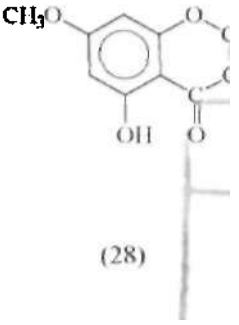


(26)

The iso-coumarins e.g. bergenin (27), are isomeric with the coumarins i.e. they also have C_9 in the basic carbon skeleton even though they are not of the form C_6-C_3 but of the form C_6-C_2 -C_1). They are more restricted in distribution, only six of them being known compared with upwards of fifty coumarins. Coumarins and iso-coumarins differ in the relative positions of the O and CO groups in the heterocycle. The coumarins and the iso-coumarins are chemically related to the chromones which also have the C_6-C_3 skeleton but are much less common. The simplest example is that of eugenin (28). It should be noted, however, that chromones are not internal esters.



(27)



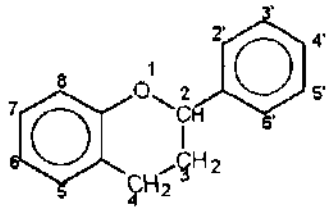
(28)

2:2:3 The Flavonoids and Related Compounds

These compounds are characterised by the C_6 - C_3 - C_6 structure in which the two benzene rings are linked by a C_3 group. (Structured C_6 - C_3 - C_6 the flavones, if "Flavonoids"). The different classes within the group are distinguished by additional oxygen-heterocyclic rings and by different patterns. The various types are distinguished by the oxidation state of the C_3 chain.

The flavonoids group may be described as series C_6 - C_3 - C_6 compounds. That is their carbon skeleton consists of two C_6 groups (substituted benzene rings) connected by three-carbon aliphatic chain.

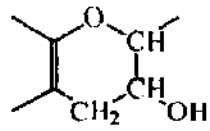
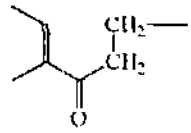
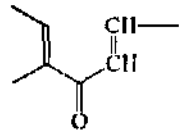
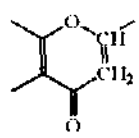
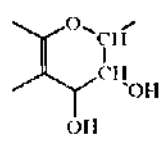
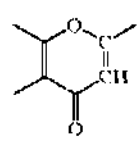
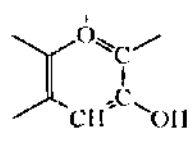
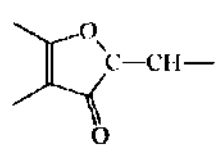
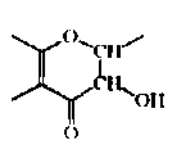
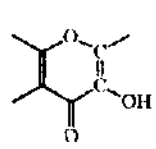
Flavonoids frequently occur as glycosides. The largest group of flavonoids is characterized by a pyran ring linking the three-carbon chain with one of the benzene rings. The numbering system for these flavonoid derivatives is given below (29):



(29)

Among the typical flavonoids having the above skeleton the various types are distinguished by the oxidation state of the C_3 chain. Going from most reduced to most oxidized, the structures and their names are as follows (only the key portion of the molecule is shown).

Table II

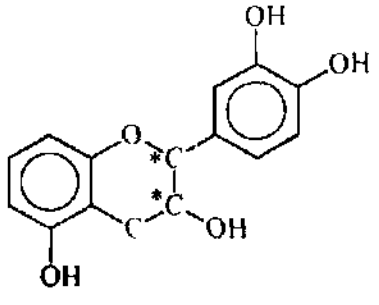
		Class	Name	Hydrogenation
A—CH_2—CHOH—CHOH—B →		Catechins (flavan-3-ols)	catechin gallocatechin	5,7,3',4' 5,7,3',4',5'
A—CO—CH_2—CH_2—B →		Dihydrochalcones	phloretin hydroxyphloretin	3,2',4',6' 3,4,2',4',6'
A—CO—CH=CH—B →		Chalcones	butein	3,4,2',4'
A—CO—CH_2—CHOH—B →		Flavanones (dihydroflavones)	naringenin butin eriodictyol	5,7,4' 7,3',4' 5,7,3',4'
A—CHOH—CHOH—CHOH—B →		Leucoanthocyanidins (flavan-3,4-diols)	leucocyanidin leucodelphinidin	5,7,3',4' 5,7,3',4',5'
A—CO—CH_2—CO—B →		Flavones	epigenin luteolin	5,7,4' 5,7,3',4'
A—CH_2—CO—CO—B →		Anthocyanidins	pelargonidin cyanidin	5,7,4' 5,7,3',4'
A—CO—CO—CH_2—B →		Aurones	sulphuretin aureusidin	6,3',4' 4,6,3',4'
A—CO—CO—CH_2—B →		Flavanonols (dihydroflavonols)	fustin taxifolin	7,3',4' 5,7,3',4'
A—CO—CO—CHOH—B →		Flavonols	kaempferol quercetin	5,7,4' 5,7,3',4'

Generally the A ring is either *meta*-dihydroxylated as in resorcinol or *meta*-trihydroxylated like in phloroglucinol (8), except in the chalcones i.e butein where one of the hydroxyls of the A ring is combined in an oxygen heterocycle of five or six atoms like in the Flavonols. The B ring is either monohydroxylated, *ortho*-dihydroxylated or *vic* - trihydroxylated. This difference has been shown to be due to the two aromatic rings being of different biosynthetic origins. Ring A is formed by the condensation of three molecules of acetic acid (The Acetate route) while B ring is derived from sugars by the Shikimic Acid route. The different OH groups may be methylated (Ribereau - Gayon 1972).

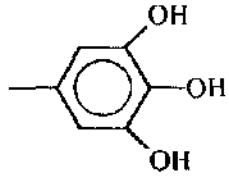
The benzenoid A rings are hydroxylated relative to C_3 chain, in the *ortho* position, through which cyclisation takes place. Consequently, such unique reactions as enolisation, elimination of water or formation of the pyrylium ions takes place without change in the oxidation of the molecule (Geissman 1963b).

2:2:3:1 The Catechins (Flavan -3 -ols)

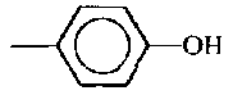
The catechins (30) are the most reduced flavonoids and they are colourless compounds, existing throughout the plant kingdom but especially in the higher woody plants. There are 3 types of catechin known, differing in the number of hydroxyl groups in ring B.



(31)

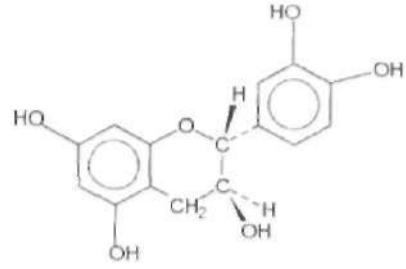


(32)

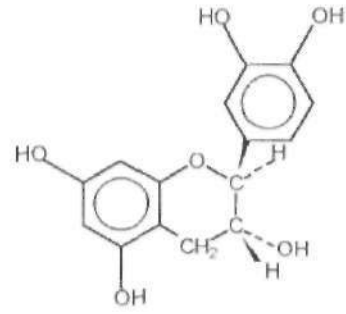


(33)

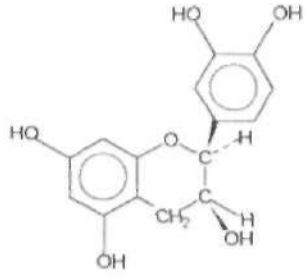
They have two assymetric carbon atoms, C_2 and C_3, and therefore 4 possible isomers.



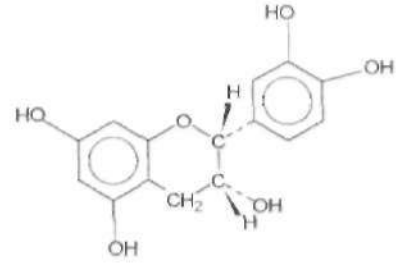
(33)



(34)



(35)

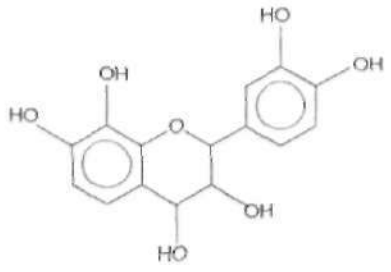


(36)

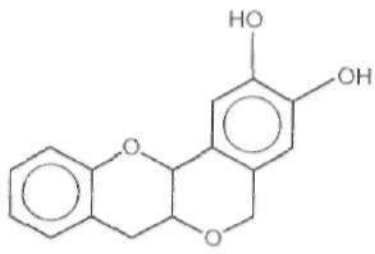
The (+) and (-) catechins (33, 34) have the C_2 and C_3 hydrogens *trans*, whereas, they are *cis* in the epicatechins (35, 36). One epicatechin glucoside has been found, and some catechins may occur as esters of gallic acid. The catechins are important because it is believed that they polymerise to form the condensed tannins.

2.2.3.2. Leucoanthocyanidins (Flavan-3,4-diols)

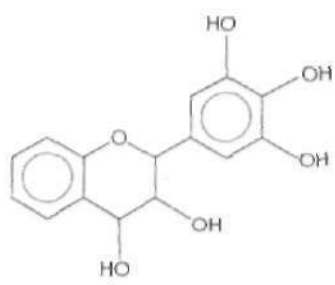
The leucoanthocyanidins are also referred to as flavan-3, 4-diols or slight variations of the structure. They are at a higher level of oxidation than the catechins. They rarely exist as glycosides although a few are known.



(37)

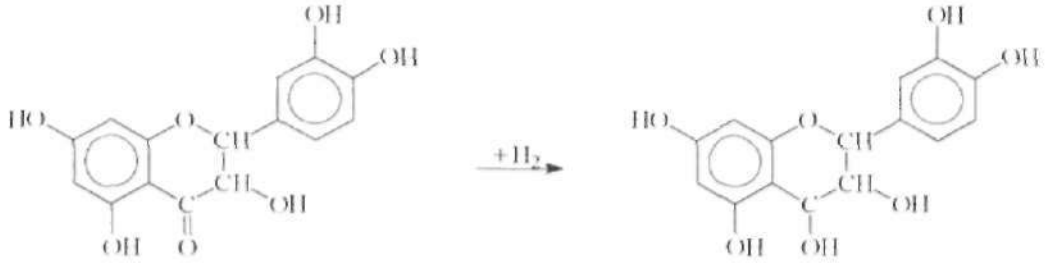


(38)

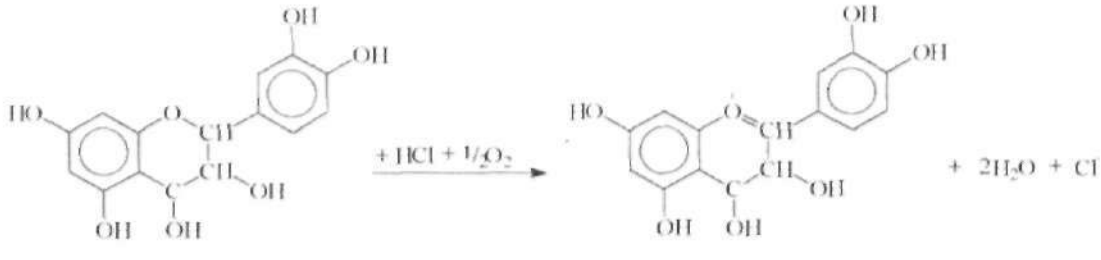


(39)

The leucoanthocyanidins, along with the other so called proanthocyanidins, form anthocyanidins on heating with acid. But more importantly, the leucoanthocyanidins, like the catechins (flavan-3-ols), polymerise to give the condensed tannins. (Michaud and Masquelier 1968)



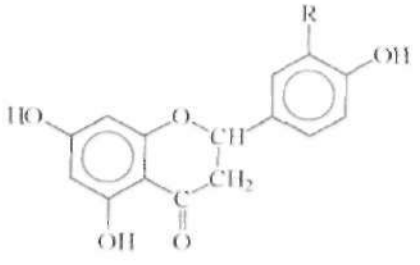
(40) (41)



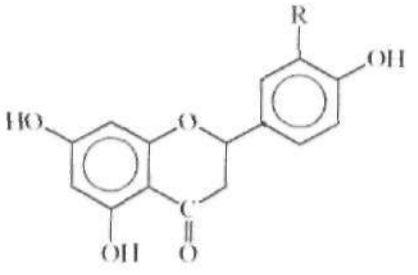
(42) (43)

2.2.3.3. Flavanones

The flavanones are at the same oxidation level as the leucoanthocyanidins (flavan-3,4-diols). They are also called 2,3-dihydroflavanols. They occur in only small amounts compared with the other flavonoids. They are either colourless or only slightly yellow.



(44) R = H
Naringenin



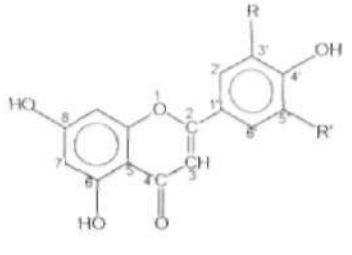
(45) R = O
eriodictyol

2.2.3.4. Flavanonols

The flavanonols are also referred to as, 3-hydroxyflavanols. They are at the same oxidation level as the flavones and the anthocyanidins. They are derived from the flavonols in the same way that flavanones are derived from flavones, by reduction of the double bond between C_2 and C_3. The flavanols may therefore be also called 3-hydroxyflavanones. Such substances e.g. taxifolin (40), occur essentially in association with tannins in the heartwoods of different species.

2.2.3.5. Flavones

The flavones have the basic structure shown below:

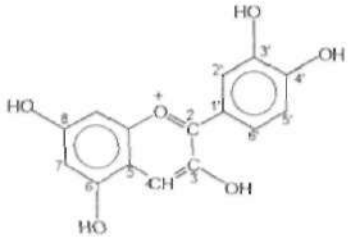


(46) R = R' = H: Apigenin
(47) R = OH, R' = H: Luteolin
(48) R = R' = OCH_3: Tricin

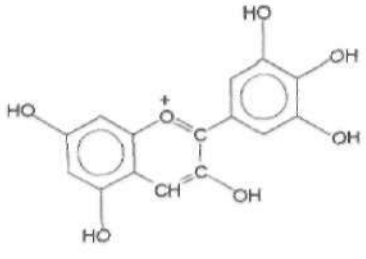
They are at the same oxidation level as the flavanonols and anthocyanidins. They are less important than the flavonols but they occur quite frequently in many families of the Angiosperms.

2.2.3.6. The Anthocyanins

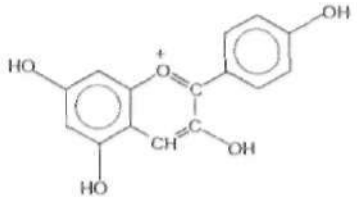
These are red pigments in acid solutions, turning blue in alkali and when they form complexes with metals. They have a C_6-C_3-C_6 structure in which the C_3 element is in the form of a pyrylium ion (they are cations). They are very widely distributed in flowers and fruits and are the most interesting of all the phenolic compounds. They occur naturally as glycosides but the aglycones from which they are dervied are quite few in number and only six are widely distributed. The structure of cyanidin (43), delphinidin (48) and pelargonidin (50) are given below. Cyanidin (43) is the most common aglycone.



(43)



(48)

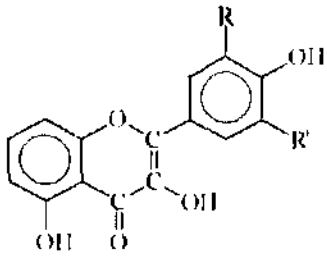


(50)

(49) R= R' = CH, Malvidin

2.2.3.7. The Flavonols (3-Hydroxy flavonones)

These are the highest level of oxidation of the flavanoids. They are the most widely distributed. The three commonest members are quercetin (51), kaempferol (52) and myrecitin (53). Quercetin (51) is the phenolic compound with the widest distribution in nature.

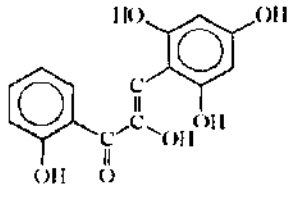


(51) R = OH, R' = H;
(52) R = R' = H;
(53) R = R' = OH;

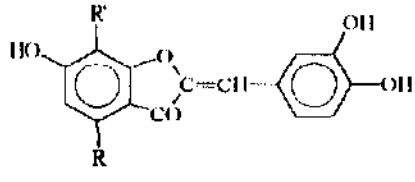
2.2.3.8. Other C_6-C_3-C_6 Phenols (Chalcones, Dihydrochalcones and Aurones)

These are all phenolic compounds having the structure C_6-C_3-C_6, but the C_3 is either not heterocyclic, or is in the form of a pentacyclic ring as in the case of aurones. Chalcones and aurones are the yellow pigments of some flowers, their colour changing to red or orange-red in the presence of ammonia. An example of chalcones is butein (54) while those of aurones are aureusidin (58) and sulphuretin (57). These are not widely distributed and are relatively few.

Not much is known about the dihydrochalcones (55) which are derived by the hydrogenation of the double bond. The best known example is phloretin (56).

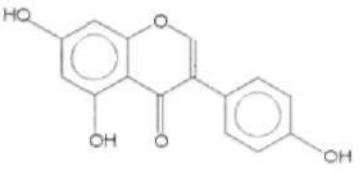


(56)



(57) R = R';
(58) R = CH, R' = H;

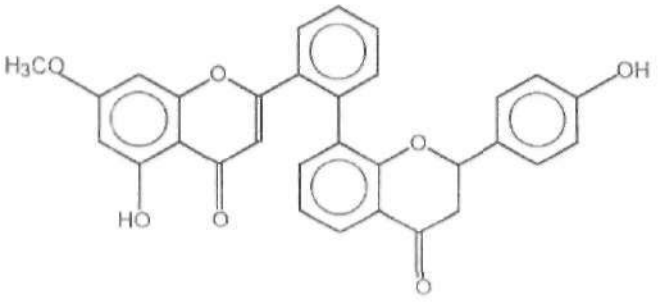
The isoflavones are included in this group mainly because the group B benzene ring is attached to the C_2 carbon instead of the usual C_3. They are much less widely distributed than the flavones, but there are many of them, and they often have structural features rarely met with in the other flavonoids. Genistein (59) is a typical example.



(59)

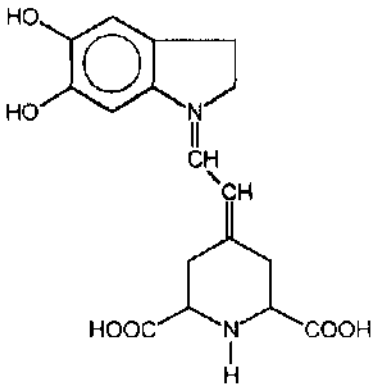
2.2.3.9. Miscellaneous Phenolic Compounds (The Biflavonyls, Betacyanins, and Betaxanthins)

The biflavonyls are C_3O dimers either of apigenin (46) (or of a methylated derivative of this flavone. Except very rarely, these are confined to the gymnosperms. Among the dozen or more known members of this group, ginkgetin (60) is the most familiar.

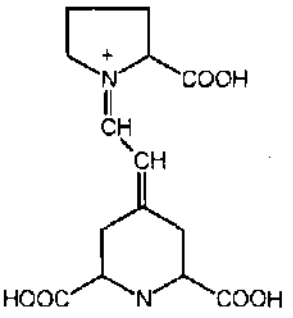


(60)

The betacyanins are red pigments responsible most notably for the colour of the red beet (Beta vilgaris). They are uniquely present in the Centrospermae and also in the Cactaceae, which are now recognised as being included along with the Centrospermae within a wider order. Owing to their resemblance to the anthocyanins, (e.g. their absorption spectra) and the presence in them of two atoms of nitrogen, they were for a long time known as "nitrogenous anthocyanins". Their structures have now been elucidated as glycosides which, when hydrolysed with acid, yield betanidin (61). They are often acetylated.



(61)



(62)

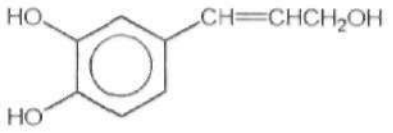
Closely related to the betacyanidin and also found in the Centrospermae are some yellow nitrogen containing pigments, the betaxanthins, indicaxanthin (62) is, however, not a phenolic compound.

2.2.3.10. Polymeric Phenols (Tannins and Lignins)

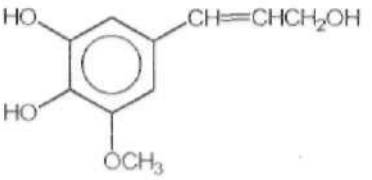
Tannins and lignins are polyphenolic compounds present as glycoside or esters in the plant tissue.

Lignins may also be defined as the non-carbohydrate moeity of all memberanes (Trease and Evans, (1972). Their composition varies according to the kind of plant from which they are extracted, and the conditions of extraction. The chemical structure is not, however, well known enough to be able to characterise the differences in terms of the origin of the plant material.

Barnoud (1965) has suggested that lignin results from the polymerisation of a C_6-C_3 compound corresponding essentially to coniferyl alcohol (63) and its methoxylated derivative sinapyl alcohol (64).

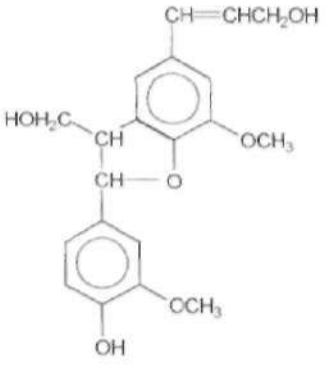


(63)

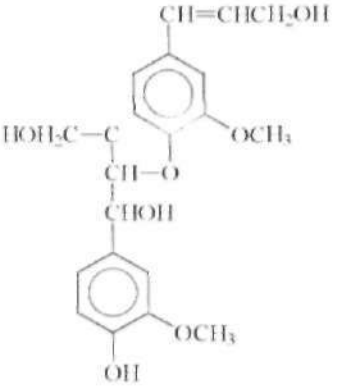


(64)

It is not possible to hydrolyse lignin, as one may hydrolyse a polysaccharide, and obtain a simple, well-defined product. This is because lignin possesses an ordered coniferyl (63) structure. Freudenberg (1959) has shown that the condensation of coniferyl alcohol (63) occurs by several oxidative pathways leading to dimers of different structures, for instance dehydrodiconiferyl alcohol (65), pinoresinol and the β-coniferyl ether (66) of guaiacyl glycerol (66) lignin being formed by the eventual condensation of these dimers, which represent the true building stones of the polymer.

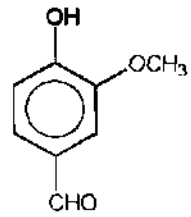


(65)



(66)

This theory accounts for the complexity of the lignin structure although it is based on a severely restricted number of basic units. Drastic chemical treatment with acids and bases could lead to the formation of small molecules like vanillin (67). Infact lignin from paper manufacture is used as a source of vanillin (67).

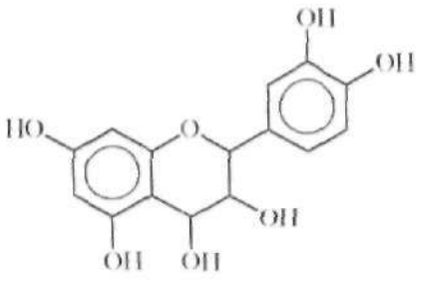


(67)

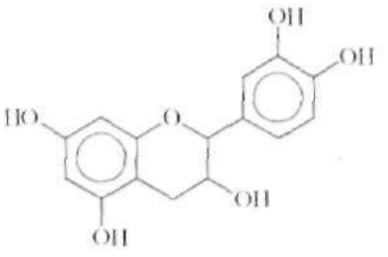
It is not easy to give a definition of the tannins, bacause this term comprises an assemblage of substances united not by analogy of structure, but by the possession of certain properties in common. Etymologically, tannins are substances used in tanning hides and skins to produce leather. Swain and Bate-Smith (1962) define tannins as phenolic compounds having molecular weight between 500-3000 and which, as well as having the classical properties of phenols, precipitate alkaloids and gelatin and other proteins.

Depending on their molecular structure, tannins are customarily divided into hydrolysable and condensed tannins. Hydrolysable tannins are complex molecules containing ester type linkages which yield, on hydrolysis, a sugar residue and a phenolic residue consisting either of gallic acid (20)or ellagic acid (68) which is a dimer of the former.

The properties of condensed tannins depend upon the nature of the molecular unit of which they are composed, the mode of linkage of these units, and above all on the molecular weight of the condensation product. It appears to be well established that they are formed by the polymerisation of molecular units having the general structures of flavonoids the most important of which are flavan-3-ols (catechins) (30) and flavan-3, 4-diols (leucoanthocyanidins) (41). A more detailed discussion of tannins will follow in subsequent sections.



(30)



(41)

2.3. The Combined Forms of the Flavonoids

Phenols in the natural state occur as esters or glycosides. The glycosidic link in flavonoids occur in selected positions. For instance, the anthocyanins are nearly always glycosylated at position, 3 and 5 while it is at position 7 for the flavones and 3 for flavonols. D-glucose is the most common sugar in the glycosidic combination. Other sugars involved are L-rhamnose, D-galactose, L-arabinose, D-xylose and several di-or trisaccharides, e.g. rutinose. Consequently, for any aglycone there may be many different glycosidic combinations, which is the reason for the very large number of phenolic compounds known to occur in the plant kingdom. Similarly, for the same reason, there may be many different consitutents to separate and identify in any one organ. It is advisable therefore, that when studying an unfamiliar plant material, to begin by studying the simple phenolic substances liberated by hydrolysis of their glycosides or esters, before embarking on the analysis of their numerous structural combinations. It is only in the flavans i.e. catechins and leucoanthocyanidins that the flavonoid residues are not combined wih sugar, but are usually polymerised to form the condensed tannins (Ribereau-Gayon 1972).

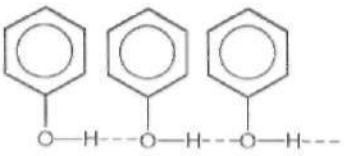
2:4 The Chemistry of Phenols.

In section 2:3, a survey of the large array of phenolic compounds was made. It would be expected that because of the large number and varieties, the chemistry of these compounds would also be complex. This complexity is due largely, to the presence of the hydroxyl group (-OH) attached to one of the six carbon atoms in the benzene nucleus (aromatic radical). This may be compared with the -OH group attached to an aliphatic radical e.g ethyl alcohol (ethanol (2)): However, that is nearly as far as the similarity goes. There is a profound distinction between the hydroxyl of aliphatic radicals and that of phenol, because of the presence of the benzene ring. Thus when studying the chemical properties of the phenols, it is necessary to consider both the effect of the phenolic hydroxyl and effect of the latter on the properties of the benzene ring to which it is attached.

2:4:1:1 Properties of the Phenolic Group.

a. Formation of Hydrocarbons bonds. Like the alcohols, phenols are the sites of intermolecular and intramolecular association by way of hydrogen bond. These hydrogen bonds modify numerous physical properties such as melting and boiling points and spectral properties such as ultraviolet and infrared spectra. The source of the hydrogen bond is essentially electrostatic. It occurs when the hydrogen atom is combined with an electronegative atom such as oxygen. The electronegative atom reducing the electron density of the hydrogen atom, as we saw in the case of phenol. In order to make up for this deficiency, the hydrogen atom attaches itself to another neighbouring oxygen atom (69) that has a high electron density. The bond

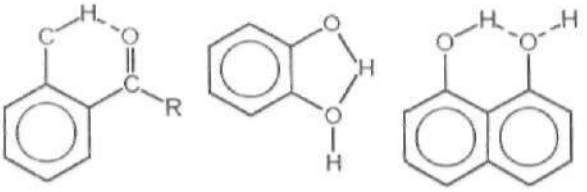
attachment is usually represented by a dotted line to show that it is a covalent bond, and molecules so associated recover their autonomy when they enter the gaseous phase.



(69)

Although the hydrogen bond is weak, it raises the boiling point because it increases the energy necessary to free a molecule from interaction with its neighbours.

In the case of natural phenols, (Thomson, 1964) intramolecular hydrogen bonds are common, notably when an O - hydroxyacetophenone (70) grouping is present. This is especially evident in the case of the flavone derivatives hydroxylated in position 5 (e.g quercetin (51) and taxifolin (40). Simarlarly, infrared spectroscopy has revealed the presence of hydrogen bonds between the -OH group in position 3 and the heterocyclic oxygen in the case of the catechins (30).

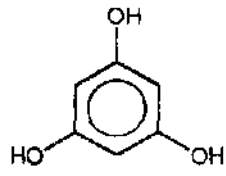


(70) (3) (71)

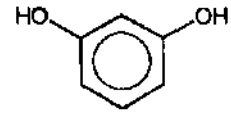
Hydrogen bonding generally reduces the reactivity of the phenolic groups, for example their solubility in alkali, and their ability to form esters and ethers.

Hydrogen bonds are stronger when they are concerned in the formation of a 6-membered "ring" e.g in the case of O-hydroxy-acetophenone (70) or 1, 8 - dihydroxy-naphthalein (71) than when the "ring" so formed has only 5 members e.g in catechol (3).

Hydrogen bond may lower solubility. For instance, in spite of having three hydroxyl groups, phloroglucinol (8) is much less soluble in water than resorcinol (4) with only two and its (phloroglucinol) melting point (219°C) is much higher than that of resorcinol (118°C). These bonds may also make it more difficult to purify phenols because they tend to form hexagonal structures arising out of six phenolic groups linked by hydrogen bonds forming cavities, (Clathrates) giving inclusion complexes with a large number of organic molecules, especially with those of the solvent used in the purification process.



(8)

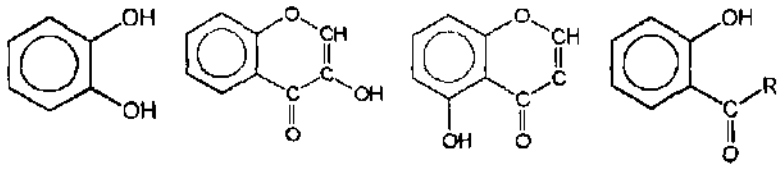


(4)

2:4:1:2 Formation of Complexes with Metals:

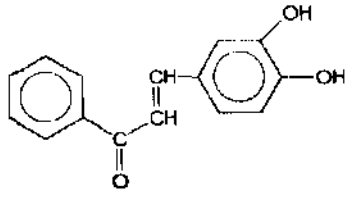
Phenolic compounds form complexes with metals (particularly iron and aluminium). These complexes are concerned in the natural colours of plants. Considerable use is made of the ability of these phenolic-metal complexes in revealing spots on chromatograms, in estimation, and most particularly in spectrophotometry.

The mechanism of the formation of the complexes has been little relatively studied, but Jurd and Geissman (1956) have pointed out that a large number of natural phenolic compounds posses certain structural groups capable of forming metal complex such as O - dihydroxy phenols, 3- and 5 - hydroxychromones and O - hydroxycabonyl residues



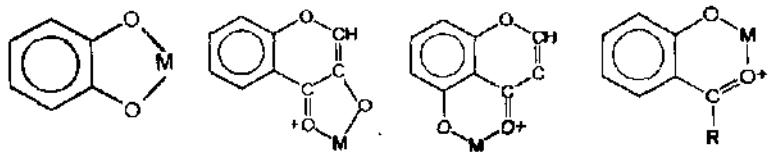
(3) (72) (73) (70)

Jurd and Geissman have also shown that the configuration of the molecule as a whole was concerned in complex - formation. For instance, aluminium chloride has much less effect on the absorption spectrum of catechol (3) than on that of 3,4 - dihydroxy chalcone (74) which has a CO group conjugated with the catechol group.



(74)

The modification of spectrum is also considerable in the presence of sodium aceate i.e under mildly alkaline conditions. This indicates the existence of a relationship betwen the formation of the complex and the ionisation of at least one of the phenolic functions. Geissman has represented the possible complexes structures thus.



(75) (76) (77) (78)

The stability of complexes depends on the structure of the groups concerened. Thus, the complexes formed with o-dihydroxy carboxyl groups (77-78) are more stable than those fomed

with o-diphenols (75-76). For this reason also, Fe and Al complex better with flavonols than with dihydroxylic anthocyanins.

In absorption spectra, metallic complexes produce changes which are quite characteristic of the substance studied. Use is also made of these complexes in the development of chromatograms and in the estimation of phenolic compounds.

2:4:1:3 Formation of Esters and Ethers:

Esters are formed by the reaction of a carboxylic acid with the OH group of an alcohol or phenol, by the removal of water from two molecules of an alcohol phenol. In nature, esters of phenols are practically unknown, apart from certain compounds of gallic acid (20), such as ellagic acid (67) which contains two lactore units.

Generally, the esters which are considered are those that are formed from the combination of an acid phenolic group and an alcoholic hydroxyl of another molecule. The phenolic compound is not in this circumstance functining as a phenol. Ester linkages are broken by alkaline hydrolysis (saponification) usually in the cold.

The formation of ethers is much more common especially methyl phenyl ethers (a reaction between CH_3OH and the phenolic group.



(79)

The methoxyl group (-OCH_3) of (79), is very stable. The OH group may be methylated using dimethyl sulphate $(CH_3)_2SO_4$ in anhydrous acetone in the presence of anlydrous sodium

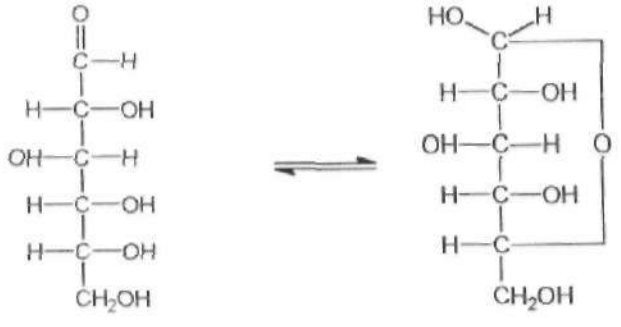
carbonate. This reaction is useful in determining the position of the glycosyl residues in flavones and flavonols.

Another bond that is closely related to the ether bond is the glycosidic or glycosyl bond. The glycosyl bond is a semi-acetal bond (82) which may be formed by the interaction of an aldehyde (80) with an alcohol (81);



(80) (81) (82)

The glycosyl bonds are not as stable as the methoxyl (79), (ether) bonds. They are ruptured by heating with acid, whereas methoxyl groups are not affected by this treatment. In sugars particularly hexoses both hydroxyl and carboxyl groups are present and this may lead to the formation of internal hemi-acetals as seen in (83) and (84).



(83) (84)



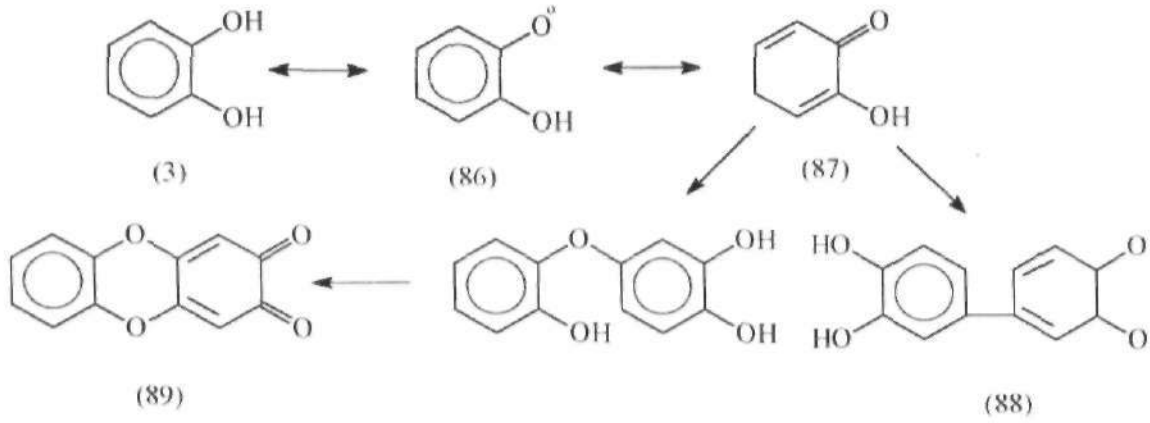
(82) (1) (85)

Acetals (85), are therefore, mixed ethers of the unstable hydrates of aldehydes (or lactones). The acetalisation reaction is reversible and this is made use of in the study of glycosides.

2:4:1:4 Oxidation

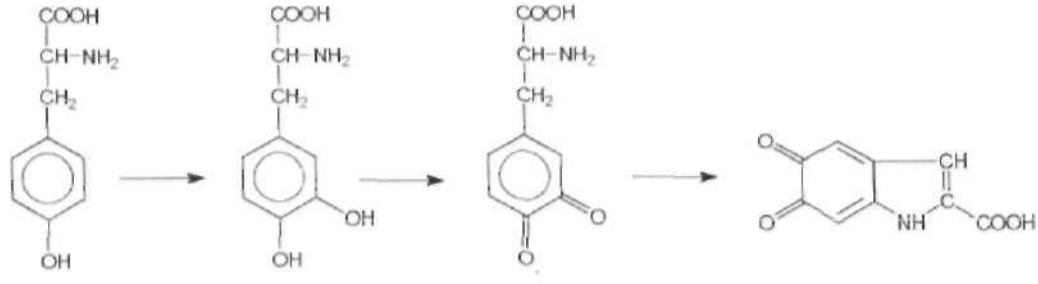
Oxidation reactions involving phenols have been little studied. The main reason is the difficulty of such reactions as has been mentioned earlier concerning the benzene ring. This notwithstanding, there are certain well known chemical and biological oxidative reaction such as the ones leading to the browning of plant tissues. The ease with which some phenols oxidise also renders them useful as auto - oxidants. Oxidative mechanisms are also involved in the biosynthesis of phenolic constituents.

It is believed that the first step in the process of attack of oxidising agents on phenols is the removal of a hydrogen atom and the formation of free radical having a singly - bound oxygen atom carrying an unpaired electron (Fieser and Fieser, 1956). Usually such a radical is very unstable and changes rapidly into one which has, in the ortho or para position, a trivalent carbon atom carrying an unpaired electron. The radical so formed can dimerise or can react with another radical forming, in order of importance, C - C, C - O, or O - O bonds (Thomson, 1964). In the case of catechol (3) (Forsyth and Quesnel, 1957, Forsyth et al, 1960) these two types of oxidations lead by way of the radicals (86) and (87) to a mixture containing several tetrahydroxy biphenyls (88) and a quinone (89).



(3) (86) (87) (89) (88)

A lot of attention has been paid to enzymatic oxidation processes. The enzymes are termed "phenolases" "phenoloxidases", polyphenoloxidases or even 'O_2 oxidoreductases'. The oxidation of tyrosine (90) (an amino acid) is affected by the enzyme called tyrosinase. The first step is the formation of dihydroxy-phenylalanine (91), which is oxidised to a quinone (92) capable of cyclisation to a red derivative (93), which then by further oxidation gives a red-brown polymerisation product melanin (Burges, 1963).



(90) (91) (92) (93)

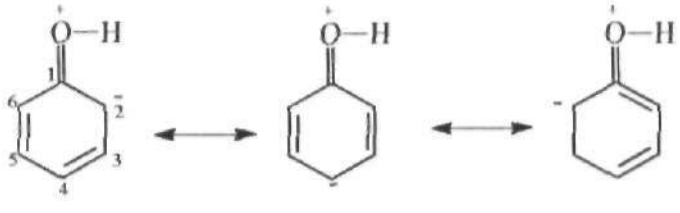
Various oxidising agents are used for the determination of phenols and for their detection on chromatographic papers. The agents include, ammonium silver nitrate, Folin - Denis reagent which is a mixture of ($H_3PW_{12}O_{40}$, phosphotungstic acid, $H_3PM_{O12}O_{40}$) and phosphomelobolic and potasium permanganate KMO_4, etc.

2:4:1:5 Specific Properties of The Benzene Ring of Phenols:

The benzene ring is very stable as has already been indicated. It undergoes substituition reactions (replacement of H by a substituent) more readily than it does additional reactions. All these substitutions are the results of the attack upon the benzene ring by an electrophilic agent i.e one defficient in electrons. This electrophilic reagent is formed from a molecule X-Y by the action of a catalyst Z.

$$X - Y + Z \longrightarrow X^{+} + YZ$$

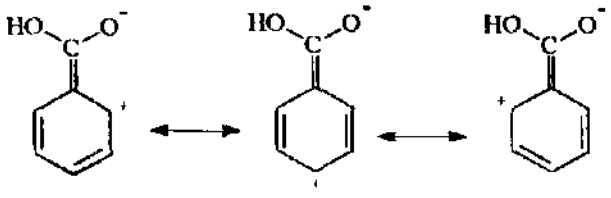
The Friedel - Crafts reaction provides the classic example in organic chemistry of electrophilic substitution in the benzene ring. It consists in the substitution of a hydrogen atom of the ring by an aliphatic radical, brought about by the action of an aliphatic halide (e.g. methyl chloride, CH_3Cl) on a benzenoid compound (e.g. benzene itself C_6H_6), in the presence of aluminium chloride, $AlCl_3$ which plays the part of a catalyst and promotes the formation of the electrophilic radical CH^{+}_{3}. In the case of phenol, the mesomeric effect accords high electron density in positions 2, 4 and 6, i.e *ortho* and *para* positions (94) to the phenolic hydroxyl. Substitution reactions that require high eletron density favour these points. It can be said that the phenolic group functions as an electron donor. The net effect is that substitution reactions are easier with phenol than with benzene.



(94)

For phenols with more than one - OH group, substitution is easier if the -OH groups are in alternate positions as in resorcinol (4) and phloroglucinol (8) than if they are adjascent to each other as in catechol (3) and pyrogallol (6). This is important because catechol (3) and pyrogallol (6a) are very common in nature especially in the flavonoids.

Other groups e.g the CO group, behave as acceptors of electrons. For example, in the case of benzoic acid, the mesomeric form involves three limiting structures (95). The electron density and hence the reactivity is decreased in position 2, 4 and 6.



(95)

The electron density is about normal at position 3 and 5. But the end result is a "deactivation" of the benzene ring and an orientation of substitution in the *meta* position. This explains why substitution reactions occur more easily in the ring of natural flavans, catechins (30) than in that of flavones and flavonols e.g , quercetin (51). In each case the A ring has the phloroglucinol pattern of substitution with one hydroxyl group in the heterocyclic bond. For example vanillin (67) reacts with catechin (30) but it does not react with quercetin (51). In either case, the B ring, with catechol type substitution, is less reactive, and does not react with vanillin (67). Substitution reactions undoubtedly occur *in vivo* in the biosynthesis leading to the formation of various phenolic constituents of plants (Thomson, 1964).

The following reactions are used in the study of natural phenolic compounds especially in their determination or in revealing them on, cromatograms: (i) coupling with diazonium salt; (ii)

condensation reactions with aldehydes e.g vanillin (iii), nitrosation i.e. Heopfner's reaction (iii); Gibb's reagent (2,6 - dibromobenzo quinone chloroimine), (iv) the use of 4 - amino phenazone etc.

Chromatography and spectroscopy are also used extensively in the study of phenolic compounds (Ribereau-Gayon, 1972).

2:5 General Biosynthetic Sources of Phenolic and other Aromatic compounds

Various routes or pathways have been used to explain the formation of phenolic compounds generally. Amongst these are:

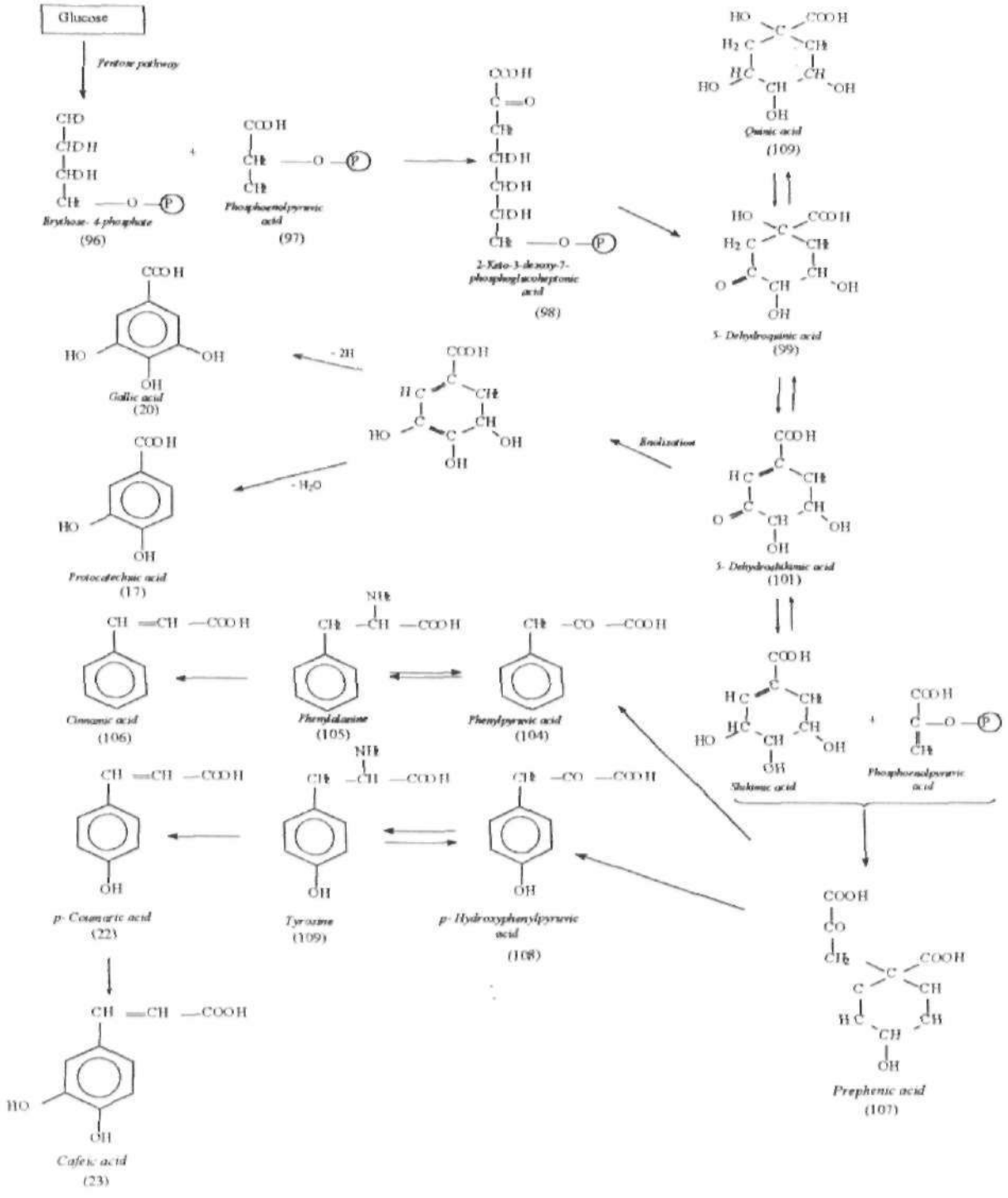
1. The Shikimic acid pathway
2. The Acetate pathway
3. The Non-defineable pathway.

2:5:1. The Shikimic Acid Pathway

This pathway explains how shikimic acid has been the major intermediate in the formation of the benzene ring, and therefore the phenolic compounds, using carbohydrates. Quinic (100) and shikimic acids are widespread in the plant kingdom, however, the transformation of shikimic acid into phenylalanine and tyrosine (90) which then give the corresponding cinnamic acids has been well demonstrated in the pathway. It will be noted that 5-dehydroquinic and shikimic acids are intermediates in the pathway (Fig. 1) but quinic acid (100) itself is not, even though it is a normal constituent of plant tissues, it appears to be a by-product of the principal pathway (Bohm 1965).

The shikimic acid pathway represents the principal mode of accumulation of phenolic compounds in plants. The aromatic amino acids, the benzoic acids, Gallic (20) and protocatechuic (17) acids) and the cinnamic acids are all formed in this pathway. The cyclisation of cinnamic acids involving the non enzymatic transformation of the natural *trans*-cinnamic acid into the cis isomer leads to the formation of coumarins. (Edward and Stoker 1967). The benzoic acids are formed by β-oxidation of the corresponding cinnamic acids (El-Basyouni et al 1964, Grisebach 1967). The reduction of ferulic acid (24) gives coniferyl alcohol (63) which is an important precursor of the lignins. Also the side aromatic B ring of the flavonoids is formed by this pathway, with cinnamic acids probably acting as intermediates.

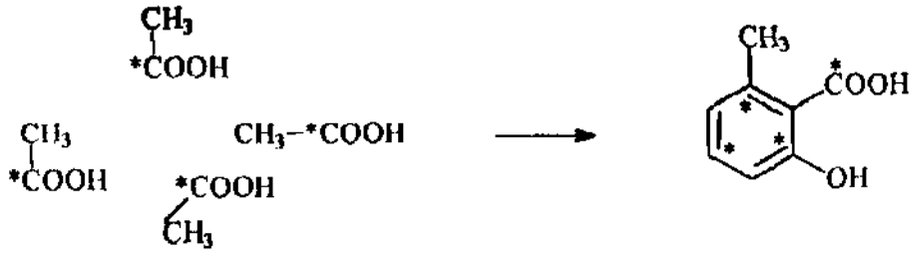
Fig 1.


Glucose
Pentose pathway
Erythose- 4-phosphate
(96)
Phosphoenolpyruvic acid
(97)
2-Keto-3-deoxy-7-phosphoglucoheptonic acid
(98)
Quinic acid
(109)
5- Dehydroquinic acid
(99)
Gallic acid
(20)
- 2H
Protocatechuic acid
(17)
- H2O
Enolization
5- Dehydroshikimic acid
(101)
Cinnamic acid
(106)
Phenylalanine
(105)
Phenylpyruvic acid
(104)
Shikimic acid
Phosphoenolpyruvic acid
p- Coumaric acid
(22)
Tyrosine
(109)
p- Hydroxyphenylpyruvic acid
(108)
Prephenic acid
(107)
Cafeic acid
(23)

2:5:2. The Acetate Pathway

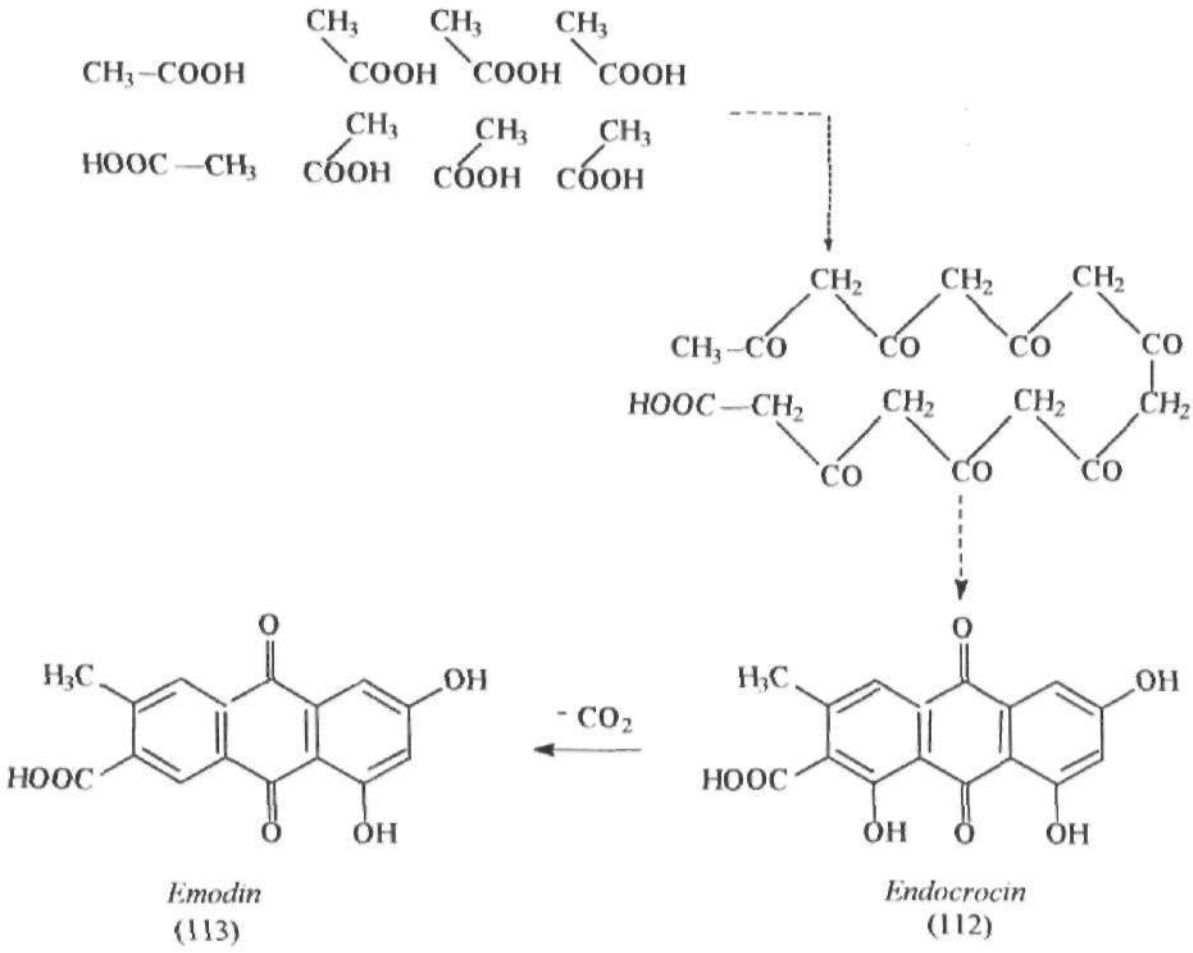
The pathway explains how acetate units condense to form the aromatic nucleus. This involves the 'head to tail' condensation of acetates in which the methyl group of one molecule is linked with the carboxyl group of another with the elimination of water.

The Shikimic Acid Pathway, although the most important route by which most aromatic compounds are formed, cannot satisfactorily account for the formation of some aromatic compounds such as the anthraquinones. The Acetate Pathway recognises the central position of acetate in relation to the general metabolism of plants and it is possible to devise many possible routes by which acetate condensation could occur to give a variety of aromatic compounds. The general validity of the mechanism has been established by the use of labelled compounds athough the detailed steps for many of the compounds remain to be established. Thus the incorporation of acetate - 1- ^{14}C into 6-methyl salicylic acid by Penicillium griseofolium takes place as below (Trease& Evans 1972)



(110) 6 - methyl salicylic acid

In this wise the production of the mould anthraquinone metabolite, endocrocin (111), can be represented as follows:



Emodin
(113)

Endocrocin
(112)

Decarboxylation of endocrocin (112) affords emodin (113).

The condensation of acetate units can be classified as an acylation reaction.

$2CH_3 - COOH \rightarrow CH_3 - CO - CH_2 - COOH + H_2O$

These reactions are common in organic chemistry and take place most readily when the hydrogen atom to be substituted is attached to an electron withdrawing group. Esterification is an acylation reaction. There is also acylation directly to carbon (C-acylation) when the hydrogen atom is sufficiently labile. The self condensation of aliphatic esters, having a hydrogen atom made labile by the presence of the α-carbon to the CO group is an example. Ethyl acetate thus yields ethyl acetoacetate $CH_3COCH_2COOC_2H_5$.

Numerous acylation reactions occur in biochemistry and usually involve thio-ester derivatives of acids with coenzyme A (HS-Co A) showing the essential functional thiol group of the type CH_3-CO~

S-CoA. The high energy bond, allows for the transfer of the acetyl (or other acyl) group. Apart from the enhancing of the lability of the hydrogens of the methyl group, the oenzyme A allows the acylation of the corresponding carbon (C-acylation). The summary of the reaction is shown below.



(114) (115) (116)

From the fundamental work on the synthesis of fatty acids it is known that acetyl coenzyme A first undergoes carboxylation to yield malonyl coenzyme A (malonic acid COOH - -CH_2 COOH). Because of the high lability of the hydrogen atoms of the CH_2, situated between the two CO groups acylation of this becomes easier. The product (118) from acetyl - CoA and malonyl - CoA is then decarboxylated to give aceto-acetyl CoA (97) which in turn can condense with a malonyl-CoA molecule.

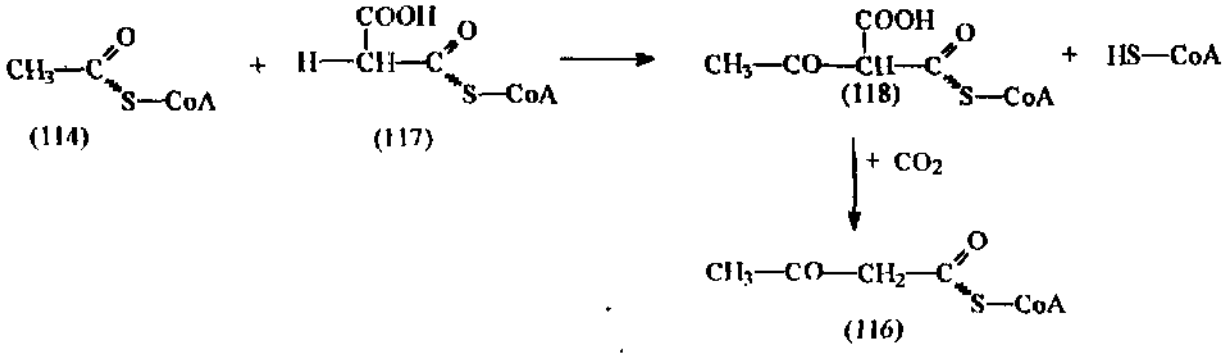
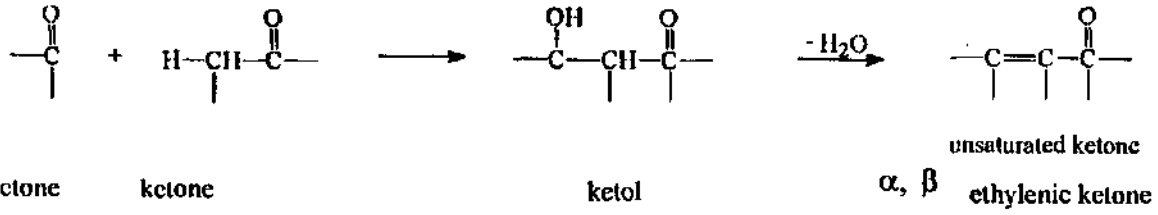
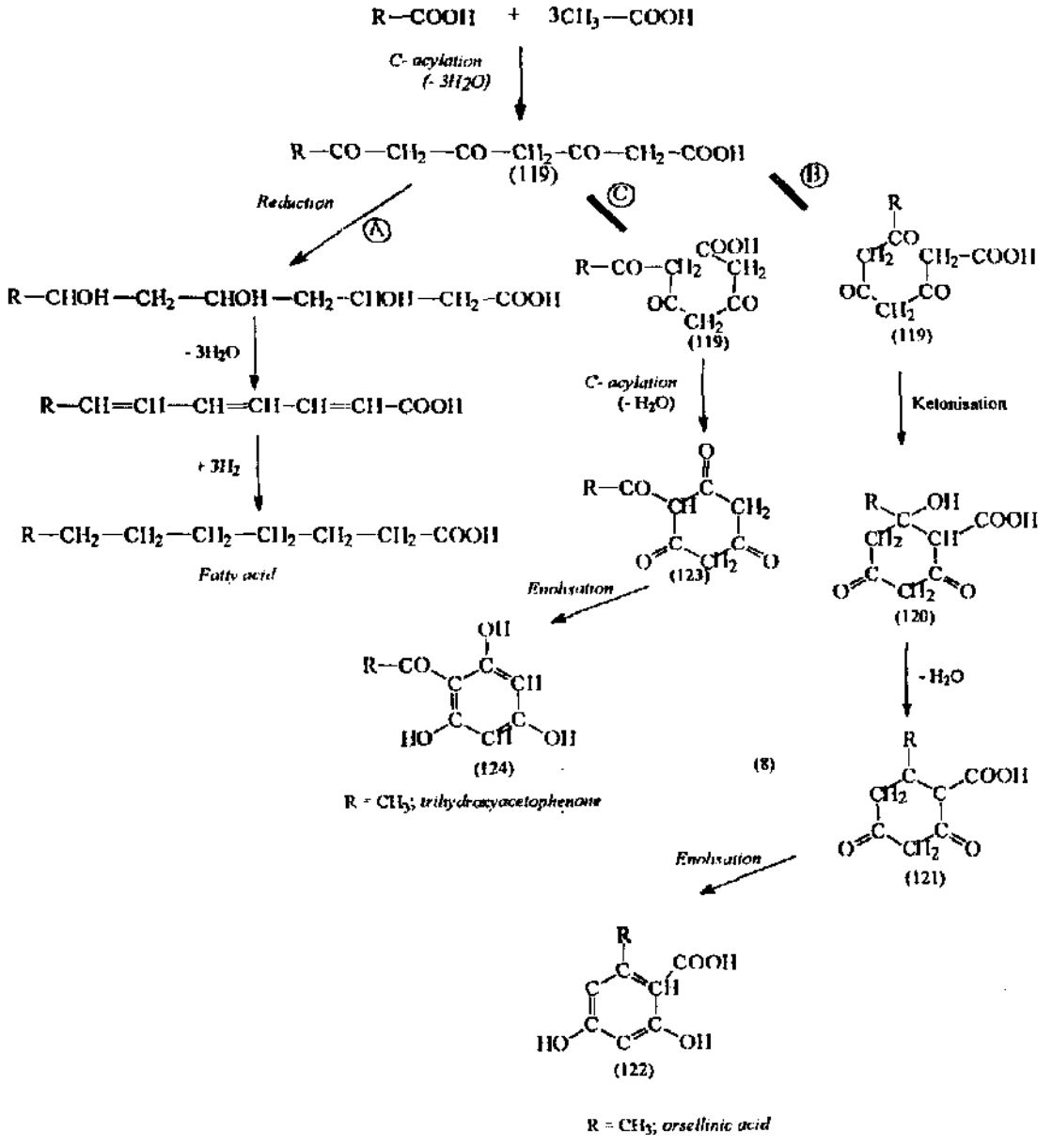


Fig 2 is a schematic representation of the reaction showing the three possible routes resulting in three types of products from the condensation of any molecule (R-COOH), reacting in the form of acyl coenzyme A, with three acetic acid molecules in the form of malonyl coenzyme A. Product (115) can be reduced, dehydrated, and reduced again to give a fatty acid as in route A. In route B, product (115) reacts in the form (116) and cyclisation takes place by an intra-molecular aldolisation reaction, that is the condensation of one CO with the α-carbon of another ketone producing a ketol (117) which readily

loses water to give an ethylenic ketone like (118). The enolisation of the two ketone groups in (118) gives the aromatic compound (119) which is orsellinic acid.



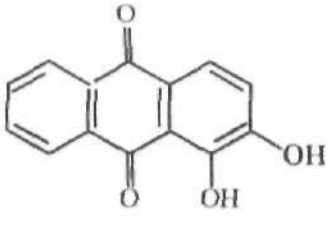


Figures 2: Schematic representation of the 3 possible routes of R-COOH condensation.

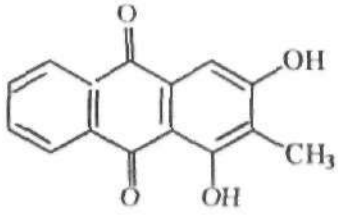
In route C, product (119) cyclises by a C-acylation reaction described above producing cylic derivative (123) which on enolisation gives an aromatic compound (122) such as trihydroxyacetophenone. If the acid involved in the initial condensation is a cinnamic acid ($R=CH_6-C_3H$) then cyclisation via route C produces chalcone having a phloroglucinol nucleus and then to different flavonoids.

2:5:3 The Non-definable Pathway:

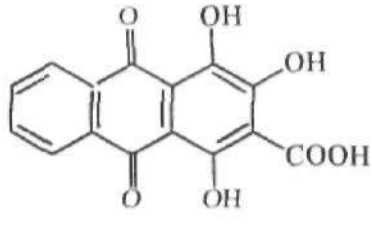
Neither the Shikimic Acid Pathway nor the Acetate Pathway can fully explain the formation of some anthraquinones. Such anthraquinones include alizarin (125), rubiadin (126), pseudopurpurin (127) etc which are pigments of the Rubiaceae and other families:



Alizarin
(125)



Rubiadinn
(126)



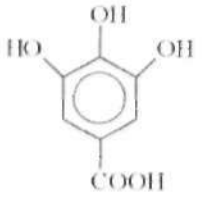
Psedopurpurin
(125)

Circumstantial evidence shows that these compounds might be formed from naphthaquinones with the participation of mevalonic acid. This was afforded by their co-occurence in teak and other plants with naphtaquinones having an isopentenyl residue. Using the plant Rubia tinctorum ring C and the carbon side chain of pseudopurpurin and rubuidin were shown to have been derived from mevalonate, (Trease and Evans, 1972). In the same plant, shikimic acid has been shown to be incorporated into rin A of alizarin

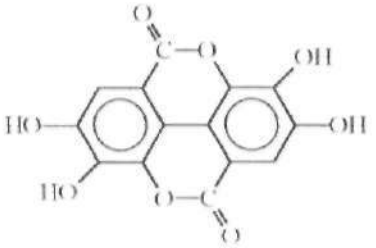
2.6 THE TANNINS

2.6.1 Introduction

As already stated, tannins are divided into hydrolysable and condensed tannins according to their molecular structure. Hydrolysable tannins are complex molecules containing ester type linkages which, on hydrolysis, yield a sugar and a phenolic residue. The phenolic residue consists usually of gallic acid (20) or Ellagic acid (68) which is a dimer of Gallic acid. Ellagic acid (68) has been identified from hydrolysates of leaves by paper chromatography (Bate-Smith, 1956).



(20)



(68)

The condensed tannins, though less studied and more important, are formed by the polymerisation of molecular units having the general structure of flavonoids, the most important of which are flavan-3-ol (catedins) (30) and flavan-3, 4-diols (leucoanthocyanidins) (41).

The properties of the condensed tannins depend upon the nature of the molecular units of which they are composed, the mode of linkage of these units, and above all the molecular weight of the condensation product (Bate-Smith, 1953, 1954b Bate-Smith and Swain, 1954, 1956).

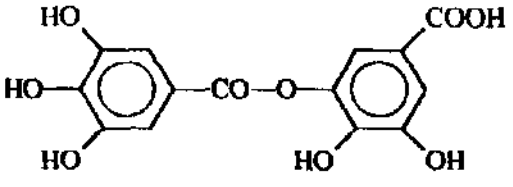
2.6.2 The Hydrolysable Tannins

Hydrolysable tannins are esters of sugars and phenolic acids or their derivatives. The sugar is usually glucose, but in some cases polysaccharides have been identified (Ribereau-Gayon, 1972).

Acidic, basic or enzymatic hydrolyses often occur spontaneously during extraction and purification. These tannins are usually divided into "gallotannins" which on hydrolysis yield gallic acid (20) as the only phenolic moeity, and the more numerous ellagitannins, which under the same conditions of hydrolysis give besides gallic acid (20) one or more of its derivatives, of which ellagic acid (69) is the most important.

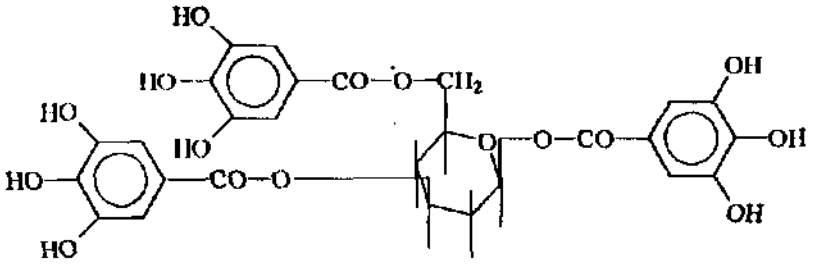
2:6:2:1 The Gallotannins.

The gallotannins are esters of glucose, or a polysacharides, and gallic acid (20) or m -digallic acid (128) which itself gives gallic acid on hydrolysis.



(128)

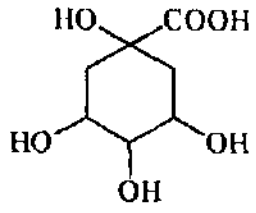
The exact structures of tannins of this type have not been elucidated but several simple galloyl glucoses have been studied, obtained either directly from natural extracts or by partial degradation of more complex molecules. Examples are 1-galloyl glucose(β-D-glucogallin), 3, 6-digalloyl glucose and 1, 3, 6-trigalloylglucose,(129).



(129)

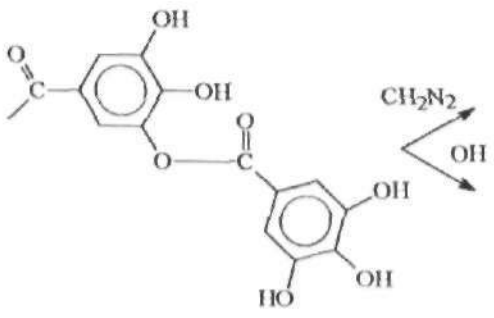
The main sources of the gallotannins are the plant galls such as the Chinesegalls, Turkish galls, Oak galls, Sumac and Tara. Other sources include, rhubarb, cloves, red rose petals, bearberry leaves,

hammamelis, chestnut and maple. Tara (Caesalpinia spinosa) contains a gallotannin in the fruit pod. The gallotannin is an ester of gallic acid (20) with quinic acid (100).

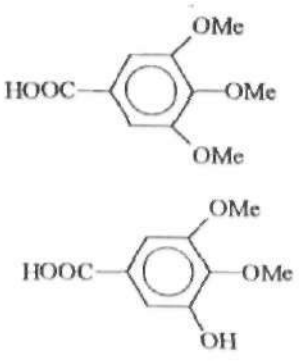


(111)

Each of the hydroxyl groups of the carbohydrate molecule is esterified by a polygallic acid in the form of a linear polymer (Bickley 1992). On hydrolysis with 5% sulphuric acid at 100°C or tannase at 33°C gives D-glucose and gallic acid (20) in a molecular proportion of roughly 1:10. The tannin of Turkish galls and of sumac are similar, and have a lower proportion of gallic acid to glucose. Chinese gall (Rhus semialata) and Turkish (Quercus infectoria) gallotannins, apart from being the first valuable commercial vegetable tannins were those whose chemical structures were first investigated. They are polygalloylglucose derivatives in which the ratio of glucose to gallic acid (20) is 1 to 9 or 10 and 1 to 5 or 6 for Chinese and Turkish galls respectively. In both gallotannins a certain percentage of the galloyl groups was shown to be bound in the form of depsides by the isolation of both 3, 4, 5 - tri -o-methylgallic (130) and 3, 4-di-o-methlygallic acid (131) after hydrolysis of the diazomethylated tannins. For chinese gallotannins the ratio of these acids was approximately 1:1 which led to the suggestion that in this substance all the hydrolxyl groups on the sugar molecule were etherification with gallic acid (20). There is also good evidence (etherification by diazomethane followed by methyl iodide and silver oxide) that Chinese and sumac gallotannins are similar - both have only D-glucose as the identifiable carbohydrate (Fischer, 1919).



(128)

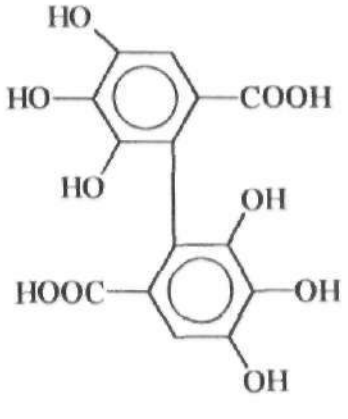


(131)

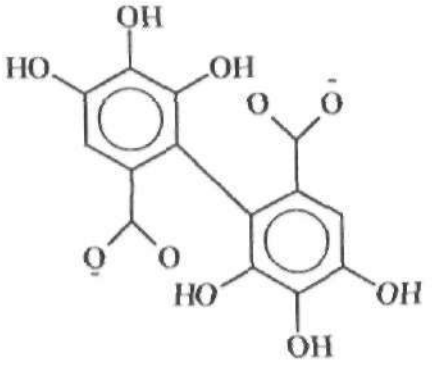
(130) 3,4,5-tri-O-methyl gallic acid

2:6:2:2 The Ellagitannins

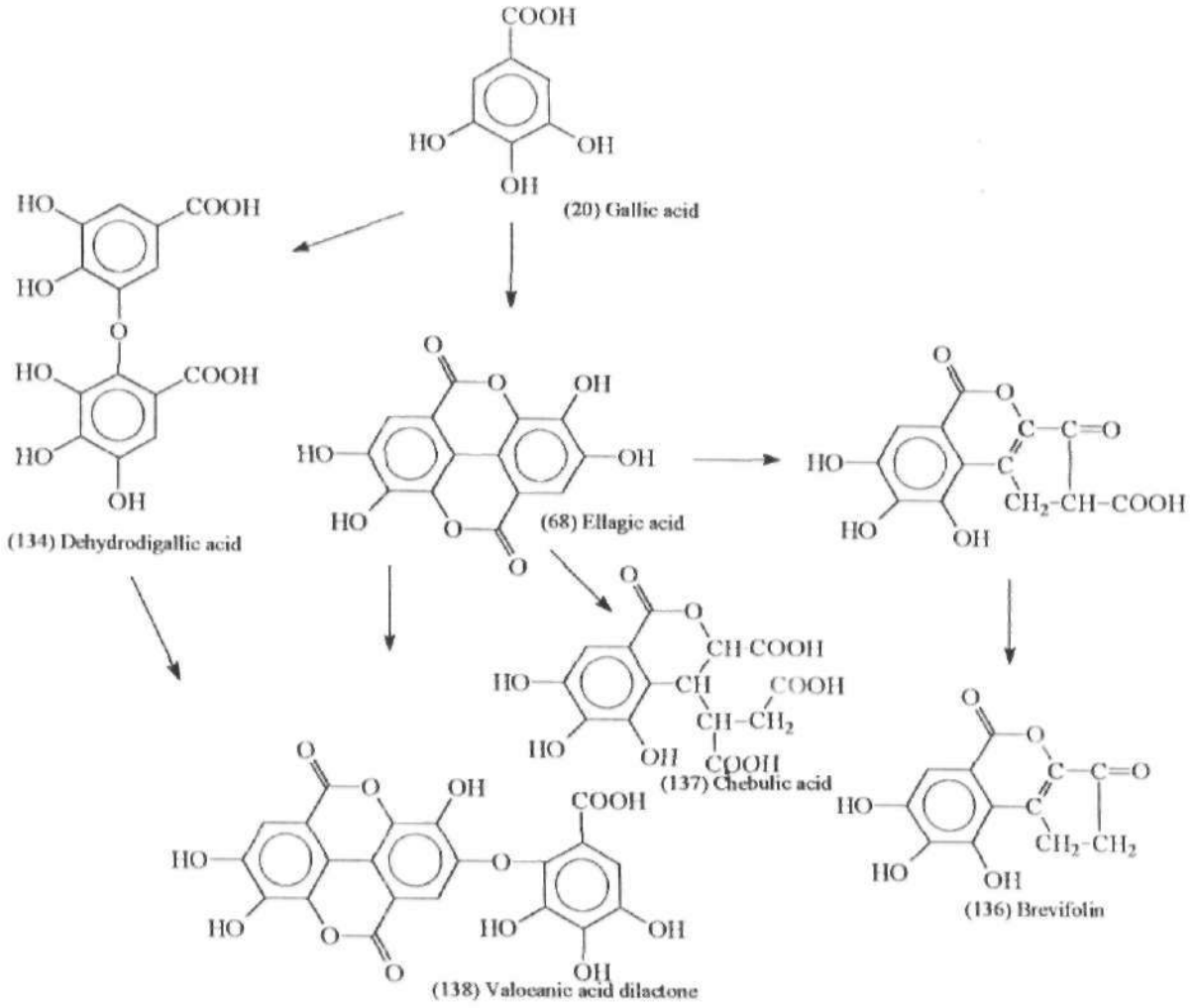
This class of hydrolysable tannins is much better known and is of wider occurence than the gallotannins. They are characterised by the fact that on acid hydrolysis they yield, besides gallic acid (20) itself, several other derivatives for example, ellagic acid (68) is the best known. Ellagic acid (68) is a depside of gallic acid (20), 4, 4^{i}, 5, 5^{i}, 6, 6^{i}, - hexahydroxydiphenic acid (132) by lactonization after hydrolytic cleavage of the hexahydrodiphenyl group (133) (Bickley 1992). This means that the presence of ellagic acid (68) in an extract is not necessarily indicative of the presence of ellagitannins.



(132)



(133)



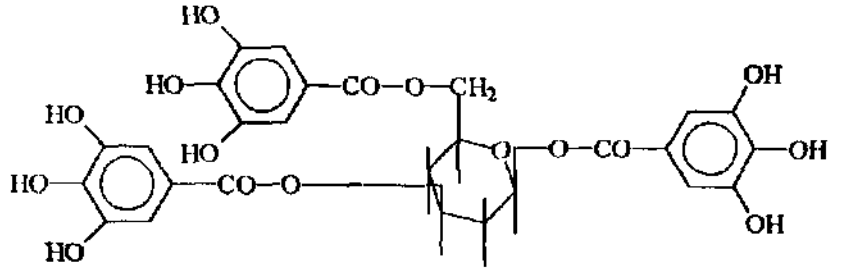
Four substances found in ellagitannins or ellagitannin extracts which bear a close structural and biogenetic relationship to ellagic acid (68) are chebulic acid (137) brevifolin carboxylic acid (135), valoneaic acid dilactone (138) and dehydrodigallic acid (134) (Bickley 1992, Heideman 1994).

As with ellagic acid (68), the first three of these substances are considered to be produced by cleavage of a particular fragment from an ellagitannin followed by lactonization. Dehydrodigallic acid (134) is isolated in the form in which it presumably occurs in the tannin.

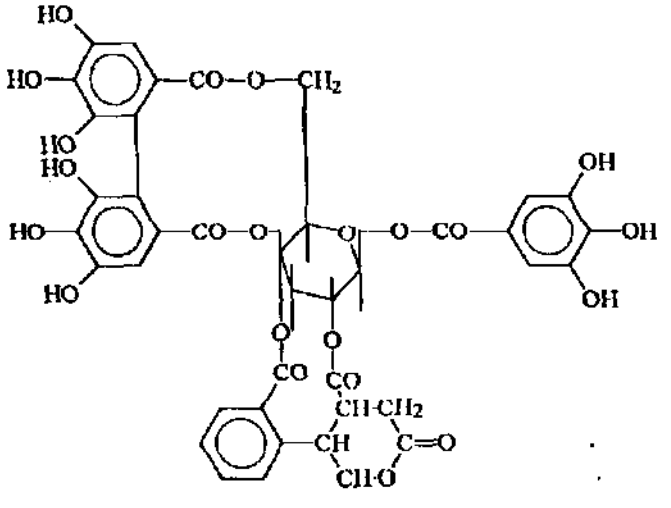
The most common sources of ellagitannins are valonea, myrobalans, Divi-divi, myrtan and algrobilla, but Bate-Smith (1956) has affirmed that ellagic acid occurs in 75 families of the dicotyledons. Other official sources include pomegranate rind, and bark, eucalyptus leaves and kinos:

oak bar and, Australian kino. Ellagic acid (68) has very low solubility in water. It can be crystallised as pale yellow needles from aqueous pyridine. It is easily identified through its distinct Rf values by paper chromatography and its light blue fluorescence under ultra-violet light.

The best known ellagitannins are chebulagic acid (140) and chebulinic acid (139). The first gives equimolar proportions of glucose, gallic acid (20), ellagic acid (68) and chebulic acid (137) on acid hydrolysis. The second tannin has a closely related structure giving two more moles of gallic acid(20) instead of the one of ellagic acid (68) due to the fact that the aromatic rings are not linked.



(139)



(140)

Thus although acid hydrolysis of corilagin, chebulagic acid (139) and ellagitannins gives ellagic acid (68), hydrolysis of the corresponding methylated tannins yielded, instead, hexamethoxydiphenic acid (132) usually in one of its optically active forms. Schmidt (1954) found hexahydroxydiphenic acid (132) to occur in divi-divi and myrobalans in the dextro-rotatory form and in algarobilla, valonea and knoppern nuts in the laevo rotatory form and proposed that the hexahydroxydiphenoyl group in

ellagitannins arose by oxidative coupling of two suitably disposed galloyl groups in a galloylated sugar rather than the alternative of esterification of the sugar by a preformed molecule of hexahydroxy diphenic acid (132).

2:6:2:2:1 Chebulic (137), Chebulinic (140) And Chebulagic Acids (139)

Chebulic acid (137) is an amorphous optically active trihydroxytricarboxylic acid $C_{14}H_{12}O_{11}$ with its structure proposed by Schmidt and Mayer (1951) and confirmed by Haworth (1954) and his collaborators. The acid has the properties of a lactone and with diazomethane gave an amorphous hexamethyl derivative which on hydrolysis gave trimethyl chebulic acid (Bickley, 1992).

Comparison of the molecular formulae of chebulic (137) and ellagic acid (68) shows that the former contains three more hydoxylgroups which led to the suggestion that ellagic acid (68) might give rise to chebulic acid (137) by a series of redox reactions since ellagic acid (68) has been shown to be derived as a result of cleavage of the hexahydroxydiphenoyl group from tannin molecules and hence the alternative formulation of chebulic acid (137) as when combined in natural tannin molecules such as chebulinic (140) and chebulagic acids (139) Schmidt and Mayer (1956).

Chebulinic (140) and chebulagic acids (139) are two crystalline tannins which are extracted from the fruit of Terminalia chebula the popular Myrobalans. Both liberate chebulic acid (137) on hydrolysis thus bearing close structural relationship one to another. It is important, however, to note here that chebulinic acid (140) does not liberate ellagic acid on hydrolysis and that it is strictly not an ellagitannin even though its chemistry is considered alongside that of chebulagic acid (139).

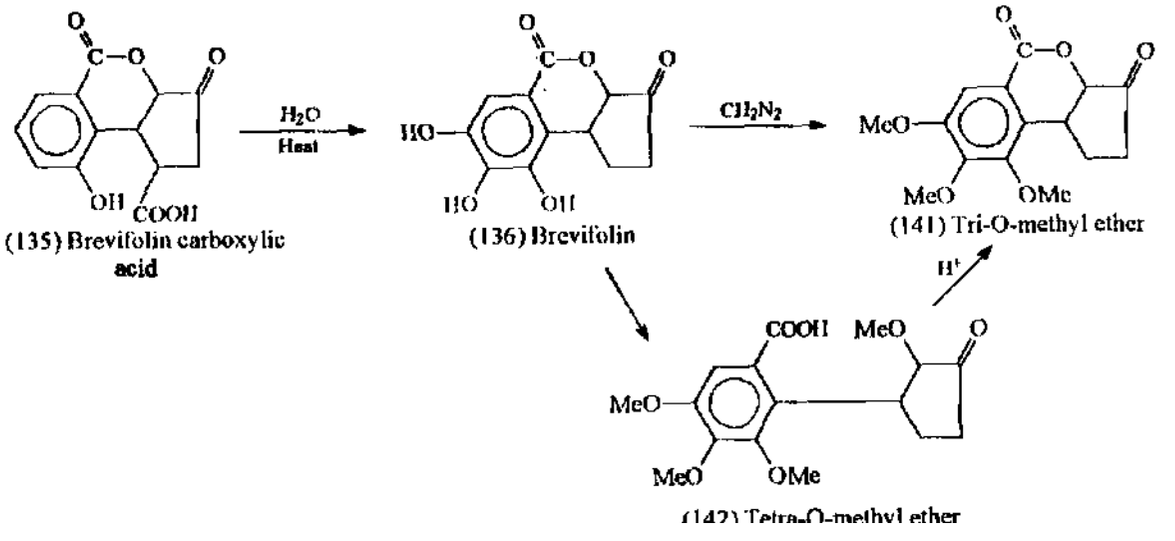
Chebulinic acid (140) the main constituent of the Myrobalans extract was the first hydrolyzable tannin to be obtained in a crystalline form. On complete hydrolysis it gives one mole of glucose, three

of gallic and one of chebulic acid while on partial hydrolysis at 100°C with water it gives a di-O-galloyl-D-glucose derivative. (Bickley 1992, Heideman 1994).

Crystalline chebulagic acid (139) occurs in myrobalans and Divi-Divi and it bears a close structural relationship to chebulinic acid (140) and corilagin. On vigorous acid hydrolysis it gives equimolar proportions of glucose, gallic (20), ellagic(68) and chebulic acids (137).

2:6:2:2:2 Brevifolin And Brevifolin Carboxylic Acid

Brevifolin (136) and Brevifolin carboxylic acid (135) were isolated and characterized from the ground fruit pods of plants of the Acacia species, usually Caesalpinia brevifolia. Brevifolin, (117) $C_{12}H_8O_6$ is the decarboxylation product of brevifolin carboxylic acid, $C_{13}H_8O_8$ (135). Brevifolin carboxylic acid (135) is a monocarboxylic acid that gives a phenylhydrazone and a tetra-O-methyl derivative. The acid, on decarboxylation by heating in water, gives brevifolin (136). With diazomethane brevifolin (136) gave the tri-O-methyl ether while with alkali and dimethylsulphate the tetra-O-methyl ether, was formed which was readily converted to the tri-O-methyl ether by treatment with acid thus substantiating the presence of an enol-lactone ring in brevifolin (136). Brevifolin carboxylic acid (135) as isolated from Algarobilla is optically inactive.



(135) Brevifolin carboxylic acid

(136) Brevifolin

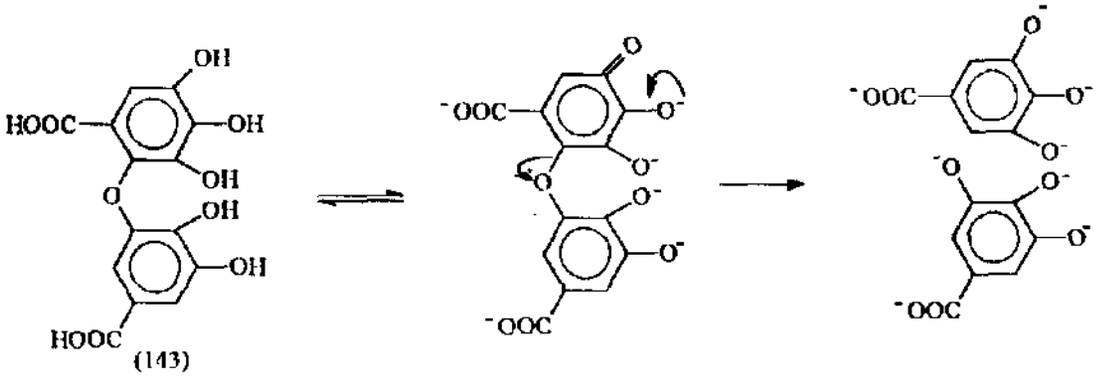
(141) Tri-O-methyl ether

(142) Tetra-O-methyl ether

2:6:2:2:3 Valoneaic Acid Dilactone (138) And Dehydrodigallic Acid (134)

Dehydrodigallic acid (134) and valoneaic acid dilactone (138) probably form parts of the structure of much larger tannin molecules in the particular plant tissues from which they have been isolated (Mayer, 1952). Their formation can be envisaged as falling within the general scheme of oxidative coupling between galloyl groups in a gallotannin molecule (Schmidt and Mayer 1956). However, in the case of dehydrodigallic acid (134), coupling is envisaged as occurring with the formation of a diphenoyl ether linkage between two galloyl groups and in the case of valoneaic acid dilactone (138), the coupling of an ether linkage between a galloyl and a hexahydroxydiphenoyl group is suspected.

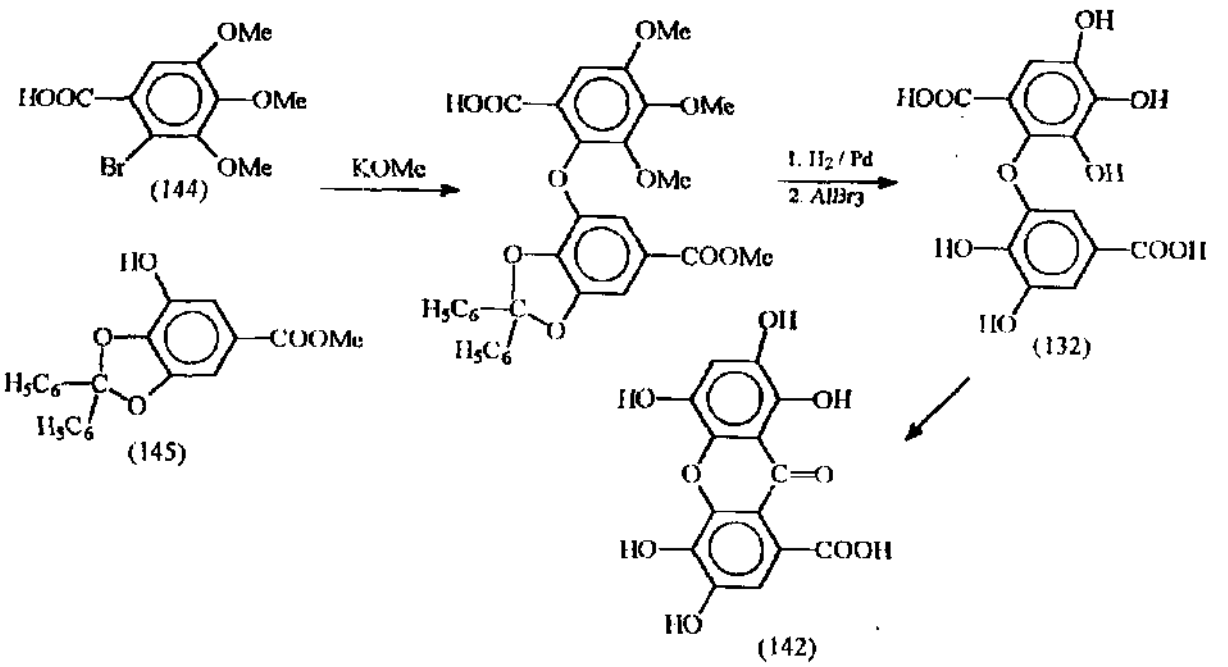
Dehydrodigallic acid (134) was isolated from the young shoots and bark of the Spanish chestnut (Castanea vesca) (Mayer 1952). It forms a penta-O-methyl ether and gives a dimethyl ester with diazomethane $CH_2N_2$and which was recovered unchanged with dilute alkali even though dehydrodigallic acid (134) itself underwent an unusual fission of the diphenyl ether linkage to give gallic acid in approximately 50% yield (Mayer et al, 1960).



(143)

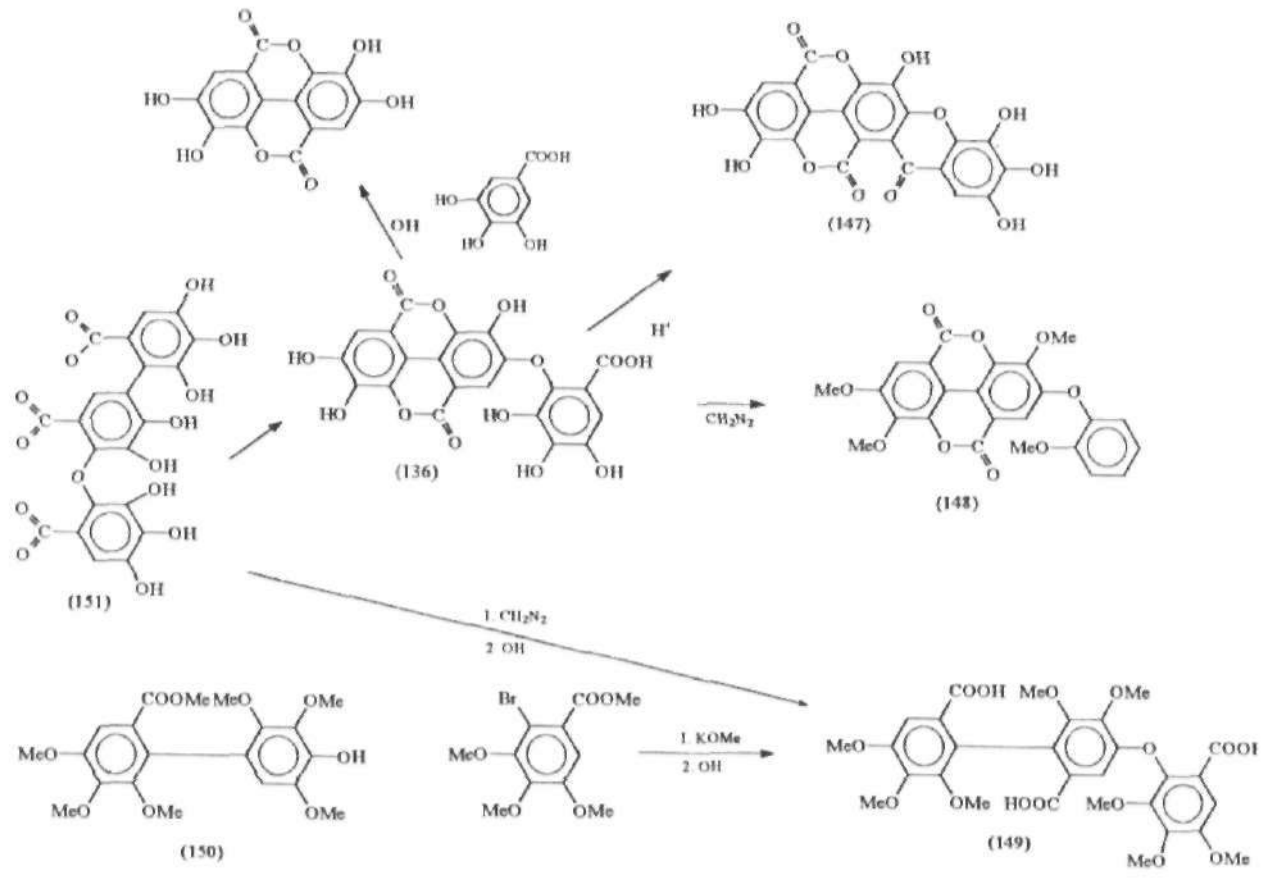
On careful study it is observed that this ether cleavage is manifested when one of the two phenyl groups contains 3 vicinal hydroxyl groups. The mechanism for the reaction is explained in terms of the β-elimination process as seen above (Mayer et al 1960).

Treatment of dehydrodigallic acid (134) with sulphuric acid gave xanthone (143) which on methylation gave a penta-O-methyl ether identical with that prepared similarly from penta-O-methyldehydrodigallic acid (Mayer and Finkentscher 1958).



On this basis the structure for dehydrodigallic acid (134) was confirmed in a synthesis of the acid and its penta-O-methyl derivatives by the condensation of 2-bromo-3,4,5-tri-O-methyl gallic acid (144) and methyl 3, 4 - diphenoyl methylenedioxy - 5- hydroxybenzoate (145), to give (146) which on hydrogenation and subsequent demethylation with aluminum bromide gave dehydrodigallic acid (134) (Mayer and Finkentscher 1960).

The hydrolysis of valonea tannin extracts obtained from acetone extraction of the acorn cups of Oak, (Quercus valonea) yielded substantial quantities of ellagic acid with an optically inactive crystalline phenolic acid, valoneaic acid dilactone (138) (Schmidt and Komarek 1955).



Its structural similarity to dehydrodigallic acid (134) was exhibited by its chemical reactions. With sulphuric acid Valonea xanthone (147) was formed while with alkali an analogous splitting of the diphenyl ether link occurred, giving gallic and ellagic acids in rather less than 50% yield. When Valoneaic acid dilactone (138) was treated with diazomethane it forms a hexamethyl ether methyl ester (148) which on further methylation with dimethylsulphate and alkali gives the octamethyl derivative (148) proving the presence of two lactone rings in the original compound. The isolation of (148) in an optically active form proved that valoneaic acid dilactone (138) bound in tannin molecules in the form (151) rather than in the form (138) further strengthening the fact that the molecule was formed by oxidative coupling of these galloyl groups in a gallotannin molecule (Schmidt and Mayer 1956).

Consequently, on this basis and its general chemical similarity to dehydrodigallic acid (134), valoneaic acid dilactone was assigned the structure (136). Condensation of the hexahydroxydiphenic

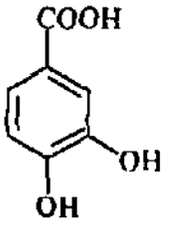
acid derivative (150) with 2-bromo-3,4,5-tri-O-methylgallic acid and hydrolysis of the product with alkali gave the product (149). (Bate - Smith, 1954).

2:6:2:2:4 Miscellaneous Ellagitannins

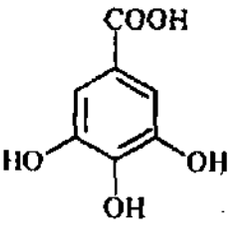
The surveys of plant species by Bate-Smith (1956) indicated that ellagic acid (68) and therefore, perhaps ellagitannins, are widely distributed in nature. Ellagitannins have been found in the cambium and woody tissues of Eucalyptus species (Hills 1960).

2:6:2:2:5 Biosynthesis of Hydrolysable Tannins

Hydrolysable tannins are direct products of plant metabolism and as such they are therefore more amenable to a normal biosynthetic study (Haslam, 1966). Gallic acid (20) and hexahydroxydiphenic acid (132) are principal phenolic constituents of the gallotannins and ellagitannins encountered in plant tissues as esters with sugars and in the case of flavan-3-ol, (36) as gallates. It should now be obvious that hydrolysable tannins are derived by the attachment of gallic and or hexahydroxydiphenic acids to a sugar molecule, commonly glucose. Therefore, a biosynthetic pathway for the two acids would lead to the understanding to that of the hydrolysable tannin. The occurence of gallic acid in plant tissues have been widely attributed to the enzymatic decomposition of the labile gallate esters during extracttion although the acid in a free estate has also been found in some plants. In an effort to establish a precursor for gallic acid (20) and protocatechuic acid (17), Brucker (1957) and Brucker and Hashem (1962) carried out culture experiments with the mould Phycomyces blakesleeanus which led them to establish that shikimic acid (102) is an intermediate between glucose and protocatechuic acid and that gallic acid may arise from simpler building units or by aromatic amino acid metabolism (Haslam 1966).

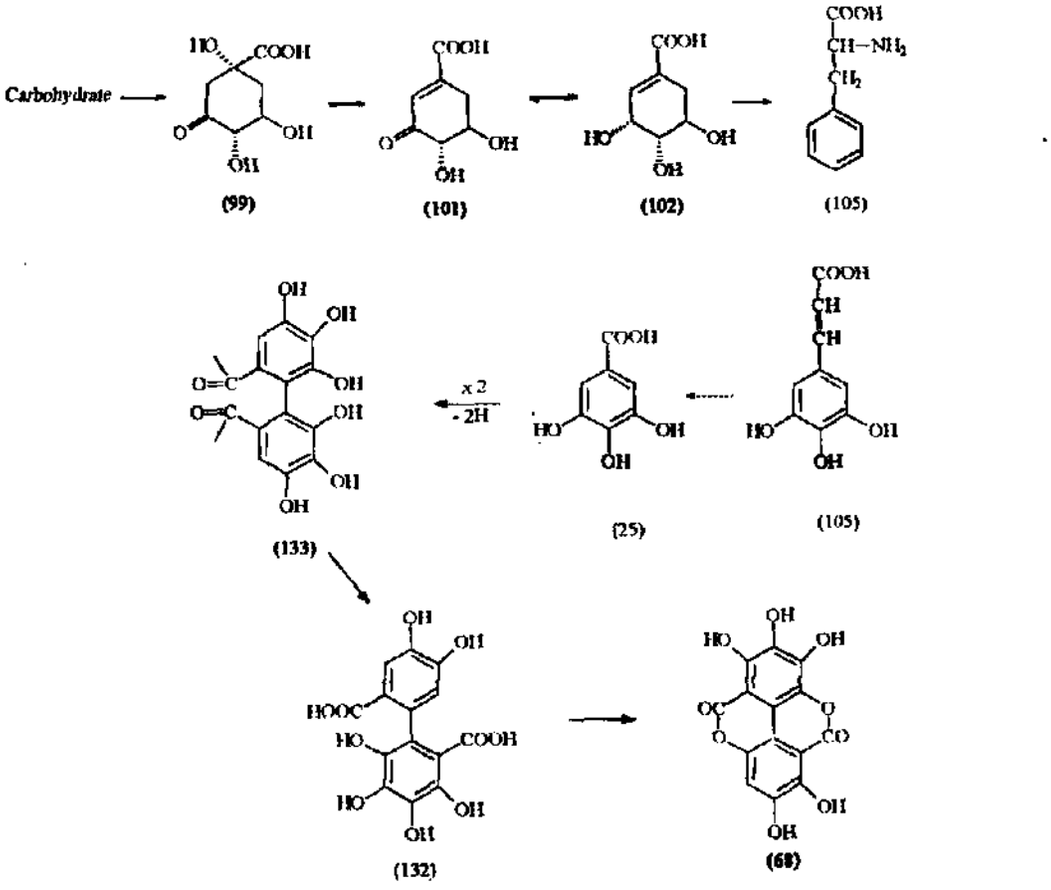


16 Protocatechinic acid



102 Shikimic acid

Conn and Swain (1961) suggested that gallic in nature was more liely to be formed directly from shikimic acid than by β-oxidation of a C_6-C_3 compound. This was because as stated by Swain, (1965) the common hydroxybenzoic acids (gallic (20), protocatechuic (17), p-hydroxybenzoic acids (16) have the same number of carbon atoms and same distribution of oxygen function as for shikimic acid, thus they are more likely to be formed from shikimic acid by oxidative dehydration. Zenk (1964) has concluded from isotopic feeding studies on Rhus typhine that gallic acid (20) was derived by metabolism of phenylaline as shown in Scheme II



Haslam and his colleaques (1961) observed that there was an increase in the concentration of gallic acid and protocatechuic acid when (102) or (101) dehydroshikimic acid was fed to the mould Phycomyces blakesleenus while Gross (1958) found that (17) was formed in selected mutants of Neurospora crassa by dehydroshikimic of (101) . It should be expected that more than natural product but the galloyl esters and the gallotannins can be assumed to be derived by esterification of the appropriate alcohol with gallic acid. Although (102) has been shown to be the immediate precusor of hydroxybenzoic acids, the methoxy-acids like Vanillic (18) and syringic (21) may be readily formed by β-oxidation of the corresponding cinnamic acids.

The biogenesis of hexahydroxydiphenic acid (132) and its congeners present a much less problem. Although it has been proposed that they could arise by condensations between hydroaromatic molecules like (102), it is more likely that they are formed as suggested by Schmidt and Mayer (1955) by oxidative coupling of gallic acid, followed by further oxidative cleavage of one of the aromatic rings to give chebulic acid (137), and brevifolin carboxylic acid (135) and hexahydroxydiphenic acid residues in tannins such as corilagin, are formed by enzyme catalysed coupling of gallic acid residues already attached to sugar, especially since it has been shown (Swain, 1965) that corilagin may be obtained by air oxidation of 1,3,6-tri-O-galloyl-glucose (129)

2:6:3 The Condensed Tannins

Although the condensed tannins are commercially more important than hydrolysable tannins, much less is known about their structure, many aspects of which remain to be elucidated.

However, there is enough evidence to show that the complex polymeric structures of many of the condensed tannins are derived primarily by the polycondensation of precusors of the flavonoid

types, namely, flavan-3-ol (30) and flavan-3,4-diol (41) (Bate-Smith 1954b, Bate-Smith and Swain 1956).

2:6:3:1 The Flavan - 3- ol (catechins)

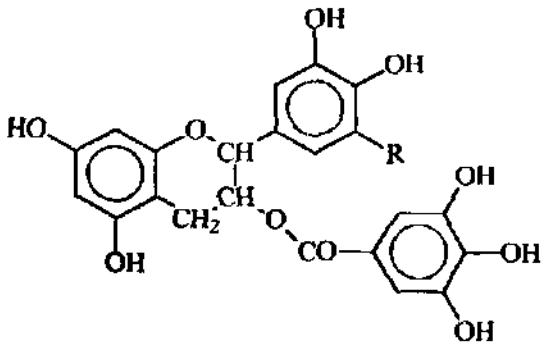
The term catechin (30) was first used by Freudenberg (1920) to describe colourless, crystalline substances commonly located in plant tissues in association with the condensed tannins as they are easily and readily converted, *in vitro*, to amorphous tannin - like materials, 1920). It was subsequently shown that catechins are derivatives of flavan - 3-ol and thus are also referred to as flavan-3-ol.

The catechins are related to the anthocyannins and flavones (the other members of the flavonoids). They do not however, generally exist in nature as glycosides. The most common members of the group differ only in the number of hydroxyl groups in the phenyl B ring and these groups are never methylated. The term "catechin" refers specifically to the flavan-3-ol which has assymetric carbon atoms at C-2 and C-3 thus giving four optical isomers. In the case of the catechin series, these configurations (+) - catechin, (-) - catechin, (+) - epicatechin and (-) - epicatechin are the common naturally occuring forms.

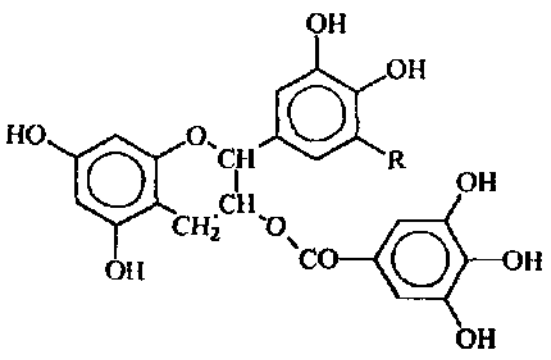
The central heterocylic ring, being saturated is not planar, the oxygen atom and C-4 are in the plane of the ring A but C-2 and C-3 lie either above or below the ring. Several consideration, notably the IR spectra, show that there is strong hydrogen bonding between the hydroxyl group at C-3 and the heterocylic oxygen and (-) - epicatechin and the configuration of these isomers is thus represented by the structures shown below:

The configuration at C-2 is the same in each case and this appears to be the same in related naturally occuring compounds. The other isomers (epimeric at C-2) can be obtained by heating the natural forms in aqueous solution.

The catechins also include gallic acid esters with the acid moeity attached to the OH group at C-3. The liberation of gallic acid on hydrolysis is thus not confined to the hydrolysable tannins.



(137) R = H



(138) R = OH

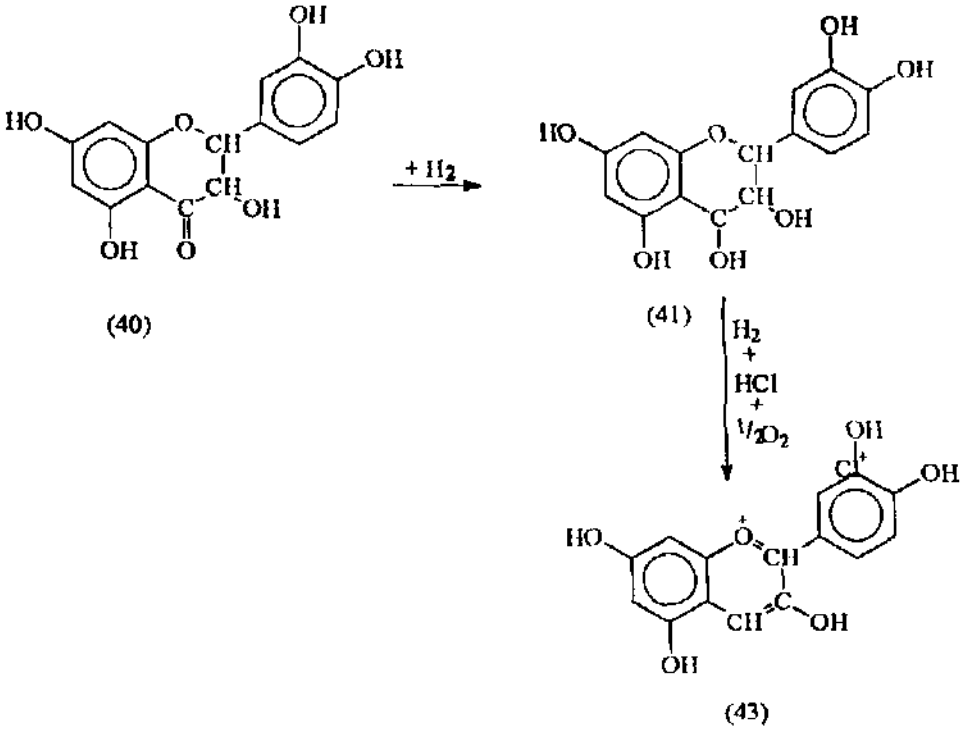
The catechins, on heating in acid solution, give yellowish-brown insoluble products (phlobaphenes) of high molecular weight. This contrasts with the flavan-3, 4-diols, which on heating give besides the yellowish-brown insoluble products some red anthocyanidins. If one wishes to examine the catechins present in natural extracts, it is essential to avoid acid hydrolysis. The catechin can be extracted with ether or ethyl acetate and separated, after concentration, by two dimensional chromatography in butanol - acetic acid and dilute aqueous acetic acid (2-15%). The compounds can be developed by spraying with a solution of 10% vanillin in concentrated hydrochloric acid which gives rise to red coloured spots.

2:6:3:2 The Flavan-3, 4-diols (Leucoanthocyanidins)

The flavan-3, 4-diols or leucoanthocyanidins were first discovered by Laborde (1910) but first made public by Rosenheim in 1920. These compounds yield phlobaphenes (yellow-brown) and anthocyanidins (red) on acid hydrolysis. The quantity of anthocyanidins formed is small compared to that of the phlobaphenes (20: 80). It had been recognised for a number of years that these compounds play an important role in the structures of tannins. The leucoanthocyanidin polymers, which

constituted the tannins, give anthocyanidins on heating in dilute acids almost as easily as do the monomers.

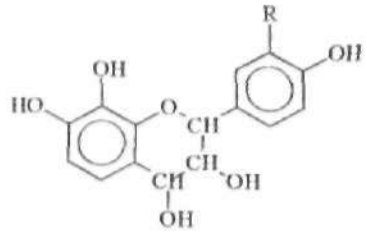
Swain in 1954, established that the leaucoanthocyanidin monomer had the structure of a flavan-3, 4-diol by reducing taxifolin (dihydroquercetin) (46) with sodium borohydride to 5, 7, 3, 4-tetrahydroxy flavan-3,4-diol and showing that this was identical in several respects with natural leucoanthocyanidins.

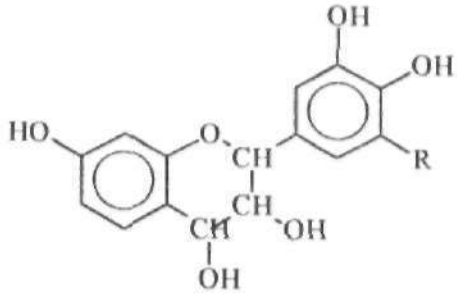


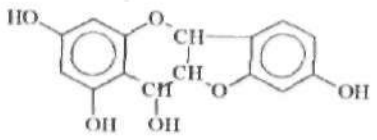
The compound was first obtained crystalline by Michaud and Masquelier in 1968. Flavan-3, 4-diols can also be synthesised by oxidation of a flavylium salt, not hydroxylated at C-3, by hydrogen peroxide followed by reduction of the product with sodium borohydride.

The condensed tannins (flavolans) are also transformed into anthocyanidins on heating in dilute acid, but the yield is exceedingly small.

Known flavan -3, 4 -diols include, leucocyanidins and leucodelphinidin which have the 5, 7-dihydroxylation pattern common to the majority of natural flavonoids, teracacidin (152) melacacidin (153), leucofisetinidin (154), cyanomaclurin (156).



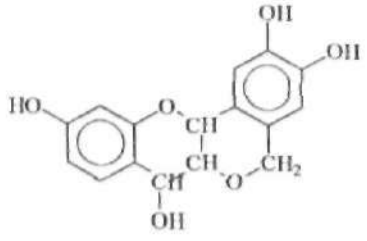


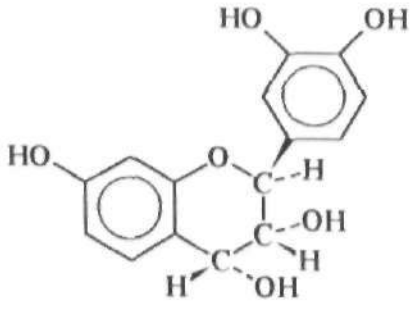


(152) R = H;
(153) R = OH;

(154) R = H;
(155) R = OH;

(156)





(157)

(158)

The flavan-3, 4-diols, have three assymetric carbon atoms (C-2, C-3 C-4) and thus can exist in eight optically active forms. The stereochemistry of their compounds is much less known than that of the catechins (flavan-3-ols). The absolute configuration of (-) - leucofisetinidin (158) is, trans, 2, 3-cis 3, 4 and several isomers of teracacidin have been obtained from acacia wood (Drewes and Roux 1966).

The seperation and identification of the flavan-3, 4-diol on paper chromatograms is more difficult than for the catechins. One of the reasons is that they readily polymerise. The second reason is that there is no suitable discriminatory spray agent since both flavan-3-ols and flavan -3, 4-diols, react equally well with vanillin. Presently, the only way of differentiating the catechins and the

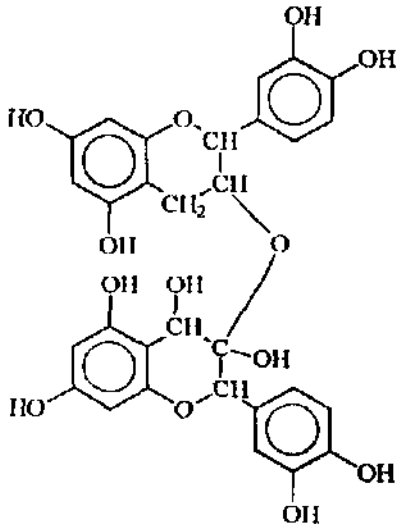
leucoanthocyanidins which react with vanillin, is to isolate the reactive spots, elute the components, effect their transformation in a test tube to anthocyanidins and then identify the latter. Using this method, it is also possible to identify the biflavans.

2:6:3:3 The Biflavans

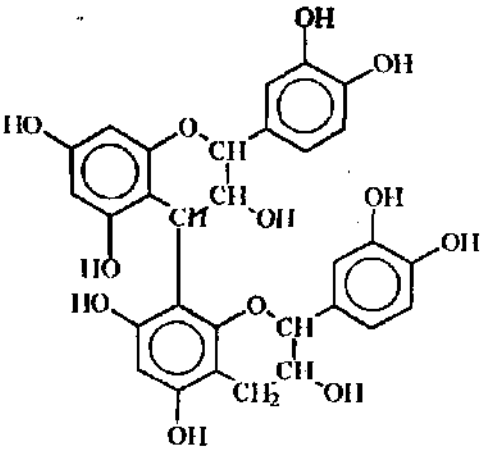
The biflavans, which occur in many fruits, are dimers in which one molecule of a flavan-3-ol is linked to a flavan-3, 4-diol, usually leucocyanidin (42) or leucodelphinidin (39). They were first identified in 1960 by Forsyth and Roberts and have since been extensively investigated by many scientists such as Geissman, Swain etc. They have been studied under the name "proanthocyanidin", a name that had been proposed by Freudenberg and Weinges (1962). The two constituent monomers are split by relatively much milder acid treatment than is required for the transformation of flavan-3, 4-diols into anthocyanidins (5 minutes in O.N HCl at 100°C is usually sufficient).

The biflavans may be intermediate in the formation of condensed tannins or they may be formed independently by a parallel route. The second hypothesis seems to be the more plausible, because polymerisation of flavan-3, 4-diol gives a polymer of high molecular weight while the reaction between the diols and the corresponding 3-ols yield only dimers (Geissman and Dittmar, 1965).

The study of the biflavans is important for two reasons: one, their study helps in understanding the structures of the condensed tannins and two, because they have molecular weight above 500, they posses certain tannin-like properties. They are, for example, astringent. Proposed structres for biflavans include the following. (159-161)

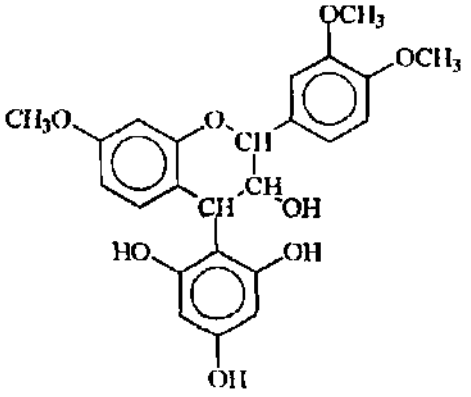


(159)



(147)

A synthetic biflavan was prepared by Geissman and Yoshimura (1966) which had the structure (160).



(161)

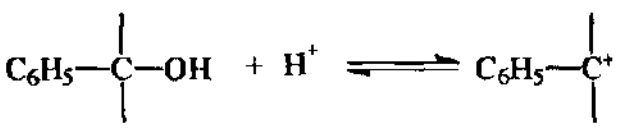
This type of structure was the condensation of phloroglucinol with flavan-3, 4-diol to give compounds such as the above (Jurd and Lundin 1968).

2:6:3:4 The structure of Condensed Tannin.

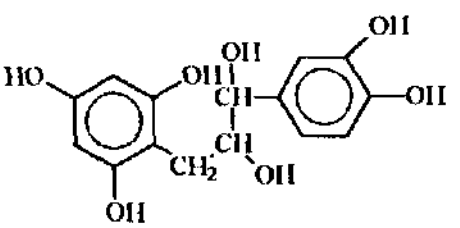
Condensed tannins are a mixture of several polymers which are condensation products of the flavan discussed above. The polymers are called flavolans. The flavan-3.4-diols play a more important role than the catechins (flavan-3-ols).

Tannins have been defined to have a molecular weight of 500-3000. The molecular monomers are made up of 2 to 10 units and could thus be called oligomers. Polymerisation plays an important role in determining the properties of the tannins, which vary depending on whether dimers, trimers or tetramers are present. Quesnel (1968) has drawn attention to the fact that the shape of the tannin polymer plays a great part in determining the properties of tannins. It is thus of particular interest to know the nature of the links in the condensed tannins.

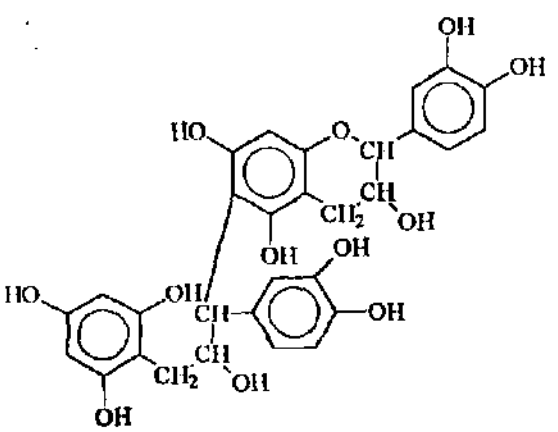
In the case of flavan-3-ols, Freudenberg and Weinges (1962) noted that there was an increase in the number of hydroxyl groups on polymerisation presumbably due to the addition of water to the O - C-2 bond of the heterocyclic ring thus giving one phenolic and one benzyl benzylic hydroxyl group. The benzylic hydroxyl group readily forms a carbonium ion (with a reduced electron density) in acid medium by the reaction shown;



The carbonium ion can then react with a strongly nucleophilic centre (one having an excess of electrons) such as is found at C-6 or C-8 of the flavan A ring of a second molecule of flavan 3-ol giving a dimer whose structure was proved by Weinges and Nagel (1968):



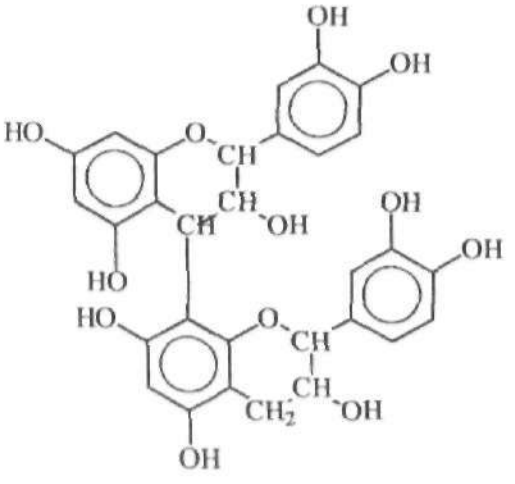
(36)



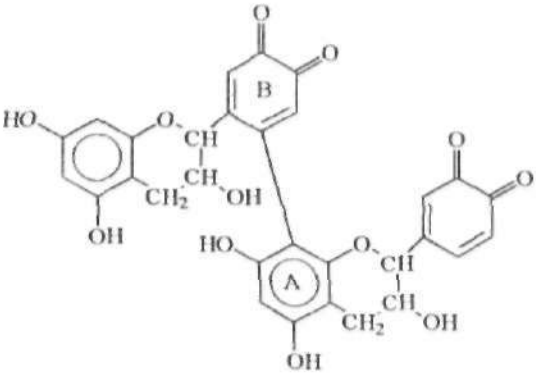
(163)

Such a mechanism could involve a large number of molecules to yield a high molecular weight polymer.

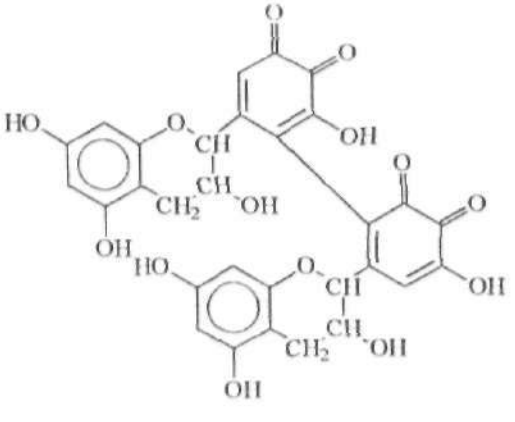
With flavan-3, 4-diols, Freudenberg and Weinges (1962) found a much greater ease of polymerisation than for the corresponding flavan-3-ols. They presumed that this was due to the fact that the hydroxyl group at C-4 was also benzylic in nature and could react with the nucleophilic centres of another hydroxy flavan to give the dimer shown below; (163)



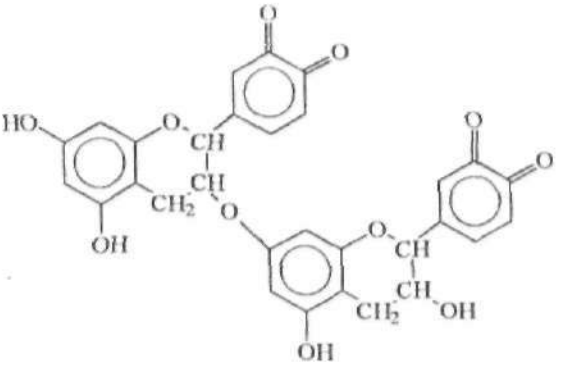
(163)



(164)



(165)



(166)

The dimer could also condense with another monomer through the free OH at C-4 in the lower ring system and thereby increasing the number flavans until an appropriate size of a polymer is reduced.

By treatment with acid, a molecule such as the dimer above gives carbonium ions and rapidly gives insoluble high molecular weight compounds, the phlobaphenes. But under the same conditions, certain bonds in the polymer can be broken giving flavan-3, 4-diol monomers which can then be transformed into anthocyanidins. These reactions account for the two main properties of the condensed tannins which are placed on flavan-3, 4-diols. It seems highly that, in any one tannin, several different types of linkages exist simultaneously, perharps involving several different monomers, like flavylium salts, anthocyanins etc.

Sources of condensed tannins include quebracho, mangrove, mimosa (wattle), Burma cutch, Myrtan, Gambier, Eucalyptus kino.

2:6:3:5 Biogenesis of Flavonoids and condensed Tannins

The classical theory of Robinson (1936b) on the biogenesis of flavonoids showed that the two benzene rings of flavonoids are fundamentally different and thus suggested two different biogenetic origins which Neish (1964) demonstrated as acetate and shikimic acid pathways. Robinson (1936b) proposed that the various flavonoid classes and their structurally related compounds are derived basically from 1,3-diarylpropane C_6-C_3-C_6 units formed by condensation of a C_6 ring (A-ring) with a C_6-C_3 (B-ring) units. Fischer and Dangschat (1935) predicted that shikimic acid and quinic acid might act as intermediates in the formation of gallic and protocatechuic acids and other natural phenolic compounds but it was Birch (1962), Birch and Donovan (1953) who suggested for the first time that the condensation of cinnamic acid and three malonate molecules would yield the chalcone as the primary compound of the C_6-C_3C_6 type of flavonoid biosynthesis. This view was favoured by Seshadari (1959) and upheld by Grisebach and Ollis (1961) and was finally confirmed Roux and Ferira (1974). Tracer experiments by Davis (1955), Sprinson (1960), Gibson and Gibson (1964a), and Gibson and

Jackson (1963) have established that the C_6-C_3 (B-ring) of flavonoids comes from shikimic acid while the C_6 (A-ring) is formed by head-to-tail condensation of three acetate molecules (Robinson, 1975; Haslam, 1977; Gupta and Haslam, 1979). Grisebach (1972) established that hydroxylation and O-methalation of cinnamic acids occur before condensation with the atoms of the A-ring but other modifications of the B-ring occur late in the pathway. The shikimic acid pathway therefore represents the principal modeof accumulation of phenolic compounds in plants since Davis (1955) had demonstrated that both benzoic acids and aromatic amino acids as well as cinnamicacids are all formed in this way while all aromatic rings with meta-hydrolxyl groups are presumed to arise from the acetate pathway. Furthermore, isotopic tracer experiments have proved conclusively that C_6-C_3 units such as phenylalanine and cinnamic acids are efficient precursors of the B-ring portion of flavonoids (Ribereau-Gayon, 1972).

The chalcone-flavonone interconversion occurs non-enzymatically while dehydrogenation of a flavanone yields the corresponding flavone. Wong (1967) established that chalcones are direct precursors of aurones and stated the involvement of an epoxide intermediate(Wong, 1968) between chalcones and flavononols earlier on proposed by Grisebach (1961b) and which subsequently act as precursors of leucoanthocyanidins, flavanols, anthocyanidins, catechins and the condensed tannins. This interconversion is demonstrated in scheme IV (Robinson, 1975).

Scheme IV: Flavonoids Pathways

2.6.4 Sources of Tannins

Tannins are of wide occurence in plants and are usually found in greatest quantity in dead or dying cells (Trease and Evans, 1972) Some sources of the different types have been indicated in

relevant places in the text. For emphasis and easy reference, these sources are brought under one heading.

2.6.4.1 Hydrolysable Tanning.

The sources include rhubarts, cloves, red rose petals, bearberry leaves, chinese galls, Turkish galls, hamamelis, chestnut and maple for gallotannins. The ellagitannins are found in promegranate bark, myrobolans, eucalyptus leaves Kuosso, some Australian kinos and oak bark.

2.6.4.2 Condensed Tannins

Some plant products like tea and hamamelis contain both hydrolysable and condensed tannins but the following are rich in condensed tanning. Condensed tannins are found in cinnamon, wild cherry, cinchona willow, acacia, oak and hamamelis barks. Krameria and male fern roots/rhizomes also contain condensed tannins. The seeds of cocoa, guarana, kola and areca are sources of condensed tannins. So also are the leaves of hamamelis and tea. Finally, extracts and dried juices of catechu, arecia and mangrove cutches, East indian kino, buteagum and eucalyptus kino also cantains condensed tannins.

2.6.4.3 Pseudo Tannins

Pseudo tannins are compounds of lower molecular weight than true tannins and they do not normally respond to the goldbeater's test. Main sources include: rhubarls (gallic acid), catechu, acacia cutch, many Australian kino, cocoa, guarana (containing condensed tannins); mate, coffee (particularly unroasted) (chlorogenic acid) and ipecacuahna (ipecacuanhic acid).

2.6.4.4 The Commercial Sources of Tannins

Commercial tannins as used in leather industry are obtained from quebracho wattle, chestnut and myrobalans trees. Other sources include the chinese and Turkish galls, pomegranate rind, pomegranate bark, eucalyptus leaves and kino, oak bark, Divi-Divi, valonea, and knoppern nuts. Commercial sources of the condensed tannins include quebraco, mangrove, mimosa (wattle), Burma cutch, myrtan and gambier.

2.6.4.5 Nigerian Tannin Sources

Traditional tanning has been in existence in many parts of Nigeria for many years. Oral evidence has shown that the pods of the plant Acacia nilotica (Bagaruwa in Hausa) have been in use for tanning in Northern Nigeria for a long time. Inspite of this fact the screening and assessment of the quantity and quality of tannins in A. nilotica and other plants did not start in Nigeria until the early 1960s when the Leather Institute established by the Food and Agricultural Organisation (FAO) of the United Nations Development Programme (UNDP) in conjunction with the Federal Government of Nigeria and the then Northern Nigerian Government. David Winters was the first person to screen Nigerian trees for tannins. Not unexpectedly, the first most detailed work on any tanning containing plant was done on "Bagaruwa' Adewoye et al 1984 characterized the tannins in the pod of the plant. He established that the pod contained 30% of tannins and 18% of non-tans Adewoye 1971. He was able to show also that the main type of tannin was hydrolysable tannin. Adewoye et al 1986, also worked on the husk of the fruit of Parkia Clappertoniana (the West Africana Locust bean) there also, the main type of tannins was the condensed tannin. The husk contained 44.68% of tannins. A. nilotica is now being used on a small scale as a source of tanning. It has infact attained international recognition and has replaced A. mimosa (mimosa) which used to be the main vegetable material and was imported.

So far the most comprehensive chemical work done on any tannin containing plant is by A.A. Nduji as reported in his PhD thesis 1986. He reported that "various tissues of Anogeissus schimperii (Combretaceae) including the leaves, bark, sapwood and heartwood were subjected to tannin analysis to determine their suitability as commercial vegetable tanning material using the Official Methods of the Society of Leather Technologists and Chemists. The analysis revealed that the leaves possess the highest percentage tannin content (15-20%). The tannins are of the mixed type and were found to be more concentrated in the very young leaves with the month of March as the peak period (23%). Investigation 'of the shelf life also showed a maximum tannin loss of 16.5% over a year indicating that leaves harvested at the peak period retain about 83.5% of their tanning potency for at least one year. Phytochemical studies of the various plant tissues using largely paper chromatography in combination with UV and mass spectroscopy led to the isolation and characterization of a large number of polyphenols and flavonoids which are known to possess tannin/filling properties (Nduji and Okwute 1987). They include a galloylated glycoside (LPE.2) whose hydrolysis products were glucose, quercetin and gallic acid together with a complex fragmentation pattern; 3,3',4'-tri-o-methyl-flaveflagic acid (m/e 360); 3,3',-di-o-methylellagic acid (m/e 344), (+)- catechin (M+1)' 291 quercetin (m/e 302), Myricetin (m/e 318), ellagic acid, chebulagic acid, chebulinic acid, gallic acid, protocatechuic acid, gentisic acid, cyanidin and delphinidin as well as a sterol, β-sitosterol (m/e 414). The isolation of 3,31,4-tri-O-methylflavellagic acid directly from a natural source as well as that of a galloylated glycoside (LPE.2) are being reported for the first time. 3,3,4'-tii-O-methylflaveflagic acid was found to co-exist with 3-3,4-di-O-mythiell . c acid and 3,31,4'-tri-O-mythlellagic acid in the bark of A. schimperii (Nduji and Okwute 1988). On the basis of the above studies, the leaves of A. schimperii are being proposed to the Nigerian tanning industry as a potential tanning material complementary to the already known and widely used Acacia nilotica pods.

2.6.4.6 The Eucalyptus as Sources of Tannins

Many Eucalyptus trees have been surveyed as possible sources of tannins (Penfold and William, 1961) notwithstanding the large number that have been surveyed, very few 'species contain sufficient tannin, material in the bark or wood to warrant commercial exploitation. The few species which are rich enough in tans for commercial purposes are confined to Western Australia. These include: E. astrigens, E. gardneri, E. talcata, E. acidentalis, E spathuleta E. brockwayi and E. dundasi. An outstanding eucalypt species of commercial importance is E. wandoo found in South Western. Australia. The relatively high yield of tannins (about 8%) in the wood, and the extent of the forests, made it practicable to make a concentrated extract (called myrtan) from the whole tree except the foliage. Very little information has been published about the chemistry of the Eucalyptus tannins beyond the fact that they consist mainly of catechol and pyrogallol types. The extract from the wood of E. wandoo contains about 60-20% of non-tannins and 18-20% of non-tannins, based on a moisture content of 18-20% of the tannins 60-65% is composed of pyrogallol and 35 to 40% catechol (Penfold 1961).

2.6.4.7 The Eucalyptus Kino

The common name, "Gum Tree" has been given to the Eucalyptus in Australia. This name is misleading because no gum in the strict sense of the word is obtainable from any species, It is true that many species yield dark-coloured, astringent exudations called "kinos", which differ considerably from true gums in chemical composition. The term "gum" is used for water-soluble substances of carbohydrate composition, whereas the name 'kino' is applied to those resinous exudatious and accretions which contain tannins.

Eucalyptus kinos are usually observed as brown or reddish lumps on the barks of trees, or sometimes as a jelly-like or glass-like mass. They are also found in veins and pocketc in cut timber, and in addition, occur as liquids in sealed resrvoirs inside the tree. When these are breached the kino flows freely as a somewhat viscious liquid and as much as 451 have been known to exude from a single reservoir in E. calophylla (Hillis, 1949). The liquid kino hardens on exposure to the air. This change is thought to be due to enzymes (organic catalyst) which cause the oxygen of the air to react with the kino to change its chemical nature soi that it becomes a hard, brittle mass.

The frequency and extent of the occurence of the kino veins and pockets, as well as free kino, varies with the species. The prevalence of these veins in such a species as E. calophylla can often limit the usefulness of its timber for some purposes. Usually, the bloodwoods such as E. calophylla contain large amounts of free kino, but in such species as E. marginata, E. regnans and E. microcorys, it is rare (Hillis, 1951).

Many of the eucalypts contain dark-coloured accretions in the cells, and these are known as cellullar kino. There is no method at present for accurately estimating this kino, but an indication of the amount present may be gained from the percentage soluble in alkali. E. marginata contains 25 to 30 per cent, and E. sideroxylon about 40 per cent (Hillis 1951).

The purpose and function of kino in the wood is not fully understood. It has been shown that the formation of kino is not due to any normal metabolic process, and that free kino collects only when the live bark is injured in some way. Kino veins in timber are caused by a variety of external factors including fire and insect damage and are often associated with knots.

The examination of Kinos from accurate botanical sources was first undertaken in a small way by Lauterer in Queensland (1895) and more extensively by Maiden & Smith (1895) and Smith (1896, 1913). However, the methods used by these workers are now of little value.

Eucalyptus kinos consist mainly of tannins (70-80 %) together with many minor constituents. According to Maiden (1889a, 1889b, 1891) they can be divided roughly into three groups as follows:

a. Ruby Group: the larger class, which give a clear ruby-coloured solution in water, e.g E. regnans and E. obliqua.

b. Gummy Group: aqueous solutions of the kino of this group precipitate a 'gum' when poured into alcohol, e.g E. siderophloia and E. sideroxylon. The so-called gum is a tannin called 'emphloin'

C. Turbid Group: crystalline compounds are deposited following the cooling of hot, concentrated aqueous solutions of these kinos. These substances are either aromadendrin or eudesmin or both, e.g E. calophylla, E. hemiphloia, E. tessellaris and E. eximia.

The composition of kinos varies according to species, and apparently in a manner determined by the order of their evolution. These Eucalypts which appear to be the more primitive, e.g the 'Bloodwoods', produce kinos belonging to the Turbid Group, whereas the kinos from the eucalypts of apparent later evolution belong to the Ruby Group.

Recent investigations by Hillis (1951), using paper chromatography, have demonstrated the great complexity of kino of E. calophylla. Other free kinos obtained from freshly opened pockets of E. gummifera E. ganiocalyx, E. obliqua, E. radiata and E. regnans, and free kinos in the solid state from E sideroxylon were also examined by Hillis (1951). Most of these were also shown to be a very complex nature as evidenced by the number of resolvable components, as follows: E. goniocalyx 11, E. gummifera 13, E. radiata 5, E. regnans 4, E. obliqua 3, and E. sideroxylon 3. It is noteworthy that the presence of a sugar was shown only in the kino of E. goniocalyx. In the above course of this work on the turbid group of kinos, some yellow crystaline compound were isolated from E. calophylla kino and identified as

kaempferol which belongs to the class of substances known as flavonols. The crystalline substances, aromadendrin and eudesmin, have also been thoroughly studied. Aromadendrin isolated from E. calophylla and E. gummifera kinos has been identified as a tetrahydroxyflavonone, and has been shown to be identical with dihydrokaempferol, and should be known by that name (Hillis, 1952). Eudesmin has been shown to be a lignan of the furanofuran type, and is chemically the same as the eudesmin from pine resin. Ellagic acid has also been isolated from the kinos of E. calophylla and E. gummifera (Hillis 1952).

Eucalyptus kino is an astringent with an action similar to East Indian kino (dried juice obtained from the trunk of Pterocapus marsupium Roxb.), but weaker and more prolonged. Liquid extract and a tincture have been employed as styptics, and as an astringent in throat gargles. It is also used internally in the treatment of diarrhoea and dysentery. Small quantities have found an unusual application in the colouring of champagne. Efforts were made during the nineteenth century to introduce eucalypt kinos into the overseas markets. Several of these kinos did not gelatinize in tincture which was the greatest objection to the kino of Pterocapus marsupium (Smith, 1904). Unfortunately, it was not practicable at the time to collect large samples from selected species, and the consequent variation in quality resulted in Australian kino falling into disfavor. A small but consistent demand resulted later when kino was obtained from a single species.

2:6.4.8 Eucalyptus citriodora "Kino "

The plant Eucalyptus is alien to Nigeria (Dashe and Shok 1993) even though many species are presently grown in Nigeria. Two species are known to exudate, E. citriodora and E. camaldulensis which are amongst the five most common. The remaining, E. tereticonis E. closean E. torealena do not exude. E. citriodora is not known to exude or produce "kinos" in its natural habitat Australia,

(Penfold and Willis 1961). Probably because Eucalyptus is alien to Nigeria, no rural tanner has tried any part of the plant for leather production. There is only very scanty information on any work being done on any species of Eucalyptus except those on work done by the National Research Institute for Chemical Technology, Zaria (Mustapha 1992); and the preliminary work on the "kinos" of E. citriodora as a source of tannins (Dashe and Shok 1993).

The present work is on the exudate of of E. citriodora. As earlier on mentioned, E. citriodora is not known to exudate "kinos" in its natural habitat Austrialia. E. citriodora is of the family Eucalyptuseae (Blakely 1973, 1980). It is amongst the seven common species of eucalyptus that grows very well in most parts of Nigeria. E. citriodora is a tall, graceful, shaft like tree 25 - 40 metres height. It is grown in poor gravelly soils from sea level to about 600 metres in rain fed areas. It grows well in almost any soil. Though it is a tropical and sub-tropical tree, it grows staisfatorily in temperate regions.

The bark is white to pinkish in colour and it is deciduous. Juvenile leaves are arranged in opposite position for 4 to 5 pairs with a few peltate. They are oblong to oblong lanceolate and have rough and bristle-like hair. Adult leaves are alternate 10 to 25 x 1 to 4 cm, narrowly to broadly lanceolate and or concolorous. Pentiolate veination is moderately conspicuous and inter-marginal vein are not distinct from margin. Lateral veins are at 40° to 60° angles to the midrib. Inflorescence is usually an axillary corymbose panicle, unbels 3 to 5 flowers on a terete. It flowers between June and July. (Pendfold and Willis, 1961).

The exudate of Eucalyptus citriodora like the exudates of other eucalyptus trees is a gummy sap called "kino" (Pendfold Willis 1961). It is a resinous material that contains tannins and it is said to be found in wood tissue called "Gum Vein". Generally, kino is rarely found in the bark. Kinos are a dark-coloured, astringent, resinous exudations and secretions that contain tannin while gums are water-soluble substances of carbohydrate composition. This is a very significant difference between

"kinos" and gum that must be properly noted for relevant application and understanding of this work This is necessary because there is a tendency to use the two terms interchangeably. The kino of E. citriodora is usually observed as a brown lump, sometimes as a jelly-like or glass-like mass on the bark of the tree. The dried kino is sometimes more crystal-like than jelly. Generally, fresh kino from eucalyptus flows freely as a viscous liquid. As much as 45 litres have been known to exude from a single reservoir in E. callophylla (Hills 1949). The experience in the Nigerian E. citriodora exudate is that it dries off in a crystal-like roundish mass most likely through a process of polymerisation. It must be noted however, that the kino collected and allowed to dry may not form the crystal-like structures.

Because E. citriodora has not been known to exudate, no work has been done on its exudate, either to determine its tannin content or its chemical components in Australia its natural habitat. However, a lot of work has been done on the tannin content of various species of eucalyptus and those that actually combine with protein (collagen) to give leathery properties are pyrogallols and ellagitannins (Steeden and Anderson, 1950) Preliminary investigation on the tannin content of the kino of E. citriodora shows that the kino possesed about 37% tannins, and 23.67% non-tan and 60% solubles on the average. It is pyragallolic in nature. It produced full leathers of yellowish colour and a shrinkage temperature of above 60° C (Dashe and Shok 1993).

2:6:5 General Properties of Tannins

Despite the long standing history of vegetable tannins, not much is known of their chemistry. However, broad term description of the nature of these plant polyphenols can be given. They are secondary metabolites widely distributed in various sectors of the plant kingdom. They are distinguished by the following general features. (Haslam et al, 1992).

(i) Water solubility: Although when pure, some plant polyphenols may be, with difficulty, soluble in water, in the natural state polyphenol-polyphenol interactions usually ensure some minimal solubility in aqueous media. Tannins are also soluble in dilute alkalis, alcohol, glycerol and acetone but generally only sparingly soluble in other organic solvents (Trease and Evans 1972).

(ii) Molecular Weight: Natural polyphenols encompass substantial molecular weigth range from 500 to 3-4,000. Sugestions that polyphenolic metabolites occur which retain the abillity to act as tannins but possess molecular weight up to 20,000 must be doubtful in view of the solubility factor (Bate-Smith 1962, Kirby and White 1954, Schmidt 1954, Haslam 1966).

(iii) Structure and polyphenol character: Polyphenols possess some 12-16 phenolic groups and 5-7 aromatic rings per 1000 relative molecular mass. This applies particularly to the condensed tannins.

(iv) Solutions of tannins precipitate heavy metals, alkaloids, glycosides and gelatin. With ferric salts, gallotannins and ellagitannins give blue-black precipitates and condensed tannins brownish-green ones. If a very dilute $FeCl_3$ solution is gradually added to an aqueous extract of hamamelis leaves (which contains both types of tannin) a blue colour is produced which changes to olive-green as more $FeCl_3$ is added (Trnease and Evans, 1972).

v. The products of acid hydrolysis of the tannins depend on the type of tannin. Hydrolysable tannins are esters of sugars and phenolic acids or their derivatives. They therefore yield sugars (mainly glucose) and gallic and ellagic acids on hydrolysis. For this reason, they are referred to as gallotannins and ellagitannins (Ribereau-Gayo, 1972n).

The condensed tannins on the other hand, are converted into coloured anthocyanidins by heating in dilute acid solution. It has been shown that this reaction is due to the presence of flavan-3 4-diols (leucoanthocyanidins) and their condensation products. The catechins

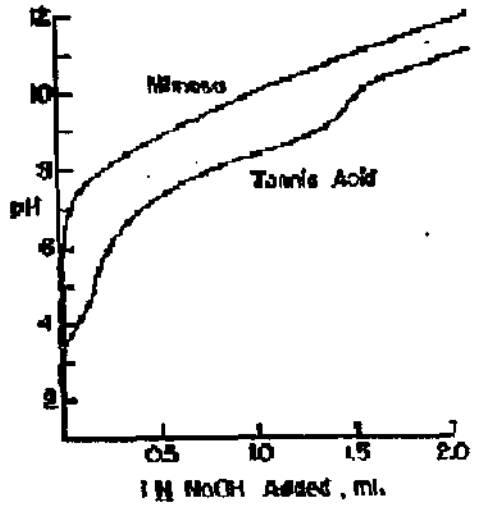
(flavan-3, -ols) give yellow brown insoluble products of high molecular weight called phlobaphenes. The leucoanthocyanidins also give the phlobaphenes but the intensity of the anthocyanidins masks them (Ribereau-Gayon, 1972).

vi. Astringency:- Tannins produce on the tongue the sensation of puckering. Substances which cause this effect are called astringent in proportion to the degree of the effect. Mainly fresh leaves (e.g guava leaves) green fruits, barks or tannin solutions cause this sensation when chewed or drank. The astringency appears to be a function of the molecular size of the tannin, the proportion of tannin total solubles, the hydrogen ion concentration of the solution, the electrical charge of the particles, the nature of anions and neutral salts present ("acid-salt ratio"), temperature, and tannin content of the solution. (Gustavson, 1956). This property is very important in vegetable tannin because it gives an indicaion as to the likely effectiveness of a vegetable tannin.

vii. The two-phase system - The solutions of vegetable tannin extracts contain a variety of substances, of which a number are probably of the same general type chemically. These constituents have various sizes (polydisparity) ranging from a few Angstrom units to some tenths of 1mm. Moreover, the constituents possess various degrees of affinity for collagen, and the disturbance of the composition of the constituents which are "peptidized" (peptidization is the production of a colloidal solution of a substance, especially the formation of a gel, this is also called deflocullation). The deposition of such peptidized substances may cause complications. One of these possible complications is that a large part of the vegetable tannins which are included in the fixed tannins may exist as a deposit without intimate chemical binding with the protein, or as one-point attached molecules, although on account of its resistance to water it is classified as fixed tannin (White 1956). In the normal tanning of hide into leather by

vegetable tannins, the actual amount of tannin combining with the hide is largely determined by steric factors, such as the accessibility of the reactive protein groups and, even more, by the volume and space between the collagen chains and fibres which can be occupied by the tannin molecule. (Gustavson, 1956).

viii. One principal difference between the hydrolysable and the condensed tannins is the presence of strongly acidic group (-COOH) in addition to the dominating weakly acid phenolic groups in the hydrolysable tannins. This points to the possibility that some of the glucoside linkages between gallic acid and the sugar involve a phenolic group, instead of the normal type, through the carboxyl group, which hence, will be free. The hydroxyl groups are the main reactive groups of the condensed tannins which, however, contain relatively few -OH groups compared to the hydrolysable tannins. The titration curves of the two different tannins, tannic acid and mimosa bring out the different electrochemical characters of these tannins i.e the presence of the strong acid group in tannic acid which titrates in the low pH range. The phenolic groups, with pK values greater than pH- 7_1 commence to be titrated at pH greater than 7 - 8 (Gustavson, 1956).



Titration curves of solutions of tannic acid and mimosa extract, containing 1.0% tannin.

ix. Tannin-Gelatin Reactions- As stated in (iv) above tannin solutions precipitate gelatin. The gelatin - tannin system has been used because the steric factors in systems with the solid fibrous skin protein as substrate and the difficulties connected with establishing the equilibrium state can be avoided. A 1% gelatin solution containing 10% NaCl has been used for these reactions. The salts particularly (NaCl) expand the range of precipitation from about pH 2 - pH8. The optimal pH range is 3.5 - 4.5 Mimosa for example, can be detected at a dilution of 1 to 200,000 at pH values of 4.0 - 4.2. The existence of tannin was known by the determination of the amount of tannin left in solution. Washing the precipitates with an acetone/water mixture (80:20) removes unattached tannin. In most cases, the ratio of attached tannins to gelatin was about 55:45 as against the ratio of 24:10 for unwashed precipitate in some cases. This reinforces the point that has been made in (vii) above that constituents other than the so called tannins may, by circumstance "participate" in the tanning process. It would seem that the fixation of vegetable tannins by gelatin, present in dilute solution, and hence without any spatial restraint and with all the reactive groups accessible, is independent of the value of the system in a wide pH range from the lowest pH up to the pH of the isoelctric point of the potein substrate. With insoluble proteins such as collagen, the effect of pH of the reacting tannin solution upon the vegetable tannin fixation is very marked. It should be noted, however, that any solid protein substrate which possesses a fairly constant amount of reactive groups for binding of vegetable tannins of the type of mimosa fixes these tannins indepenently of the pH value of the system in such a wide pH range as 2 - 8. Such substrates are hydrated modified polyamides and formaldehyde-tanned hides (Gustavson, 1956).

Hydrogen ion concentration: The hydrogen ion concentration of the system is probably the most important factor in tanning. This effect is very intricate because, firstly, it involves the

influence of the hydrogen ion concentration on the collagen substrate as regards its degree of swelling. The degree of swelling determines the accessibility of reactive protein groups, and the internal resistance by the hide structure to the diffusion of tannins. Secondly, the pH effect involves also the influence of the protons on the tannins in regard to their charge, ionisation, degree of aggregation and secondary chemical reactions (which are especially noticeable at high and low pH values). This effect is however, not as important as the first one. This is because swelling tends to make the reactive groups of protein accessible to the tannins by wideninig the intermolecular spaces and above all, by rupture of crosslinks, preferably hydrogen bonds, due to the concentration of the protein chains and widening of the gap between adjacent collagen chains in certain regions (Gustavson, 1956).

2:6:6: TESTS FOR TANNINS

2:6:6.1 Goldbeaters Skin Test

Goldbeater's skin is a membrane prepared from the intestine of the ox and behaves similar to an untanned hide. A piece of the skin is soaked in 2 percent hydrochloric acid then rinsed with distilled water before placing in the solution to be tested for 5 mins. This is then washed again with distilled water and transferred to a 1 percent solution of ferrous sulphate. A brown or black colour denotes the presence of tannins. (Trease and Evans 1973).

2:6:6:2 Gelatin Test

Solutions of tannins of about 0.5 - 1 percent precipitate a 1 percent solution of gelatin containing 10 percent sodium chloride. Gallic acid and other pseudotannins precipitate gelatin if the solutions are sufficiently concentrated, (Trease and Evans 1973).

2:6:6:3 Phenazone Test

Add 2.5g of sodium acid phosphate to about 5ml of the aqueous extract, warm, cool and filter. To the filtrate add 2 percent of phenazone. All tannins are precipitated to a bulky and sometimes coloured precipitate. (Trease and Evans 1973).

2:6:6:4 Test for Catechin

Catechins on heating with acid form phloroglucinol. They can thus be detected by the modified popular test for liginin. Dip a matchstick in the plant extract, dry, moisten with concentrated hydrochloric acid and warm near a flame. The phloroglucinol produced turns the wood pink or red. (Trease and Evans 1973).

2:6:6:5 Test for Chlorogenic Acid

An extract containing chlorogenic acid when treated with aqueous ammonia and exposed to air gradually develops a green colour. (Trease and Evans 1973).

2:6:6:6 Test for Anthocyanidins

Acid-catalysed cleavage of the interflavanoid bond with subsequent oxidation to yield anthocyanidins has been one of the more important reactions of condensed tannin chemistry and has been used as an indicator for the presence of condensed tannins in plant tissues. A Forestal solvent made up of acetic acid-water-concentrated hydrochloric acid in the ration 30:10:3, V/V/V is used.

About 10mg of tannins is dissolved in 20ml of n-butanol-concentrated HCl (95:5, V/V) and heated for 40 min at 90°C to produce intense red colored solutions. Recent work has recommended

the addition of ferric ion as a 2 percent solution of ammonium ferric sulfate to a methanolic solution of the tannin in the originally proposed n-butanol - HCl solution. This gave a substantial increase in anthocyanidin yield and improved the reproducibility of the results (Hemmingway 1978, Trease and Evans 1973).

2:6:6:7 Ferric Ammonium Citrate Solution Test

Add 5ml of 0.25 percent solution of ferric ammonium citrate plus 0.5-1.0g sodium acetate to adjust pH to about 8. Boil, cool and filter. A purple violet or black precipitate is formed and is insoluble in hot water, ammonia or alcohol, (Balboa 1976).

2:6:6:8 Bromine Water Test

To the tannin extract add bromine water and observe the formation of a precipitate. Condensed tannins would give a buff-coloured precipitate while hydrolysable would not. The bromine probably reacts in the same way as it does with certain mono-and dihydric phenols i.e. substitutes in the aromatic nucleus, (Balboa 1976).

2:6:6:9 Formalin Test

Add 3 drops of formalin i.e. 40 percent formaldehyde in water, plus 6 drops of condensed tannins, this produces a precipitate called phlobaphenes which is insoluble in hot water, alcohol and 5 percent potassium hydroxide.

2:6:6:10 Ellagitannins Test

With sodium nitrite ellagitannins give green color while other tannins give a brown color. Add a few crystals of sodium nitrite to the tannin extract and it gives a green colour, (Balboa 1976).

2:6:6:11. Gallotannins Test

When the extract is added to lime water it gives a blue-grey colour or precipitate which indicate gallotannins, (Balboa 1976).

2:6:6:12 Paper Chromatography

Of the many and varied methods of the analysis of vegetable tannin extracts, paper chromatography has proved to be the most powerful (Harborne 1959). Although the tannins themselves give poorly defined patterns, paper chromatographic methods are ideal for the analysis of the co-occurring flavonoid compounds since these have a wide range of solubilities and are readily detected on chromatograms by means of specific spray reagents and in certain cases by their distinctive fluorescences in ultra-violet light. Several solvent systems have been employed in the paper chromatography investigation of plants extracts, this technique being the most widely used. In the two dimensional chromatography, dilute acetic acid is used for the first direction of development and a partitioning mixture of butan-2-ol, acetic acid and water. Butan-2-ol is preferred to butan-1-ol since it undergoes esterification less readily making and the composition of the solvent phase to remain constant over long periods. The separation is based on physical properties as in the principles of paper chromatography. In dilute acetic acid, separation is ascribed both to differential absorption by the cellulose medium and to differences in solubility in the aqueous medium, while in the organic phase, separations are due to the repeated partitioning of the solute between the solvent and the microscopic stationary aqueous phase of the paper (Seikel 1962). Flavones, flavonols, anthocyanidins, aurones and chalcones have been determined in terms of the low solubility of these compounds in water and to their

planar configuration which permit adsorption via hydroxyl groups to the cellulose. In the organic partitioning phase, the Rf. values within a particular class of flavonoids decrease with increase in the content of phenolic or alcoholic hydroxyl groups whereas in the aqueous phase of development, phenolic and alcoholic hydroxyl groups have opposing effects on the Rf values of a flavonoid molecule, thus generally, increase in the former lowers the Rf but increase in the content of alcohol hydroxyl groups has the reverse effect (Haslam 1966).

Various spray agents have found useful applications in the identification of typical orientations of hydroxyl groupings in flavonoids. A few common ones are ferric chloride-potassium ferricyanide (Kirby et al 1953), ammoniacal Silver nitrate (Partridge 1948), bis-diazotised benzidine and p-toluenesulphonic acid (Roux 1957). Roux has used the chromatographic methods with a little modification to prevent the formation of phlobaphenes which interfere with chromatographic analysis, to observe that the concentration of flavan-3,4-diols decreases with increasing age of the heartwood of the tree (Roux et al 1960).

2:6:6:13 Glycosides and Esters

This involves isolation of constituents in the forms they exist in plant tissues. As much as possible hydrolysis is avoided. It is advantageous to use ethanolic or methanolic extract which can easily be concentrated if necessary as against aqueous extracts.

The basic solvent is the butanol-acetic acid. However, if satisfactory separation is not achieved, water may be employed, using 2-dimenstional chromatography if necessary. Consequently to avoid trailling, use a weakly acid solvent, usually acetic acid at a concentration between 2 and 15% (Ribereau-Gayon 1972). In 1953, Ribereau -Gayon, used an aqueous solvent for the separation of anthocyanidins, that is a solvent in which partition between two immiscible solvents is not the only

phenomenon involved in the separation of the constituents. Factors of absorption and differential solubility are also involved. The lower layer of the butanol- acetic acid - water mixture, which is in effect an aqueous solution of acetic acid saturated with butanol was used. It was found to be better for the separation of anthocyanidins (Ribereau-Gayon 1953).

The solvents Forestal and toluene acetic acid for the aglycones, butanol-acetic acid-water for the glycosides, are undoubtedly the most valuable and represent the common solvents for the study of phenolic compounds (Ribereau-Gayon 1972).

The ease of detection of phenolic compounds on paper is one of the reasons for the success of the use of this method in the investigation of these substances. Some flavonoids i.e. anthocyanidins, aurones chalcones are immediately evident because of their phenolic compounds are coloured under ultraviolet light, mostly in Woods lamp (366nm) and also at shorter wavelengths (253.7nm). Some are infact florescent. Luminescence is strongly intensified at low temperatures i.e. when the chromatogram is plunged into liquid nitrogen (-196°). Many of the benzoic acids, which are invisible at ordinary temperatures in ultraviolet, become intensely blue when so treated (Ribereau-Gayon 1972). Similarly, phenols, on account of their acidic character, have different colours in acid and alkalis. This property is used in the examination of chromatograms in the vapour of concentrated ammonia (sp. gr. 0.925, 20%NH_3).

2.6.6.14 Aglycones or Simple Phenolics

The procedure comprises the extraction of the plant materials and then hydrolysis separately each of two fractions with acid and alkali. The hydrolytic products are acidified using HCL, prefarably with cooling. The aglycones in the acid solutions so obtained are extracted with immiscible solvents (ether, ethyl acetate, isoamyl alcohol). It is necessary, therefore, to operate in aqueous solution.

However, if alcohol is used for the extraction this has to be removed by distillation *in vacuo* and the residue redissolved in water.

The solution resulting from alkaline hydrolysis, contains the phenolic acids; This can be extracted with ether after reacidification with just sufficient acid to produce a supernatant phase after shaking. The ether extract is spotted on the chromatographic paper and developed in butanol-acetic acid (upper layer of a mixture of n-butanol: acetic acid: water: :4:1:5 or in Forestal solvent (acetic acid-conc. HCl-water::300:30:100); or for preference, toluene-acetic acid (upper layer of a mixture of toluene acetic acid water::4:1:5). The last solvent is especially recommended because it gives a good separation of the phenolic acids which, in other solvents, do not separate well and become crowded at the solvent front. With toluene-acetic acid mixture, it is necessary to equilibrate the paper for some hours with the aqueous phase of the solvent mixture before beginning the separation proper. The substances are revealed either by viewing in ultraviolet light or by spraying with diazotised nitroaniline (Rebereau-Gayon, 1972).

The aglycones resulting from the acid hydrolysis are treated differently according to the presence or absence in them of anthocyanidin because these red pigments may mask the presence of other constituents.

1. If anthocyanidins are absent, the hydrolysate is extracted once only with isoamyl alcohol, which extracts all the phenolic compounds present.
2. If anthocyanidins are present, remove all the other phenolic compounds by extraction with ether, then the anthocyanidins with isoamyl alcohol. There are thus two extracts to be analysed.

As in the case of the alkaline hydrolysis the solution is treated with just sufficient solvent from a supernatant phase, which is then applied to the chromatographic paper. The Forestal mixture is the

best solvent and while toluene-acetic acid may also be used, butanol- acetic acid is useless. Consequently butanol-HCl (upper layer of a mixture of n-butanol : 2N HCl: 1:1) may be used.

Though good separation of anthocyanidins, flavones, and flavonols is achieved in Forestal, many of the either phenolic constituents like benzoic and cinnamic acid derivatives and coumarins, have high Rf values hence crowd together near the solvent front, where any glycoside which may have escaped hydrolysis and brown oxidation products of some of the phenols may be found. This is resolved by using toluene-acetic acid which gives a good separation of these constitutents with high Rf values in Forestal solvent.

Where one-dimensional chromatography fails to effect a satisfactory separation, two-dimensional chromatogram with butanol-acetic/forestal of butanol-HCl/Forestal may, be used when anthocyanidins are present. Aglycones present on the chromatogram can be located in U.V. light (Ribereau-Gayon 1972).

In the same vein ellagic acid can be readily identified in hydrolysed plant extracts by paper chromatography using Forestal solvent (Bate-Smith 1956a). Gallic acid and the other constituents of hydrolysable tannins have now been identified and isolated in a pure state and their structures determined. Most investigators have used 5-10% acetic acid and butanol-acetic solvent in two-dimensional paper chromatography and revealed the tannins with ammonia, silver nitrate or ferric chloride - potassium ferric cyanide.

2:6:6:15 Thin Layer Chromatography

Paper chromatographic methods provide, perhaps the most suitable technique for the analysis of plant polyphenols with predominantly hydrophilic character. However, thin layer chromatography has been found to be more useful for similar phenols with significant lyophilic nature. It is m ore useful

because of speed, sensitivity and efficiency of separation. The method has not been extensively used as yet for the identification of phenolic compounds in plant extracts (Randerath 1963). From previous work it is interesting to note that chromatography of phenols on polyamide powder, similar to silica gel on thin layer chromatography, is dependent on the formation of hydrogen bonds between the phenolic groups and the amide groups of the polymer (simulating to some extent the natural process of tannage) and the ability of a particular solvent to displace the phenols from their points of attachment to the amide linkage. There seems to be a very bright future for thin-layer chromatography methods for the analysis of plant phenols either to supplement other techniques or as a sole method of analysis replacing existing techniques. Gas liquid chromatography has not been as useful as thin-layer or paper chromatography in the isolation and identification of phenolic compounds of plant extracts. Paper chromatographic analysis in addition to being an efficient method by which the complexity of the tannin extract may be assessed also provides a useful guide to the selection of a particular isolation procedure. Thus substances that are separable in the aqueous phase of paper chromatographic analysis can usually be separated by column or thin layer chromatography on cellulose. Similarly a mixture of substances whose Rf values in the organic partitioning phase of paper chromatographic analysis are quite different may be separated by column or Thin layer chromatography on cellulose using a suitable organic solvent as eluent, or, by counter-current distribution between water and an organic solvent such as ether, ethyl acetate or butan-1-ol, since paper chromatographic separations in the organic partitioning phase arise essentially from repeated partitions of the counter-current type. Where the Rf values of substances in the aqueous phase of paper chromatographic development are approximately the same, the distribution coefficients in the counter-current distribution increase with increasing value of the Rf in the organic phase of development.

2:6:6:16 Infrared Spectroscopy

Studies of infrared spectroscopy of the flavanoids is not as advanced as in the visible and ultraviolet regions. However, it is valuable for the identification of substrances which are complex but closely related, because each substance has its own characteristic spectrum. The other advantage is that each peak of absorption is associated with the presence of a specific chemical group. The use of the spectrum of a reference compound may lead to the production of, or help to determine the structure of an unknown constituent.

Infrared spectra are usually measured in solution in a transparent solvent such as carbon tetrachloride or carbon disulphide, but flavanoids are not very soluble in these solvents, so that it is necessary to work with dispersions of the product in Nujol (highly purfied liquid parafin) or better still in discs of potassium bromide which require lesser amounts of material (1mg) (Ribereau - Gayon and Josien, 1960). It is important to note that the solid state spectra do not always agree with those in solution (Lebreton 1962).

The following observations have been made (Lebreton 1962)

a. Free OH groups are responsible for absorption in the region of 3,300cm^{-1}. When they are hydrogen bonded i.e. in position 3 or 5, the frequency of this bands is reduced to lower, - 3, 100cm^{-1}.

b. Absorption due to CH_3 groups occurs at 2,930cm^{-1}. It is often masked by absorption due to OH groups as indicated in (a) above.

c. Absorption due to the CO group of the heterocycle occurs at 1,685cm^{-1} in the case of flavanones, where the carbonyl group is conjugated only with the A ring. This is lowered to 1,650cm^{-1} in the case of 5 - hydroxyflavanones.

In the case of flavones the carbonyl group is conjugated with both A and B rings. The absorption due to the CO group in 5, 7-dihydroxyflavones is at 1,655cm^{-1} compared with that of the homologous flavones.

d. Aromatic double bonds absorb between 1,500 and 1,600cm^{-1}, a second peak is present at 1,585cm^{-1} when the double bond is conjugated with another benzene ring.

e. Phenolic hydroxyl groups have strong absorption at about 1,360cm^{-1} with a secondary peak at 1,200cm^{-1}.

f. A peak at 1,165cm^{-1} is due to *meta*-dihydroxy substitution (5,7-dihydroxy).

g. Below 1,000cm^{-1}, however, absorption due to the hydrogen atoms of the benzene ring may be observed.

The absence of a carbonyl group from the central heterocyclic ring makes the interpretation of the infrared spectra of anthocyanidins simpler than for other flavanoids. However, because of their poor solubility the solid state method, either by dispersion in Nujol or preferrably in the form of potassium bromide discs are used (Ribereau - Gayon and Josien 1960).

The band, well-defined at 1,6372cm^{-1} is due to the conjugation between the heterocyclic double bond and the benzene ring. Between 1,600 and 1,500cm^{-1} are benzene ring absorption bands. Thus in all anthocyanidin spectra, there are two main peaks, varying in their intensity, the first at 1580cm^{-1}, the second at 1520cm^{-1} . The band at 1,450cm^{-1}, is due to C-H vibration of a methyl group of the methylated anthocyanidins; at 1,464cm^{-1} for peonidin, 1,460cm^{-1} for petunidin and at 1,464cm^{-1} malvidin. A band at 1,430cm^{-1} is due to the methyl group absorption while at 1,445cm^{-1} is due to the benzene ring usually masked in the methylated anthocyanidins by the bands at 1,460 and 1,430cm^{-1}. (Ribereau-Gayon 1972)

2:6:6:17 High Pressure Liquid Chromatography (HPLC)

High pressure liquid chromatography (HPLC) has found useful application in the qualitative analysis of vegetable tannin extracts. An Alphasil ODS column (4.6 X 250mm), UV detection at 280nm, solvent system -0 to 40% acetonitrile, 0.5% aqueous acetic acid over 20 minutes, 2.0cm^3 1 minute flow rate was used (Haslam et al 1992) in the determination of gallotannins in aged vegetable tanned leathers. The result, however, suggested that the polyphenol had probably been transformed by oxidative processes with little evidence of hydrolytic breakdown of the gallotannins.

The formation of multiple peaks in HPLC by hydrolysable tannins that possess dehydrohexahydroxydiphenoyl groups due to the formation of adducts with methanol used to dissolve the annalytes can be overcome by preparing solutions using acetonitrile as solvent prior to chromatography (Hatano, et al 1988). Flavanoids of Rhus coriaria were separated by HPLC with methanol/water gradient elution and UV detection at 350nm or 280nm (Vau Loo et al 1988). Risch and co-workers (1988) analysed catechin and epicatechin by HPLC after benzoylation using benzoyl chloride in the presence of pyridine with 180 octane-diethyl ether--acetonitrile (150: 60: 10 V/V/V) and isocratic elution and UV detection at 231nm. Tannic acid and its phenolic metabolites in biological fluids have been determined by HPLC after extraction with ethyl acetate under acidic condition (Zhu et al, 1992).

2:6:7 Possible Function Of The Vegetable Tannins In Plants

Despite the interest shown by early workers in the biological function of tannins, this aspect is not receiving much study presently considering that tannins usually account for more than 25% of the dry weight of the plant in which they occur. The suggestion that they are metabolic waste products deposited in various tissues in order to get rid of them cannot be properly evaluated until we know

more about the exact nature of typical tannin molecules and can trace their derivation. The high tannin content of the heartwood is observed to be associated with the longevity of the species. Similarly there is a high increase in tannin secretion in response to injury or to insect or disease attack by the plant. It is true that tannins occur more extensively in trees in tropical and semi-tropical areas than in the temperate zone, which is closely related to a greater frequency of occurrence of bacteria or fungi in these areas or possibly to a greater need for synthesis of precursors fluorescing polyphenols in the leaves to convert the high UV content of the sunlight into less damaging visible radiation.

Whether these observations are correct or not there is now a reasonable amount of information indicating that tannins do modify the effect of bacteria, fungi, and viruses on plants, and this may well be one of the main reasons why plants produce these substances. Whereas from these investigations it appears that tannins are not toxic to micro-organisms, they are able to inactivate both viruses and extracellular enzymes by insolubilising these, just as they will any typical protein molecule possibly by chelation thereby creating conditions unfavorable to enzyme action. Thus we can see how tannins could prevent simple mechanical transfer of viruses, or attack by bacteria or fungi dependent on extracellular enzymes for converting a substrate into a soluble assimilable form. Consequently it is easy to understand the need for a relatively high concentration of tannins in a plant tissue to provide adequate protection, since their protective value seems to depend not on specific toxicities, but on their capacity for insolubilising protein by a reversible, easily dissociated, cross-linkage (White 1956).

Because of the paucity of information on this aspect it is difficult to state whether work has been on tannins in the true sense of the term or on lower molecular weight polyhydric phenols which cannot tan or both. It has been shown that low molecular weight phenols can interfere with this inhibition by competitively occupying combining sites on the virus particles without insolubilising or "tanning" the virus. Similarly it is evident that oxidative condensation of such low molecular weight

phenols makes them also capable of producing virus inhibition. From the foregoing it is evident that the production of tannins in plants is to provide resistance against diseases. The degree of resistance varies with different tannin extracts which is in line with the variations in the properties of different tannin extracts (White 1956).

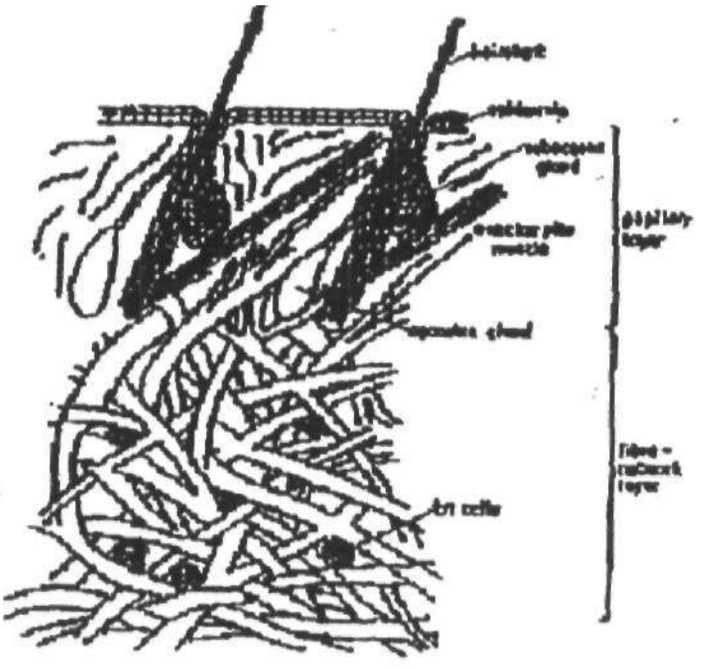
PART II
TANNING

2: 8 INTRODUCTION

Tanning is the process by which hide/skin is converted to leather. Leather is the product of the reactions between the protein of hide/skin with tannin. These reactions alter the chemical composition and physical properties of the protein, making the hide/skin resistant to chemical and microbial attack and also according it some additional physical strength.

2:8:1 The Hide/Skin.

The skin is the covering of animals. It is the raw material for the production of leather. For ease of identification the word skin usually refers to the outer covering of smaller animals like sheep, goat, pig, fish or swallow bird. Hide, on the other hand, refers to the covering of bigger animals like cow, horse, elephant, etc. However, whether it is the skin of a cow, sheep, sardine fish or swallow, it is histologically divided into three principal parts viz:- the epidermis, the dermis and the hypodermis, going from the outermost to the innermost layer, as is shown in the figure below.



2:8:1:1 ***The Epidermis.***

The epidermis is the outermost layer of the skin. It is very thin compared with the thickness of the underlying dermis. It consists of layers of epidermal cells which are differentiated into a number of regions which may be recognised using the light microscope. The outermost region is the stratum corneum which consists of keratinized or cornified layer. The innermost region is the stratum Malphigi which is the living basal cells which also includes the melanocytes. The basement membrane is part of the epidermal-dermal junction which separates the stratum Malphigi from the so called papillary layer of the dermis.

From the stratum Malphigi are produced all the epidermal structures including hair and granules of the pigment melanin. No blood vessels or lymph ducts extend from the papillary layer over the basement membrane unto the epidermis. The basal cells derive their nourishment by the diffusion of materials from the dermis via the basement membrane. Many basal cells have finger-like intrusions into the papillary layer and so aid in the adhesion of the epidermis to the dermis. During life, the stratum corneum is shed in thin sheets and is replaced by upthrusts from the cells below.

The hair has its origin deep in the papillary layer. It consists of the bulbs (root) and the shaft, the long projecting part. The lower portions of the shafts are contained in follicles which are sheaths of epidermal cells, continuous with other parts of the epidermis and completely surrou- nding the hair bulbs. Structures associated with the hair are the sebacious glands which secrete oil that lubricate the hair and sweat glands (sudoriferous glands) which are involved in temperature control of the body. In the majority of skins handled by the tanners, papillary and the more numerous club are present. Of these, the papillary hairs are the more deeply seated and firmly anchored in the papillary layer, so that their removal becomes difficult. They may persist throughout the unhairing and subsequent operations. The epidermal layer including the hair is made up largely of protein called keratin. Keratin has a high percentage of sulphur containing amino acids such as cystine and cysteine. Along with the hair, there are fatty cells, and other globular proteins like albumins (water soluble) and globulins (water insoluble).

2:8:1:2 The Dermis:

The dermis, also known as the corium, is the leather making portion of the skin. It consists of densely packed, fibrous and relatively stable layer of the skin. The dermis, like the epidermis, has identifiable regions but there are only two as against about five in the case of the epidermis. The two regions are the sub-epidermal papillary layer and the fibre network layer. In the papillary layer, the fibrous components form a series of papillae which surround and embed the epidermal structures which intrude into this upper layer of the dermis. The region also contains blood vessels, sweat glands, hair follicles and their associated structures, sensory bodies etc. The connective tissue in this layer consists mainly of collagen and some reticulin fibres, but loose networks of elastin - staining fibres (containing the protein. elastin) are also present. The collagen fibres are very closely packed, especially in the upper region, presumably to support and protect the many delicate tissues which are present. This closely packed region is employed by the tanner to produce the well-prized and firm grain of finished

leather. In its lower layers, about the level of the hair bulbs, the papillary layer gradually becomes free from other tissues and merges imperceptibly into the connective tissue of the fibre network layer.

The fibre network layer is the lower and main region of the dermis and forms the major part of leather (almost all of the epidermis and hypodermis of the hide/skin having been removed). It consists almost entirely of connective tissue fibres the main component being tissue fibrils which are organised in long, wavy bundles which in width vary from 1 to 20μ. These interpenetrate to form a complex fibre network on which many of the characteristic qualities of leather depend. They provide shape and mechanical strength. The fibres are arranged in three dimensions with many consisting of carboxylic and amino groups available for many reactions. Some reticulin fibres are also present, whilst elastin-staining fibres are also sparsely but evenly distributed throughout this region. A small member of cellular structures, fibroblasts (which are thought to produce the fibres of connective tissue.) and mast cells, may occasionally be seen, especially in growing skin. In some animals, e.g sheep and goat, the connective tissue of the fibre network layer is infiltrated by fat cells, giving depositions of dermal fat. Such excessive depostion of fat downgrades the skin for leather-making purpose.

2:8:1:3 The Hypodermis:

The hypodermis is the layer of tissue between the densely fibrous dermis and the skin muscle. It contains more of fatty, elastin and muscle tissues. In flaying (the process of removal of hide/skin from a dead animal) the body wall is separated from the subcutaneous tissue and the hypodermis about the level of the skin muscle (if this is present). If the fibre-network layer is damaged accidentally or carelessly during flaying, the skin is downgraded because it would not produce a good quality leather.

The general structure of the hide\skin (epidermis, dermis and hypodermis) discussed above not withstanding , the relative proportion and arragement of the different layers and structures in each type of animal may vary considerably and the tanner must be aware of such variations. Sex and age for the

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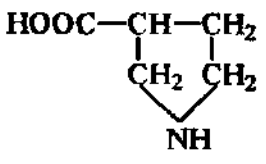
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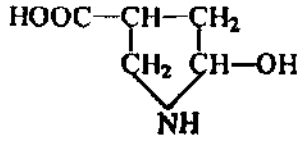
same animal also have influence on skin character and quality. All these factors are important in selecting hides and skins and also parts of individual skins, for different methods of processing into leather of various kinds.

2:8:2 Proteins and Amino Acids.

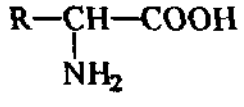
Protein is a macromolecule made up of many amino acids. Amino acids are organic compounds which have a carboxylic group (-COOH) and an amino group (usually a primary amino group $-NH_2$) but in a few cases the amino group may be secondary as in proline (173) or hydroxy proline (174):

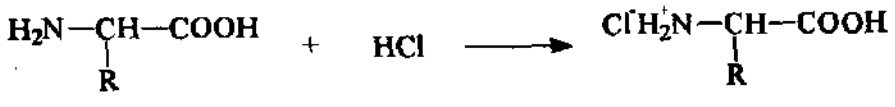


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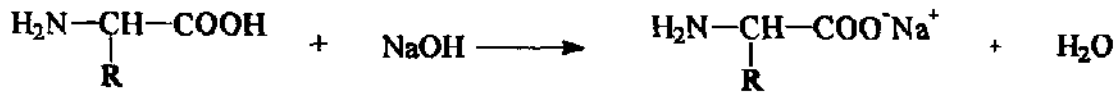


(174)

The general formula may be given as  where R stands for different groups ranging from hydrogen (-H) in the case of glycine, $-CH_3$ in alanine, $-CH_2SH$ in cysteine. Generally, R may be polar or non-polar. The non-polar groups are essentially hydrocarbons in nature and have little or no affinity for water (i.e are hydrophobic) e.g. as in alanine, and phenyl alanine. On the other hand, the polar groups vary but all have some degree of attraction for water (i.e are hydrophilic) e.g. cysteine, cystine, methionine, etc. Amino acids are, generally, amphoteric, compounds. This means that in an acidic solution the amino group forms a salt (175) while in an alkaline solution the carboxylic group forms a salt (176).

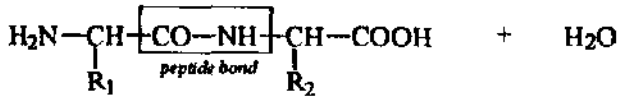


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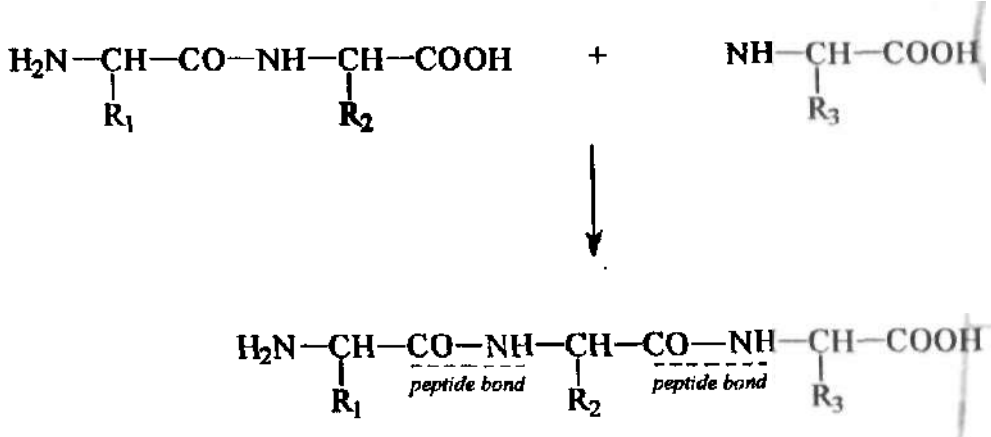
(176)

This property of amino acids has a lot of implications in the behaviour of proteins. The amino acids in proteins are joined by peptide bonds. A peptide bond (177) is formed when two amino acid molecules combine, the acid group in one molecule reacting with the amino group (basic group) in the other with the elimination of one molecule of water:



(177)

The product above is called a dipeptide (177) because two amino acids have been involved in the reaction. Note that the dipeptide (177) still has one free amino group ($-NH_2$) and one free carboxyl group (-COOH). The dipeptide can, therefore, react with another amino acid to form a tripeptide (178):



Tripeptide

(178)

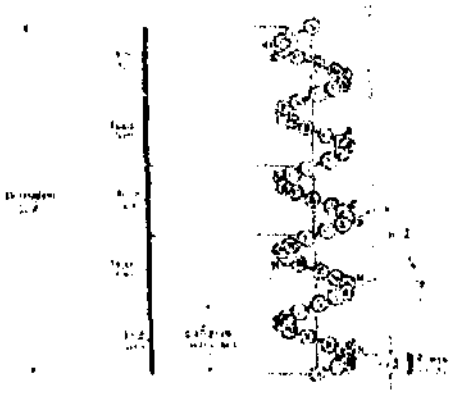
The type of protein or peptide (177) obtained depends on the types of amino acids involved in its formation and also the number of polypeptide bonds. In general, about twenty one amino acids have

been found to be involved in the formation of most natural proteins. Even then, not all the twenty one amino acids may be necessarily involved because some amino acids are used repeatedly in the formation of the peptide bonds (177) of a particular protein or peptide.

As would be expected, the types of groups found in protein units exert some structural consequences, indeed, four basic structural levels of proteins are known - the primary, secondary, tertiary and quartenary structures.

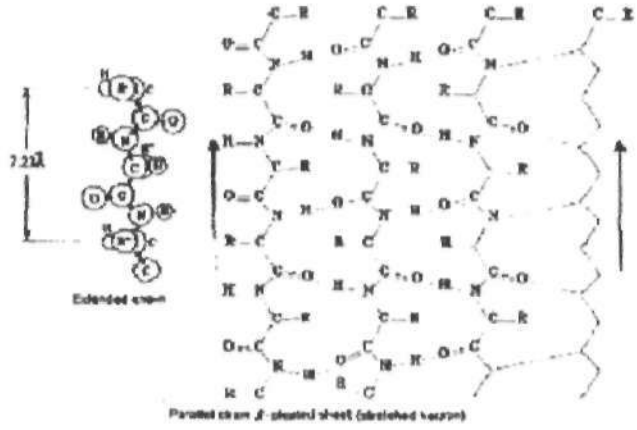
i. Primary structure

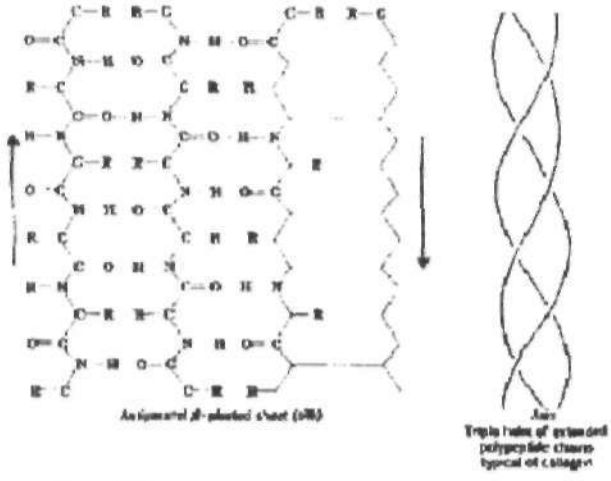
This is the linear sequence of amino acid residues making up the polypeptide chain. The peptide linkage between each amino acid is the main force binding the polypeptide unit.



ii. Secondary structure

As a result of the amino acid residues lying fairly close to one another in the primary structures, interactions through hydrogen bonds are possible. The carboxyl and the amino groups of the peptide bonds interact this way. This is as seen in the α - helical/spiral structures. This interaction gives the stability required.





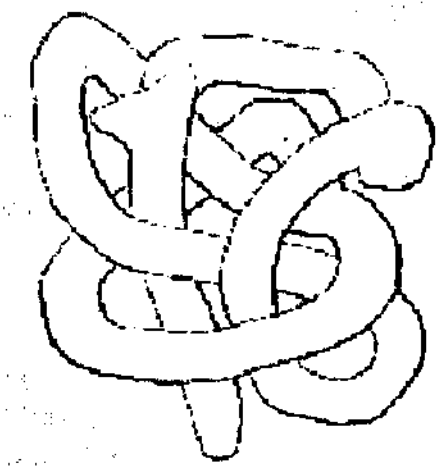
iii. Tertiary structure

As a result of the interactions between amino acid residues relatively far away in the sequence, the polypeptide chain undergoes extensive folding and coiling to produce a complex, somewhat rigid structure stabilised by the reactivities associated with the R groups in the amino acid residues.



iv. Quartenary structure

This is the structure resulting from inter-actions between separate polypeptide units of a protein containing more than one sub-unit (Conn and Stampf 1976).



2:7:2:1 Keratin

Keratin is a protein that is found in fur, hair, claws, hooves, feathers and of course the epidermal layer of skin. It consists mostly of an α - helix, three of which form a left-handed coil or a protofibril which is stabilised by cross-linking disulphur containing amino acid. This is why the epidermis of a skin has a high percentage of sulphide for example fibre consists of a very large number of polypeptide chains, held together by hydrogen bonding and cross-linking, disulphide bridges embedded in an insoluble protein matrix. When α - keratin is exposed to moist heat and stretched, it is converted to a different conformational form, namely, β- keratin. The hydrogen bonds stabilising the α -helical structures are broken under these conditions and an extended parallel β - pleated sheet conformation results.

2:8:2:2 Collagen

It has already been stated that collagen is the main protein of the dermis. Collagen possesses the basic tripple helix based on multiple repeats of the simple tri-peptide, Glycine (Gly) - X - Y which

varies in length for different supra - molecular aggregates to achieve optimum function for particular tissues. X is often proline and Y sometimes hydroxyproline. The major function of collagen is to provide shape and mechanical strength and the latter is achieved by intermolecular cross-linking of the collagen molecules in the supramolecular aggregate. The cross-linking occurs through an enzymic mechanism involving the oxidation of specific lysine and hydroxylysine residues providing divalent cross-linking which subsequently matures to multivalent cross-links. Later on stability is obtained through the adventitious accretion of glucose which is non-enzymic. (Bailey et al 1997).

All amino acid side chains project radially from the rod-like tripple helix and the quarter staggered array of the molecules allows highly specific intermolecular cross-linking with bifunctional reagents. Reactions with basic or acid groups can therefore be carefully controlled. Synthetic cross-links bind the molecules closer together and increase intermolecular interactions, thus increasing the shrinkage temperature and resistance to enzymic degradation (Bailey, et al 1997).

Collagen is classified into 4 main groups:-

i. Fibrous Collagen

ii. Non-fibrous Collagen

iii. Filamentous Collagen

iv Fibril associated Collagen

2:9 Tanning - Definition and tanning agents

As has already been stated the collagen of the dermis is the fibrous collagen. Oxford dictionary define the word "tanning" as the conversion of skin into leather by impregnation with an infusion of tannin. This definition recognises the need of an additional agent, the tannin, to convert the readily putrescible animal skin into the useful fabric, leather. As earlier mentioned the term "tannin" was used

to describe the extractable matter in certain plant tissues with capacity to convert animal skin into leather (Sequin 1796).

The term "tanning" refers to a process of converting putrescible or unstable raw hide or skin into leather with adequate strength properties and resistance to various biological, chemical and physical agents. During the process the tanning agent is introduced into the hide or skin which reacts with and forms additional cross links into collagen binding the active groups of the tanning agents to functional groups of the protein (Bienkiewiez 1983). The protein functional groups are thus inactivated through an irreversible reaction with the tanning agent thereby reducing the water-binding capacity and the swelling tendency of the collagen. The reaction of the tanning agent with collagen, stabilises the collagen thus improving its resistance to heat, proteinases, and swelling agents and preventing the glueing together of fibres upon drying without detrimentally affecting the mechanical strength of the original hide or skin structure.

From the foregoing, tannage is said to have been achieved, on satisfying the followng tests:

i. Shrinkage temperature of leather in water which is 100°C for 3 minutes.

ii. The resistance of the tanned collagen in the moist state to attacks by enzymes (proteinases);

iii. The chemical stability of the material upon storing (leather) should not deteriorate under normal conditions of use and on storage.

iv. The retention of the physical properties of the fibrous structure of the native hide or skin, particularly referred to as the degree of splitting up of the fibres (Gustavson, 1956).

Tanning can thus be said to be the treatment of collagen in conditions optimal for the reaction with substances, every molecule of which may react with several identical or different polar groups of peptide chains, forming supplementary water - resistant bridges between them and changing at least one of the essential properties of the protein (Bienkiewecz, 1983). From what has been said tanning

agents may be of diverse chemical nature and do not form a distinct class of compounds. Some examples are, basic chromium salts, vegetable tannins aldehydes, syntans. (Certain condensed phenols containing sulphonic acid groups) and unsaturated oils. Of these, the most commonly used are basic chromium salts and vegetable tannins. For several economic reasons syntans have become very popular too, the recent formulation incorporating certain metal ions. Tanning agents can be divided into two groups.

i. mineral tanning agents.

ii. tanning agents of organic nature.

In the second group the most important are the natural vegetable tannins which are polyphenolic compounds and among the oldest tanning agents. They are chemically heterogeneous and are the most important in the popular vegetable tannage. Chrome tannage from the first class is also very important.

The conversion of the putrescible collagen of the raw hide/skin into non-putrescible collagen called leather proceeds via several physio-chemical treatments. These treatments are aimed at preserving some of the inherent functional properties and characteristics of the raw hide and skin i.e flexibility and water repellency. The processed hide/skin should not putrefy on drying and wetting back, neither should it be hard and brittle after drying, but should be flexible and workable (Haslam 1992; Reeds, 1966).

2:9:1 The Pretanning Processes:

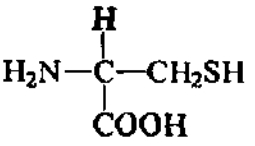
The pretanning processes are aimed at preparing the hide/skin for the tanning process proper. These processes include the removal of hair and other unwanted substances from the epidermis, flesh and other unwanted substances in the hypodermis. Both physical and chemical means (and in some cases biochemical when enzymes may be used to remove hair) are used in the pretanning processes.

The pretanning processes expose the dermal layer and modify the side groups making it receptive to tannins and other chemicals during tanning and post-tanning processes. The pretanning processes may be likened to the physical and chemical methods that are used in preparing and purifying reagents in the laboratory for the production of other substances. Some of the physical processes that may be utilised in purifying reactants include crystallisation, distillation etc. while chemical processes may include oxidation of alcohols or esterification of alcohols as protection against unwanted reactions etc.

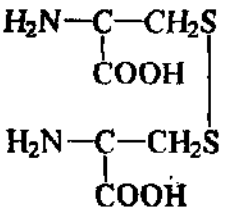
2:9:2 Epidermal Layer (Removal) Treatment.

Usually, the hide/skin received in the tannery is in the dried state. It is also dirty (dust, dung etc) and may be salty as a result of curing. The dried, dirty hide/skin is soaked in clean water containing a wetting agent and a bactericide to facilitate quick soaking back and to check bacterial action respectively. The aim of soaking is to re-hydrate the dried hide/skin so that it would be in a state close to when it was removed from the animal. Soaking also removes the dirt and any salt that might be present.

When soaking back has been effectively completed and the hide/skin adequately cleaned, it is placed in a lime-sulphide solution. The lime-sulphide solution is expected to remove hair and other epidermal structures. The major chemical action here is the disruption of the disulphide bonds of keratin and elastin which contain the amino acids cystine and cysteine thereby modifying the structure and rendering the epidermal layer (including the hair) easily removable. The amino acids greatly affected are the cysteine (179) and cystine (180) In the few cases where the removal of hair is not needed (i.e when hair-on leather is to be produced) this process is skipped.



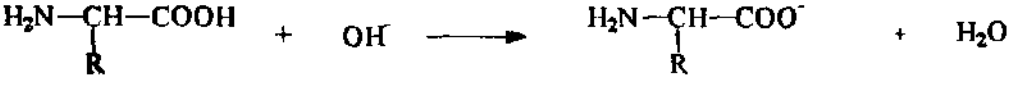
(179)



(180)

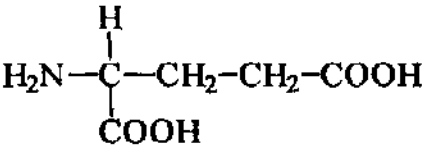
The unhaired hide/skin is treated (re-limed) with a 10% solution of calcium hydroxide overnight (12 hours).

Re-liming is aimed at completing the removal of the epidermal structures, saponifying the natural fats, and chemically and physically modifying the dermal layers of the raw hide/skin as may be shown in the alkali reaction.

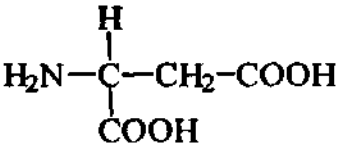


(181)

Glutamic (182) and aspartic acids (183) are the amino acid groups that are most affected in this treatment.



(182)



(183)

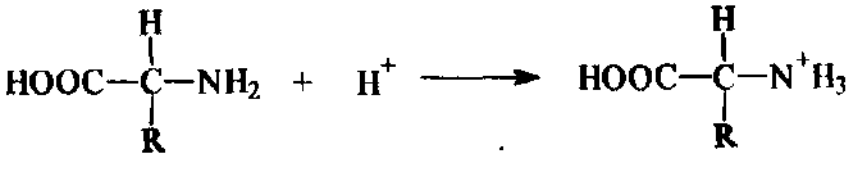
2:9:3 Hypodermal Layer Treatment

The hypodermal layer contains mainly fats, elastin and muscle. The liming process is not adequate to remove it so as to expose the fibrous dermal layer adjacent to the hypodermis but the

action swells (or plumps) the hide/skin making the removal of the unwanted flesh (this is called fleshing) easy. The swelling is a physical modification and it opens up the fibre structures which makes for easier penetration of the tanning material.

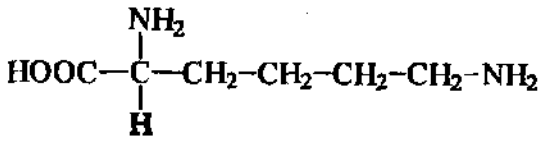
2:9.4 Vegetable Tanning.

The "purified" (hide/skin) fibrous collagen layer is swollen and is ready to take up tannin without too much resistance from peripheral structures like the epidermis and the hypodermis. In this state the raw hide/skin would react aggresively with any cationic material. When placed in a fresh solution of vegetable tannins, the rate of fixation would be high and the grain of the hide/skin becomes drawn and unsightly, resulting in a phenomenon called "case hardening" in extreme cases. Case hardening can be avoided in two ways, either by the adjustment of the pH or by decreasing the concentration of tannins in the solution. The adjustment of the pH is done by the addition of sulphuric acid, a process known as "drenching" (pH 4-6) if the pH of the acid is high and "pickling" if the pH of the acd is below (pH 2-3). The main reaction is as shown below.

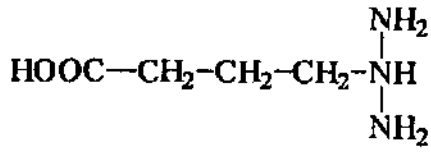


(184)

This changes the surface charge allowing for good and uniform penetration (Dashe and Shok 1994). The amino acids that are usually modified in the above reaction are mainly the basic amino acids, lysine(166) and arginine (167).



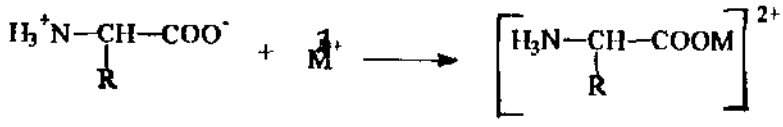
(185)



(186)

The reactive sites for both mineral and vegetable tanning agents are activated and the tannin and collagen form irreversible bonds giving "collagen tannate" or leather, as may be seen below:

Basic (cationic) tanning material.



(187)

Acidic (Anionic) tanning material



(188)

Anionic tanning reaction can effectively commence after alkali treatment. This process is popularly referred to as "Liming". It is important to note that the raw hide/skin which was neutral prior to tannage is now predominantly polar as a result of the tanning agents used. The swollen condition of the hide/skin encourages the penetration of tannins and their deposition in the voids, increasing the uptake of the tannins resulting in improved degree of tannage. This is why vegetable tannage is done with the hide/skin in swollen condition.

Vegetable tannins are extracted from plant leaves, barks, roots, pods etc and consist of large polyphenol molecules with some acidic groups and high secondary valency potentials i.e the dipole H-bonds (Bienkiewiecz, 1983; Heidmann 1993). The acidic groups may combine with the basic groups

of the protein, displacing the hydrated water and the numerous secondary valencies, dipole or hydrogen bond to the peptide groups displacing their hydrated water. Consequently vegetable tannage could be considered as dehydration of the wet protein, replacing the water molecules by a sheath of vegetable tannin molecules. On drying these will create some mechanical impedence to the fibres shrinking together and the presence of the vegetable tannin will obstruct the formation of the cross-links. The type and quantity of vegetable tannin introduced into the skin will dictate the degree and level of softness obtained. The higher quantities of vegetable tannins given to sole leather produces a firmer leather as a result of filling action of the vegetable tannins and the possibility of aggregate formation between the fixed tannins and the fibres. Whereas this is a common phenomenon with the two classes of vegetable tannins, the hydrolysable and the catechols, produce harder and firmer leathers than hydrolysable or pyrogallol (Sharphouse, 1983; Heldmann, 1993).

Generally, acid conditions favour vegetable tannin fixation by increasing the ionisation of the protein basic groups, as in reaction (184). This is the situation at pH below 5.0. for well- limed hide, which are attracted to hence its ionic attraction for ionised acid groups in the vegetable tannin molecules. This attraction between cationic basic groups and anionic tannin molecules should favour penetration. This is the treatment of limed hide/skin with acid as in drenching or pickling.

The vegetable tannin acids are weak acids, except the sulphited ones, therefore the greater the acidity or the lower the pH, the less they will be ionised and the less the ionic charge they would carry. The significant effect of this is that as the ionised groups in the tannin molecule decrease, the dipole and H-bond forces increase. This is why penetration is not favoured but fixation of vegetable tannins on the protein fibre is greatly favoured this time not by ionic but by H-bonding which predominates at any given pH depending on the constitution of the particular vegetable tannin used. The increase in dipole bonding force does not only increase the bonding of tannin to protein fibre but of one tannin molecule

to another with consequent increase in viscosity resulting in the accumulation of tannin on the surface of the skin giving a phenomenon known as "case hardening" which has already been explained. Increase in temperature, however, reduces these secondary forces with consquent reduction in viscosity and fixation to the skin and inproved penetration of tannin into the skin (Sharphouse, 1983).

Vegetable tannins are used for the production of two types of leathers:

1. Heavy leathers - these include, sole, belting, industrial, saddle and harness leathers. These are usually from cattle hides, and are sold by weight.
2. Light leathers - these include shoe uppers, (crust form), lining, book binding, and hat bands leathers. They are usually from calf, goat, and pig which are usually sold by area i.e. price per square foot.

Heavy leathers are produced or manufactured using similar procedure except for the amount of tannins offered, this being dictated by the type of leather to be produced and the level of rigidity and solidity expected of it. Usually after fleshing the limed pelt is given a light acid wash with boric acid, a process earlier on referred to as "Drenching", before tannage with vegetable tannin by the counter current system of battery or vat or pits. The lowest battery or vat or pit contains the lowest tannin concentration while the highest vat contains the highest tannin concentration from where the tanned leather leaves. The choice of the type of vegetable tannin material is very critical as heavy leather is sold by weight. The choice vegetable tannin extract must have ability to give weight, increased solidity and rigidity. Mimosa and Quebracho which are catechol (condensed) tannins are usually preferred. The catechols usually exhibit a higher tendency for secondary dipole forces hence higher rate of aggregation and this is encouraged to take place in the pelt filling the void and cross-linking

2:9:5 Post Tanning Analysis of Leather

The Post Tanning analysis of Leather (tanned hide/skin) are meant to determine, primarily the extent to which tanning has taken place and whether the resultant leather has desirable properties or not. Various chemical and physical standards have been established for such purpose. The chemical standards include: the determination of nitrogen and hide substance, the degree of tannage; volatile matter (moisture), substance extractable by dichloromethane; total (sulphated) ash; water soluble matter (inorganic and organic) pH value and difference figure of an aqueous extract. The physical standards include: tensile strength, % elongation, stitch tear (resistance) strength, ball burst or grain strength and flexing endurance.

2:9:6 Determination of Nitrogen and Hide substance (SLTC, 1996)

Schroder and Passler (1905) have shown that hides/skins of animals have constant nitrogen content (for certain types of animals) which varies from animal to animal. This can however be falsified by certain nitrogenous substances like fixative synthetic tannins, cationic dyes and fatliquors which have to be removed before the determination of the nitrogen content. This has been used as a means of determining the hide substance of the hide/skin.

Thus the Hide Substance is defined as the content of nitrogenous substances calculated from the nitrogen content of leather by multiplication by the factor 5.62 (SLTC, 1996).

The principle is based on the decomposition of the leather by the Kjeldahl method, the ammonia is distilled and determined titrimetrically.

3g of the ground leather sample taken in accordance with the official method of analysis is transferred quantitatively to the Kjeldahl flask, 30 ml of oleum or sulfuric acid is added along with approximately 5g of appropriate catalyst mixture which is heated to boiling and allowed to digest first with a low and later with a high flame for 1 hour till all the carbon has been oxidised.

The digest is cooled and diluted with approximately 50 ml of water, cooled, and transfered to the distillation flask. The Kjeldahl flask is rinsed twice with 20 ml of water each time and a few drops of phenolphthalein indicator are added and the mixture made alkaline with an excess of 35% (m/m) sodium hydroxide solution (a total, at least, of 100ml). The distillation apparatus is assembled and steamed. When direct heating is to be used and digestion has been done in a 750ml Kjeldahl flask, cool, diluted with 250ml of water, and a few granules of anti-bumping material and a few drops of phenolphthalein indicator added. The mixture make alkaline with an excess of 35% (m/m) sodium hydroxide solution (about 100ml) and the distillation apparatus assembled distillation.

Ammonia and water vapour are distilled into a receiver containing 100ml boric acid solution with the cooling tube dipping into boric acid solution. The distillation is continued until the volume of the distillate collected is 150 - 200ml. Shortly before ending the distillation, the receiver is lowered so that the cooling tube no longer dips into the boric acid solution. The distillation is continued for approximately 3 minutes more, and the end of the condenser is rinsed with water into the receiver. The combined distillate and rinsings is titrated with 0.5 N sulfuric acid, or hydrochloric acid to pH 4.6.

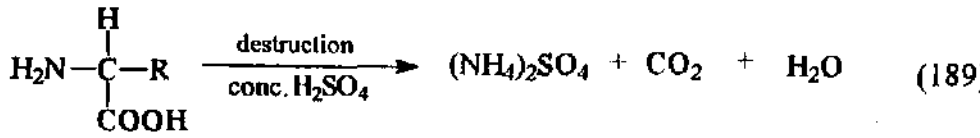
A blank test in the reagents alone including the catalyst using sucrose in place of ground leather following the above procedure is also carried out.

Hide substance, percentage by mass = $\frac{V_2 \times M_2 \times 14 \times 5.62 \times 100}{\omega}$

where v_2 = Volume of acid
M_2 = molarity of acid
ω = weight in mg of sample of leather
N = 14

The reactions are as follows:

Destruction



(189)

Distillation

$$(NH_4)_2 SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_3 + 2H_2O \quad (190)$$

$$NH_3 + H_3BO_3 \rightarrow (NH_4) H_2BO_3 \quad (191)$$

Titration

$$2(NH_4) H_4BO_3 + H_2 SO_4 \rightarrow 2H_3BO_3 + (NH_4)_2SO_4 \quad (192)$$

2:9:7: Degree of Tannage

The percentage of fixed tannin in the leather cannot be determined directly. For vegetable tanned leather, fixed tannin is taken as the difference between 100% and the sum of the percentages of volatile matter, oil and fats, matter soluble in water, hide substance and the ash of the matter insoluble in water. The degree of tannage is thus defined as the number of parts of fixed tannin per 100 parts of hide substance or

$$\text{Degree of tannage} = \frac{\text{percent fixed tannin} \times 100}{\text{percent hide substance}}$$

It is thus correct to define fixed tannin as combined organic matter. (SLTC 1996). In the case of combination tannage this calculation will give a high value of fixed tannin. Various correction factors have been proposed from time to time (Kanagy 1961).

2:9:8 Volatile matter (moisture) SLTC 1996

The exact determination of the moisture content in leather is not possible, as at elevated temperature other volatile substances escape and tannins and fats can be oxidised. Some absorbed water may be left in the leather after drying.

Volatile matter can thus be defined as loss of mass by leather when dried to constant mass at 102 ± 2°C as described below.

3g of the sample prepared and ground according to Official Method of Analysis is weighed to the nearest 0.001g into a tared weighing vessel and dried at 102± 2°C for 5 hours. The vessel and contents are cooled for 30 minutes in a desiccator and weighed. The drying, cooling and weighing is repeated but with drying times of 1 hour until further loss in mass does not exceed 3 mg (i.e 0.1% of the sample mass) or till the total drying time is equal 8 hours. The final mass of sample and weighing vessel is recorded from which the dried mass of sample is calculated as follows.

$$\text{Volatile matter, percentage by mass} = \frac{100\ (M_1 - M_2)}{M_1}$$

Where M_1 = mass of sample before drying

M_2 = mass of sample after drying

Duplicate determination should not differ by more than 0.2% calculated on the original mass of leather.

2:9:9 Substance Extractable by Dichloromethane

Not all fatty and similar substances can be extracted from leather with organic solvents; they may be in part soluble and partly bonded to the leather. However, the solvent can dissolve non-fatty substances e.g; sulphur and impregnants both of which cause difficulty in the determination of the fat. Consequently, extractable substances can thus be defined as fats and other soluble matter which can be extracted from leather with dichloromethane.

Weigh 10 ± 0.1 g of the prepared sample were weighed and press evenly into the filter paper thimble or into the glass bell. Cover the leather with a thin layer of cotton wool. Dry the extraction flask with two glass beads in it by heating for half an hour at 102±2°C. Weigh after cooling in a desiccator. Extract continuously with dichloromethane till after at least 30 change of solvent before distilling the dichloromethane from the flask containing the extract. The extract is then dried in the oven for four hours at 102±2°C. Remove any visible drop of water by adding 1-2ml of ethanol before drying. Weigh after cooling for 30 mins in a desiccator. Repeat the drying, cooling and weighing at least twice more, but with drying periods of 1 hour, until either the further loss in mass does not exceed 10 mg or the total drying time equals 8 hours.

The Extractable substances is calculated as follows:

Extractable substances, percentage by mass = $\frac{100\ M_1}{M_0}$

Where M_1 = Mass of extract

M_0 = Mass of sample used

The extract can be used for the determination of the acid and saponificaton values of the fats or the free acid content of the leather; while the extracted leather may be required for the determination of water - soluble matter.

2:9:10 Total (Sulphated) Ash

The amount of mineral substances found by ashing can differ from the actual content owing to decomposition, reduction, or the escape by volatilisation of certain salts. Treating the ash with sulphuric acid the salts and oxides are converted into sulphates before determination. It must be noted even then that some salts can still be transformed into oxides at the selected temperature of ignition. To determine the total mineral content in leather analyses, therefore, it is necessary that the water - soluble and water-insoluble substances be calculated separately. Sulphated total ash is thus defined as residue obtained from burning leather in an open crucible after sulphating.

2.5g of the sample prepared in accordance with the official method of analysis is carbonised over a low flame in a crucible which has been previously heated to 750° C, cooled and weighed, so that the leather burns with a small flame. Fatliquor leather must be carbonised carefully to allow the grease to burn slowly. The ash is moistened properly with sulphuric acid and heated over a low flame until sulfur trioxide fumes are no longer visible. Heat more vigorously then ignite in the furnace and weigh. Repeat the addition of the acid, heating, cooling and weighing until the mass of the residue is constant.

Calculate sulphated total ash, percentage by mass $= \frac{100M}{M_0}$

where M_1 = mass of sulphated total ash

M_0 = mass of the original leather sample.

Duplicate results should not differ by more than 0.1%

2:9:11 Water Soluble Matter - (Inorganic and Organic).

The result obtained for the content of water-soluble matter in leather depends on the extent to which the leather is ground, the temperature, the extraction period and the ratio of leather to water. Any ammonium salts in the filtrate are condensed as part of the water - soluble matter and are then decomposed on ignition contributing to water-soluble organic substances result.

Water-soluble matter can thus be defined as substances which under certain conditions are dissolved out of the leather by water. These substances are principally organic tannins non-tannins and mineral salts. Water-soluble inorganic matter on the other hand is the sulphated ash of water soluble matter, while water soluble organic matter is the difference between total water-soluble matter and water-soluble inorganic matter.

Adequate extraction under specified conditions of a prepared sample is followed by determination of the water-soluble matter in the extract by evaporating and drying at 102±2°C.

Sulphating and ashing of the residue at 750°C yield the water-soluble inorganic matter and the water-soluble organic matter is derived by difference. The results are calculated as follows.

Total water-soluble, percentage by mass $= \frac{1000M_1}{M_0}$

where M_1 = the mass of dry residue

M_0 = the mass of original sample of leather.

Water - soluble inorganic matter, percentage by mass $= \frac{1000M_2}{M_0}$

where M_2 = the mass of sulphated residue M_0

M_0 = the mass of original sample of leather

Duplicate results should not differ by more than 0.2%

2:9:12 pH Value and Difference Figure of an Aqueous Extract

Leather always contains a certain amount of acid and sometimes alkali which determines its pH value. With time free strong acid or alkali could damage either the leather or possibly other materials associated with it.

The difference figure is defined as the difference between the pH value of the aqueous extract and the pH value of a tenfold dilution of the extract.

The pH value of the aqueous leather extract and difference figure both of which measure the acidity or alkalinity of the leather, depend on the quantity and strength of the acids or alkalis present and on the presence of buffer salts in the leather. Where the pH value of the extract is below 4.00 or above 10.00 the difference figure is.

i. Large in the presence of acid or base, buffering salts being absent

ii. Small in the presence of strong acid or base, buffering salts being present in considerable quantities.

iii. Small in the presence of weak acid or base, independent of any buffering salts present..

From the foregoing it is clear that both the pH value and the difference figure determine whether the leather contains dangerous amounts of free strong acid or base. Consequently, it is important that their determination separately must be accurately done.

The aqueous extraction of the prepared sample is done under specified conditions and the pH determined. The pH of the tenfold dilution is also measured representing the difference figure. This becomes necessary if the pH is lower than 4.00 or above 10.00. (SLTC, 1996).

2:9:13 Tensile Strength and Percentage Elongation.

The combination of tannin with protein apart from preserving the protein impart to it some level of strength. One method of determining this strength is tensile strength measurement.

The specimen is cut from the specified sampling position and conditioned according to the official method of analysis. The cross sectional area is determined accordingly by multiplying its width by its thickness. The jaws of the tensile strength machines are set 50mm apart. The leather specimen is clamped such that the grain surface lies in one plane. The machine is then run until the specimen breaks and the load at this point which is the breaking load is recorded. The tensile strength is calculated by dividing the breaking load by the area of cross-section of the specimen.

Tensile strength has direct relevance with the level of cross-linking by the tanning material % Elongation at break (Official Method 1996).

The percentage elongation is very directly related to the tensile strength hence the procedure is the same as highlighted above.

Measurement of the distance between the pairs of the jaws at the point when rupture of the specimen occurs. This will be the length of specimen at break .

Elongation at break is thus calculated by taking the difference between the initial length and the length at break expressed in percentage.

The result of the percentage elongation is more informative on strength of the leather and therefore the level of cross-linking than the tensile strength measurement. It is very important therefore to strictly keep the procedure and sample methods.

2:9:14 Stitch Tear Resistance (Tearing Load)

Most leathers are usually sown or stitched in converting them to products like shoes, bags, belts etc. For the products to be considered as of good quality they should not tear at the stitching point hence this measurement. This also is an indication of the strength of the leather.

The specimen is sampled and conditioned according to the Official Method of Analysis. The specimen is fixed on the tensile machine already fitted with the specimen holders and the machine run until the specimen is torn apart and the highest load reached at the tearing point is recorded as the tearing load in Kgf while the thickness of the specimen in millimeters (JSLTC 1987).

2:9:15 Distension and Strength of Grain.

This is referred to as the Ball Burst Test. The grain of the skin is the first part of the skin along with flesh side that comes in contact with various chemicals from the pretanning to tanning and post-tanning processes. Reactions usually start here before penetration into the whole skin. Some of these reactions could be vigorous while some materials like tannins and particularly vegetable tannins could remain on the surface - actually on both surfaces. Their effects vary considerably from the flesh to the grain which is mostly hard hit. This in most cases affects the grain adversely thereby weakening it. For shoe upper and other similar leathers, the Ball Burst Test measures this effect.

The specimen is prepared and conditioned according to Official Method of Analysis. It is then clamped in the lastometer with its flesh surface adjacent to the ball and to grain surface flat. The distension is increased at a rate of approximately one-fifth of a millimeter per second while the grain surface is being watched for the occurrence of any crack. Note the load and distension at crack and continue loading with as little delay as possible till bursts and record the load and distension at burst. Delay must be avoided because delay brings about the relaxation of stresses in the specimen resulting in the falling of the load reading.

2:9:16 Flexing Endurance of Light Leathers.

This test will also always include surface finishes of the leathers. A shoe on the leg experience forces at various angles according to the weight of individual and his structure or form. Consequently, as we walk there is folding of the leather (the shoe upper leather). With time this folding will affect the surface finishing and the leather at the folding point. The Flexing Endurance attempts to measure the ability of the leather to withstand this effect.

A machine simulating the walking of human being is produced and called Flexometer. The specimen is prepared and conditioned according to the Official Method of Analysis . The sample is folded and clamped at each end to maintain it in a folded form in the machine. One clamp is fixed and the other moves backwards and forwards causing the fold in the specimen to run along it in the same way as it occurs in shoes while we walk. The motor is switched on and after 100, 1000 and 10,000 cycles the motor is switched off and the leather finish is examined to see whether there is any damage. The nature of the damage and the number of cycles at which the damage occurs are recorded. After 2, 4, 6, 8, 12, 16, 24 and 32 hours of flexing, the leather itself is examined and the type of damage, its nature and the number of cycles at which it occurs are recorded.

Types of damage expected on the finish includes, change of shade (greying), crazing of the finish (cracks), loss of adhesion of the finish to the leather, loss of adhesion of one finish layer to another and powdering or flaking off of finish. On the other hand damage to the leather may include, development of coarse grain folds (pipey grain), loss of an embossed grain pattern, cracking of the grain layer, powdering of the fibres (usually on the flesh side) leading to an empty feel, and development of a hole through the entire thickness of the leather.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

The exudate of Eucalyptus citriodora was collected first in October through December 1993 at the plantation behind Layinzomo Kwangila on the way to Samaru in Zaria. The identity of the plant Eucalyptus citriodora was confirmed at the Savannah Forestry Research Station in Samaru-Zaria. The exudate was identified at the Department of Pharmacognosy at the Ahmadu Bello University Zaria. The exudate after drying was crushed into powder using normal laboratory porcelain mortar. The exudate was collected from four identified trees throughout the investigation. Some exudates were collected during the very hot and cold seasons and at the peak of the raining reason, usually between July and August. A full year monthly collection was also made and all these were carefully analysed.

The pH meter used was Digital supplied by the NAAFCO Scientific Ltd Lagos, Nigeria, while the tinctometer was the Lovibond Tinctometer supplied by the Tinctometer Ltd Salisbury England. Standard hide powder grade and were by Hopkin and Williams Ltd., Essex, England and were of SLTC standard grade. Purified, filtered sand (40 - 100 mesh) was supplied by BDH, Poole, England. The solvents used, acetone, methanol, ethanol, ether, ethyl acetate, and methyl acetate were all of Analar Grade and were supplied by BDH, Poole England. The filter papers used are ashless Whatman Numbers 1, 2, 3, 4, supplied by Hopkins and Williams Ltd Essex England. Glacial acetic acid, hydrochloric acid, n-butanol, n-butan-2-ol, and ferric chloride used are of Analar Grade and supplied by BDH Poole England. Pure samples of tannic and gallic acids were supplied by BDH of Poole England. The raw skins, goatskins medium size second

Grade, were supplied by Alhaji Abdu Namalawa of Tudun Wada, Zaria. The tannin agent chromosol and auxillary tanning chemicals like ammonium chloride/sulphate, sulphuric acid (industrial grade), Bate, dyes, common salt were supplied by BASF - Bayer Germany and Yorkshire Chemicals Yorkshire England.

The HPLC system is Backman product USA with wavelength of 254nm and scanning range of 200 -300nm.

The IR was the Mattson Analytical Technology Incorporated Fourier Transformed Infrared Spectrometer using Nujol.

The flow rate was 1cm^3/minute while the column was ODS dp 5 u, 4.6mm ID X 25cm. The instrument used for this test is called the Lastometer Flexometer, Instron and Tensometer were supplied by SATRA, Northants England.

3.2 METHODS

PART I

3.1` Qualitative Analysis of The Tannins of Kinos

3.1.1 Solubilities in Various Solvents.

The solubility of kinos was detemined in various solvents as described below.

Solubility Test: About one gramme of sample was introduced in 10cm^3 of solvents Ethanol methanol benzene acetone Petroleum ether (40-60), butanol, water ethylacetate etc.) and shaken at room temperature. The result is recorded in Table 1.

3.1.2 Ferric Chloride Test

5g of the kinos was dissolved in 250ml of water and allowed to warm up till all the kinos have dissolved. 5ml was taken and 5 percent ferric chloride solution was added to it drop by drop and the colour change was noted. The result is recorded in Chapter 4 Part I.

3.1.3. Gelatin Test

A solution of gelatin containing 10 percent sodium chloride was prepared and a few drops of the kinos prepared in 3.1.1 above was added. The result is recorded in chapter 4 Part I.

3.1.4. Ferric Ammonium Citrate Solution Test

1g sodium acetate, was added to 5ml of 0.25 percent solution of ferric ammonium citrate adjust the pH to 8 before addition of the kinos solution. This mixture was boiled, cooled and then filtered. The result in recorded in Part 1 of chapter 4.

3.1.5. Bromine Water Test

A solution of bromine water was produced in the fume cupboard. This was added to 5ml of the kinos solution and the colour change observed.

3.1.6 Formalin Test

5 drops of formalin plus 6 drop of 10 percent hydrochloric acid was added to 5ml of the kinos solution and boiled for 1minute. The result is recorded in section A of chapter 4.

3.1.7 Sodium Nitrite Test (Ellagitannins)

A few crystals of sodium nitrite was added to 5ml of the kinos solution and the colour change was observed.

3.1.8 Lime Water Test (Gallotannins)

To 5ml of lime water was added a few drops of the kinos solution and the colour change was observed.

3.1.9 Test for Anthocyanidin

100mg of kinos was dissolved in 20ml of n-butanol - concentrated HCl at the ratio 95:5, V/V. To this 2 percent solution of ammonium ferric sulfate was added and the mixture heated at 90°C for 40 min. The colour change was observed. The result is recorded in section A of chapter 4.

3.10 Infrared Spectroscopy

The infrared spectra of the kino was done using Analytical Technology Incorporated Fourier Transformed Infrared Spectrometer. This procedure was done for all the kino collected on a monthly basis for one year. The results are recorded in Annex II.

3.2 Paper Chromatographic Analysis

i. Brownish coloured Eucalyptus citriodora exudate was grounded and a concentrated solution of it made in 70% acetone. A spot of the solution was applied to Whatman No. 1

paper (30x14 cm). The mobile phase comprised of glacial acetic/n-butanol/water (1:4:5) mixture. It was left to run for at least 7hrs.

After separation, the paper was dried in air and sprayed with 5% ethanolic $FeCl_3$. The results are recorded in Fig. 1

Eucalyptus exudate was shaken in ether and equilibrated with 2% NaOH at room temperature. The aqueous phase was separated and acidified with dilute HCl. This action regenerated the tannin as phenol. The solution was concentrated and a drop of it applied to Whatman No.1 paper. The mobile phase was acetic acid/HCl/Water (3:3:10) mixture. This was left to run for about 7hrs. It was then dried and sprayed with $FeCl_3$- $K_3Fe(CN)_6$ solution. The results are recorded in Fig. 2

After the separation in No.2 above, a 25cm^2 Whatman No.2 paper was used for the chromatographic separation (2-dimensional). $FeCl_3$ + NH_3- $AgNO_3$ was the spraying agent. Bluish-black spots were observed.

Paper Chromatography of exudate was done using the solvent systems: acetic acid, hydrochloric acid and water (3:3:10 V/V) (Haslam 1990), Water, n-butanol, and glacial acetic acid (5:4:1. V/V) (Haslam 1990). Chromatograms were run at room temperature (25 - 27°C) using Whatman Nos 1-3 papers at various stages and development was by alcoholic ferric chloride. The results are recorded in Fig. 3.

Eucalyptus exudate was hydrolysed in 2M HCl and in 4M NaOH solutions for 4hrs. The products of acid hydrolysis were extracted separately with isoamyl alcohol and ether and run with Forestal solvent (acetic acid: Conc.HCl:Water:30:3:10). The solution from

alkaline hydrolysis was acidified with dilute HCl and the Chromatogram obtained using Whatman No 1 paper in upper layer of a mixture of toluene: acetic acid: water (4:1:5). The spots were developed using diazotised p-nitroaniline or viewed under UV-light.

3.3 High Pressure Liquid Chromatography (HPLC) Analysis

20mg of kino dissolved in 50cm^3 redistilled methanol in 100cm^3 volumetric flask and the solution sonicated using ultrasonic bath for 10 minutes. The solution was made to the volume with HPLC water and filtered.

20mg of each of the reference standards, tannic and gallic acids were dissolved and treated exactly as the test sample.

Two mobile phases were used. HPLC grade acetonitrile and disodium hydrogen phosphate in the ratio 40:60. The pH was adjusted to 3.1 with phosphoric acid. The second mobile phase was redistilled methanol and HPLC water in the ratio of 20:60.

The results are recorded in figure 2.

3.4 ANALYSIS OF THE RESIN OF THE KINO

3.4.1 Acid Number

This is expressed as the milligram equivalent of potassium hydroxide required to neutralize one gramme of test sample.

0.001gramme of the exudate was carefully and accurately weighed and dissolved in 25cm^3 of 80% ethanol. It was then tirtated with 0.1M alcholic potassium hydroxide. The change in pH was measured with a pH meter because the phenolphthalein end point would be blurred and

practically unclear as a result of the brown colour of the reaction mixture. The equivalent point appeared at about 1.5cm^3 of titrant.

3.4.2 Saponification Value (Reed 1966)

This is defined as the number of milligramme equivalent of potassium hydroxide required to saponify one gramme of test sample.

4grams of kino were accurately weighed into a 200cm^3 conical flask and dissolved in 50cm^3 of 0.5M alcoholic potassium hydromide. The mixture was refluxed for about 1hr. The hot solution was titrated with a 0.5M hydrochloric acid and the equivalent point monitored with a pH meter.

A blank determination was also carried out at the same time involving every other reagent and conditions but the substance being tested.

3.4.3 Molecular Weight determination

The method of Lamber et al, (1973) was used for this determination as follows:

An ignition tube was weighed empty and then with 0.8g of camphor. About 0.08g of Kino was then added and the ignition tube and its contents reweighed. A thermometer (0 - 360^{0}C) was inserted into the tube and secured with a rubber cork. The ignition tube was placed in a boiling-tube containing olive oil and warmed gently until the mixture melted. The ignition was removed and the thermometer adjusted to dip into the molten mass. The tube was replaced and allowed to cool while in the oil. The temperature at which the camphor became cloudy was taken

as the freezing point. Another ignition tube was used to determine the freezing point of camphor. The molecular mass of kino was calulated using the cryoscopic method.

3.5 Tannin Analysis

A quantity of the exudate (7g) was weighed and dissolved in warm water and made up to one-litre to give between 3-5g per litre of tannins, and tannin content was estimated according to the Official Method of Analysis (SLTC, 1996). It was not necessary to leach as the exudate dissolved completely in hot water between 40-60°C. The liquor was allowed to cool overnight and analysed for tannins, non,-tannins, solids, solubles, pH, color and total salts and total salts of weak acids. The results are shown tables 1.3

3.5.1 Determination of Total Solids (SLTC 1996)

50cm^3 of the unfiltered tan liquor was measured and evaporated to dryness over a water bath in triplicate. The residues were dried in an oven at 105°C for 1hr before drying to constant weight with cooling in a desiceator. The average percentage of residue was calculated as total solids from the equations: $\%\ \text{Total Solid} = \frac{\text{Weight of residue}}{\text{Weight of extracts}} \times \frac{\text{(Vol of extract)}}{\text{(Vol evap.)}} \times 100$

3.5.2 Determination of Total Solubles

The stock solution was filtered and 50cm^3 of the filtrate was measured and evaporated to dryness over a waterbath in triplicate according to the official methods of analysis (SLTC 1996). It was then dried in an oven at 150°C for 1hr before drying to a constant weight with cooling in a

desicator. The total solubles was calculated as a percentage from the average of the weight of the residue according to the equation % Total Solubles =

$$\frac{\text{Wt of residue}}{\text{Wt of sample}} x \frac{\text{(Vol of extract)}}{\text{(Vol of extract evap)}} x\ 100$$

3.5.3 Determination of Non-tans (SLTC 1996)

(6.25g) of dry hide powder was weighed out and ten times its weight of distilled water added and shaken for 1 hr before pretanning with (1cm^3) of 3% chrome alum per gram of dry hide powder by shaking for 15mins then allowing to stand overnight. The following morning the hide powder was squeezed in a filter cloth then (110cm^3) of distilled water was added to the hide powder stirred for 15mins before dropping to drain and before squeezing again. This was repeated four times so as to remove all uncombined chrome alum ions. The hide powder was weighed into a conical flask and its weight made up to 26.25 (per gram of dry hide powder) with distilled water. The unfiltered homogeneous tan-liquor (100cm^3) was added into the conical flask and shaken with mechanical shaker for 20 mins. The content of the conical flask was transferred to a filter cloth to drain, then thoroughly squeezed. Kaolin (1.0g) was added to the filtrate, stirred and filtered till the filtrate was clear. The filtrate (50cm^3) was taken in a preweighed crucible and evaporated to dryness over a water bath. The residue was dried in an oven at 105°C for 1hr till a constant weight was obtained. The non-tans % was calculated according to the equation.

$$\% \text{ Non-tans} = \frac{\text{Wt of residue}}{\text{Wt of sample}} \times \frac{\text{Vol of extract}}{\text{Vol of extract evap}} \times 100$$

3.5.4 Determination of Total Salt content by Sulphated Ash Method. (SLTC 1996)

50cm^3 of the filtrate of the tan-liquor was measured into a preweighed crucible and evaporated to a small volume of (5.0cm^3). 5.0cm^3 of 2.5M H_2SO_4 was added to it and the crucible swirled to ensure that the acid came in contact with the residual tan-liquor. Evaporation was then continued to completion and the residue was transferred to a hot plate in a fume cupboard and heated to drive off excess acid before ashing in a muffle furnace at 600°C for 1hr, allowed to cool then weighed to a constant weight. The residue was calculated as % total salt content per 100g of the total solubles according to the equation

$$\% \text{ Total Salt Content} = \frac{\text{g sulphated residue}}{\text{Vol (original wt of sample)}} \times 100$$

3.5.5 Determination of Total Salts of Weak acids Content by the Ash Method (SLTC 1996)

The filtrate (50cm^3) was pipetted into a preweighed crucible and evaporated to dryness on a water bath and weighed. The weighed residue was ashed in a muffle furnace at 600°C for 4hrs and allowed to cool. O. 1M HCl (25cm^3) was added and the crucible swirled faintly to ensure complete dissolution of the residue and the solution transferred to a 500cm^3 beaker before dilution with distilled water (25cm^3). The resulting solution was titrated with 0.2m NaOH with phenolphthalein as indicator and 0.1m HCl (25cm^3) diluted with distilled water (50cm^3) as blank. The salts of weak acid was calculated in 100g total soluble as milligram equivalent NaOH.

3.5.4 Determination of Total Salt content by Sulphated Ash Method. (SLTC 1996)

50cm^3 of the filtrate of the tan-liquor was measured into a preweighed crucible and evaporated to a small volume of (5.0cm^3). 5.0cm^3 of 2.5M H_2SO_4 was added to it and the crucible swirled to ensure that the acid came in contact with the residual tan-liquor. Evaporation was then continued to completion and the residue was transferred to a hot plate in a fume cupboard and heated to drive off excess acid before ashing in a muffle furnace at 600°C for 1hr, allowed to cool then weighed to a constant weight. The residue was calculated as % total salt content per 100g of the total solubles according to the equation

$$\% \text{ Total Salt Content} = \frac{\text{g sulphated residue}}{\text{Vol (original wt of sample)}} \times 100$$

3.5.5 Determination of Total Salts of Weak acids Content by the Ash Method (SLTC 1996)

The filtrate (50cm^3) was pipetted into a preweighed crucible and evaporated to dryness on a water bath and weighed. The weighed residue was ashed in a muffle furnace at 600°C for 4hrs and allowed to cool. O. 1M HCl (25cm^3) was added and the crucible swirled faintly to ensure complete dissolution of the residue and the solution transferred to a 500cm^3 beaker before dilution with distilled water (25cm^3). The resulting solution was titrated with 0.2m NaOH with phenolphthalein as indicator and 0.1m HCl (25cm^3) diluted with distilled water (50cm^3) as blank. The salts of weak acid was calculated in 100g total soluble as milligram equivalent NaOH.

PART II

3.6 TANNING TRIALS

Twenty (20) pieces of raw goat skins were taken after careful selection for quality and mindful of any blemishes that might affect the tanning trials. This number was taken because three types of tanning methods were envisaged, that is:

i. Full tannage with Kinos

ii. Full tannage with Bagaruwa

iii. Retannage with chromium salt.

This choice of tanning trials is to allow for meaningful comparison between leathers produced from kinos and those from Bagaruwa which is an already fairly established tanning material in Nigeria and whose leathers are acceptable internationally.

The twenty skins were weighed and then processed together till they were ready for tanning. That meant they were soaked, unhaired, limed and delimed together. After that they were separated into four groups of 5. Each group for the three types of tannages

i. Full kinos

ii. Full Bagaruwa

iii. Retannage with chromium salt

3.6.1 Laboratory Tanning Trials

One piece of delimed pelt was taken and given an acid treatment referred to as drenching to adjust the pH to between 4 and 5 close to the pH's of both kinos and bagaruwa. The pelt was then divided into halves along the back bone line. The pieces were weighed separately and their

individual weights used to prepare the respective tanning liquors for tanning trials in beakers. These trials were done at various pHs to obtain the optimum pH that would give good rate of penetration resulting in good exhaustion of the tannins in the liquor. This is necessary because the amount of free acids in the resultant leather is very critical to the life of the leather. The result of these laboratory trials would determine the best pH for tannage. That is the pH that would give good penetration rate.

Four pieces each of the delimed pelts were weight and pH adjusted accordingly and tannage with kinos and Bagaruwa were done as follows.

3.6.2 Tannage with Kinos

Pelts meant for this tannage were divided into 3 different lots of 3 pieces each and reweighed. 30% tannin liquor was prepared by weighing an amount of kinos corresponding to 15%, 25%,30% tannins and dissolved in warm water as kino is not easily soluble in cold water. This warm liquors of 20-25°C were maintained and added to the pelt in the experimental drum in three lots of 5, 5, 10 or 5, 10, 15 or 10, 15 drumming intermittently and testing for penetration till tannage was completed. Addition of the tannin liquor were made after every hour. At the completion of tannage the leathers were washed and fatliquored with 3% fatliquor on the weight of the pelts. These leathers were horsed up to drain before drying by hanging.

3.6.3 Tannage with Bagaruwa

The same numbers of pelts were taken treated with Bagaruwa tannin liquor in exactly the same way before fatliquoring and drying. The only major difference was that Bagaruwa dissolved very well in cold water.

3.6.4 Retannage with Chrome

Two pieces of pelt previously divided along the backbone line and each half treated with equal amount of kinos and Bagaruwa following the same process were retanned with 3% chrome salt (chromosol) in the same drum after proper washing. The retanned leathers were then basified with 1.5% sodium bicarbonate administered in 3 lots at 15mins interval till the completion of basification. The resultant leathers were washed with clean water before fatliquoring with 3% fatliquor on the weight of the pelts. The leathers after drying and physical observation were sent in for full analysis.

3.7 Leather Analysis (Chemical Analysis)

3.7.1 Determination of Nitrogen and Hide Substance (SLTC 1996)

This method is based on the fact that the grease free, ash free, dry substance of the pelts of various animals has different, but practically constant nitrogen contents for some animals 0(Schroder and Passler 1905). This can however, be adulterated by other nitrogenous substances like fixatives, synthetic (resins) tannins, cationic fats and dyestuffs.

Hide substance can be defined as the content of nitrogenous substances calculated from the content of leather as decomposed by the kjeldahl method, the ammonia is distilled off and titrated.

(i) Digestion

Sampling and grinding were done in accordance with the Official Method of Analysis. 150mg of the leather tanned with the "kinos" of Eucalyptus citriodora was weight into a suitable conical flask and 1gm of the catalyst mixture previously prepared by mixing 10gm $CuSO_4$ and

90gm K_2SO_4 was added. This was followed by a few boiling stones. The glass stopper was moistened with the same concentrated sulphuric acid and the flask closed with it before heating to boiling with low heat first, allowing to digest, then higher heat was offered for half an hour after all carbon had been oxidised.

(ii) Distillation

Boric acid, an indicator which is a mixture of methylene blue and methyl red (at 40/60 ratio) and concentrated sodium hydroxide were prepared according to specification (SLTC, 1996) 1-2 drops of methylene blue - methyl red were added to $25cm^3$ of saturated boric acid solution till the colour turned pink. This was put at the receiver end so that the cooling tube dipped into it to deliver the ammonia. The digested sample was quantitatively transferred into the distillation room of the steam distiller and $25cm^3$ sodium hydroxide added till the colour turned blue. The ammonia was then distilled into the boric acid solution continuously for 4 mins before lowering the receiver so that the cooling tube no longer dipped into the boric acid solution. The distillation was continued for another one minute before the distillate was removed and the distiller washed. The distillate which was green, was titrated against previously standardised sulphuric acid. The Hide substance was calculated according to the equation:

$$\% \text{ Hide substance} = \frac{V_2 \; {}_2 \, x 14 \, x \, 5.62}{W} x \; 100$$

Where V_2 = Vol of sulphuric acid

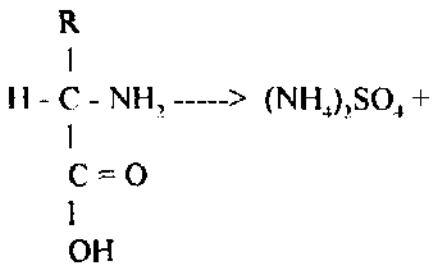
M = Molarity of the acid

W = Weight in mg of sample

N = 14 = atomic weight of Nitrogen

The reactions involved are as follows

Destruction:



$$H - C(R) - NH_2 \text{ -----> } (NH_4)_2SO_4 + CO_2 + H_2O \quad (189)$$

Distillation:

$$(NH_4)_2 SO_4 + 2NaOH \text{ -----> } Na_2SO_4 + 2NH_3 + 2H_2O \quad (190)$$

$$NH_3 + H_3BO_3 \text{ -----> } (NH_4) H_2BO_3 \quad (191)$$

$$2(NH_4)H_2BO_3 + H_2SO_4 \text{ -----> } 2H_3BO_3 + (NH_4)_2SO_4 \quad (192)$$

The results of the % hide substance are recorded in table IV

3.7.2 Degree of tannage

This is defined as the quantity of tannins bound to 100 parts hide substance. Given by the equation:

$$\text{Degree of tannage} = \frac{\%\ \text{bound organic subs}}{\%\ \text{hide substance}} \times 100$$

This is usually applicable to vegetable tanned leathers only.

Content of bound organic substances is calculated in % as difference of the sum of % hide substance, water soluble organic substances, total ash, substances extractable by dichloromethane and volatile matter. i.e. bound organic substances.

= 100 - % hide substance + water soluble organic substance + total ash

Substances + total ash + substances extractable by dichloromethane+ volatile matter. (Kanagy 1961).

The results are recorded in table IV.

3.7.3 Determination of Volatile Matter (Moisture) (SLTC 1996)

Volatile matter content refers to the weight lost by the leather when dried at 102 ± 2°C for 12 to 16 hrs. This can be done for any kind of leather but wet leathers must be first dried. It should be noted that an exact determination of the moisture contained in leather by drying is not possible, because at elevated temperature other volatile substances escape while tannins and fats can be oxidised and some adsorbed water may be left in the leather after drying.

The samples prepared according to official methods of leather analysis were weighed to approximately 3g and weighed after cooling in a desiccator for 30mins till constant weight. Volatile matter was calculated as

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W} X\ 10($$

Where W_1 = weight of

W_2 =

The results are recorded in table IV.

3.7.4 Determination of substance Extractable by Dichloromethane (Fats and other Solubles) (SLTC 1996)

Substances extractable by dicholoromethane usually refers to fats and other solubles. These can be determined for any type of leather.

Dichloromethane has been found to be most suitable for the extraction of all fatty and similar substances from leather, other solvents may not be able to remove all fatty substance bound or unbound but could also remove non-fatty substances like sulphur and other impregnants.

10g of the leather was weighed and press evenly into the filter paper thimble and covered with a thin layer of cotton wool that is grease free and sintered glass plate.

The extraction flask was dried by heating with two carborundum beads in it for one hour at 102 ± 2°C and weighed after cooling in a desicator for 30min till constant weight. The dichloromethane was then added until after 30 changes of the solvent. Dichloromethane was then distilled from the flask contaning the extract. The extract dried in the oven for one and half hour at 102 ± 2°C, cooled in the desicator for 30 minutes and weighed. This was repeated till constant weight was achieved. Extractable substance was calculated as follows:

$$\% \text{ Extractable substance} = \frac{\text{g extract}}{\text{g original sample}} \times 100$$

The results are recorded in table IV.

3.7.5 Determination of Total (Sulphated) Ash (SLTC 1996)

Total Ash is defined as the residue left from burning leather in an open crucible at 800°C after sulphating. This excludes ammonium salts.

The leather sample is obtained and prepared according to Offical Method of leather analysis.

5g of the leather sample was weighed out and carefully carbonised over a low flame in a previously weighed crucible that has been heated to 800°C so that the leather was burned with a small flame. This was important so that grease in the leather burned slowly. The residue was carefully moistened with concentrated sulphuric acid and heated over a low flame until sulphuric

acid fumes were no longer visible. The crucible was cooled and the fuming with sulphuric acid was repeated before the residue was ignited in the furnace at 800°C until completely ashed. The residue was cooled in a desicator and weighed repeatedly until constant weight was obtained. Total ash was calculated as follows:

$$\% \text{ Total Ash Content} = \frac{\text{g total ash, (sulphated)}}{\text{g original wt of leather}} \times 100$$

The results are recorded in table IV.

3.7.6. Determination of Water Soluble organic and Inorganic substances in Leather (Water Soluble matters) (SLTC 1996)

Water soluble matter is define as the quantities of all those subtances, which under certain conditions are dissolved out of the leather by water. They are principally organic tannins, non-tannins and mineral salts.

Water soluble inorganic substance is the sulphated Ash of water soluble substances, while water soluble organic substance is the difference between total water solubles and water soluble inorganic substances. The method is applicable to all leathers.

The principle is based on the fact that the extent to which a leather is ground, the temperature, the extraction period and the ratio of leather to water, influence greatly the amount of water soluble extracted and hence the results in total of the water soluble test. Test conditions must therefore be strictly adhered to.

10gm of leather previously extracted by dichloromethane was weighed out and 500cm^3 deionised water at 18°C was added to it in a flask. This was shaken in a mechanical shaker with 50± rev. per minute for 2hours. The content was filtered with an ash free filter paper discarding

the first 50cm^3 of the filtrate. 50cm^3 of the filtrate were than evaporated on water bath till dryness in a previously heated and weighed platinum dish according to specification. The residue dried in an oven at 102 $\pm$ 2°C for 2hrs then quickly weighed. This drying and weighing were repeated till a constant weight was obtained. Total water solubles were calculated as follows:

$$\% \text{ Total water solubles} = \frac{\text{g (dry residue)}}{\text{g original (wt of leather)}} \times 10 \times 100$$

The factor 10 is dilution ratio i.e. 50cm^3 used for extract.

The results are recorded in table IV

To obtain the sulphated ash of water solubles the residue used for the calculation of water solubles could be sulphated directly if not 50cm^3 of the fitrate is taken evaporated before sulphation. The sulphated residue was then ignited in a furnace at 800°C after all the fumes have been expelled. The white ash obtained was cooled in a desicator and quickly weighed till constant weight was obtained. Sulphated Ash of water solubles was caluclated in percentage as follows:

$$\% \text{ Sulphated Ash of Water Solubles} = \frac{\text{g sulphated residue}}{\text{g original wt of leather}} \times 10 \times 100$$

The results are recorded in table IV

Water Soluble oraganic substance was calculated as follows:

% Water Soluble Organic Substance

= % Total Water Solubles - % Sulphated Ash of Water Solubles.

The results are recorded in table IV

3.7.7 Determination of the pH Value and Difference Figure of an aqueous Leather Extract (SLTC 1996)

The pH is a measure of hydrogen ion activity or hydroxyl ion activity. The difference between the pH of solution and the pH of its tenfold dilution is referred to as the Difference figure. It is a measure of the strength of acids and bases. It should never exceed 1 and when it is between 0.7 and 1.0 then the solution contains a free strong acid or base. Ionisation of the weak acids and bases increase with dilution and the Difference figure acts as a criterion for the presence of free strong acid or base in aqueous extract with pH values below 4 and above 10. These principles apply to all types of leather and at all stages of dressing.

There is alway some amount of acid but less often alkali in leather which dictates its pH. Free strong acid or alkali damage leather with time and any other material treated with it. The pH value of the aqueous leather extract and its Difference figure, serve to measure the acidity or alkalinity of the leather. Both depend on the quantity and strength of the acid or base contained in the leather and on the presence of buffer salts. If the pH value of the extract is below 3.5 or over 10, then the Difference figure is (i) large in the prensence of strong acid or base and absence of buffering salts. (ii) small in the presence of strong acid or base buffering salts being present in considerable quantities. (iii) small in the presence of weak acid or base independently of any buffering salt present.

5gm of leather already sampled and ground according to the official method was weighed out in a bottle and 100cm^3 deionised water added and the content shaken by hand for 30 mins before transfering to the mechanical shaker for 24 hrs. The pH of the unfiltered extract was

measured with the pH meter at 20 ± 2°C. 5cm^3 of the filtrate was then diluted 10 fold and the pH measured by the same pH meter to determine the Difference figure.

The results were recorded in table IV.

3.8 Leather Analysis (Physical Analysis)

One of the major aims of producing leather or preserving raw hide or skin by tanning is to impart to it some level of strength. It is expected therefore that after the skin has been properly tanned it should show some reasonable increase in strength over the raw one. Methods of evaluating this strength include:

i. Tensile strength measurement and % Elongation

ii. Stitch tear (resistance) strength

iii. Ball burst or grain strength.

3.8.1. Determination of Tensile Strength (SLTC 1996)

Tensile strength is actually the strength of the leather. The Principle is to apply load to a piece of leather until the leather breaks. The maximum load required to break the leather was determined using a Tensometer suppled by SATRA. The leather sample was cut according to the Official Method of Analysis and all test conditions were maintained. Tensile strength was calculated as follows.

$$\text{Tensile strength} = \frac{\text{Shear Load x } gNm^{-2}}{\text{Width of strip x Thickness}}$$

The results are recorded in table V.

% ELONGATION (SLTC 1996)

This also determine the strength of the leather and most importantly its elasticity. It is the ability of the leather to extend under stress without being damaged. This is important if we note that this is one of the properties of the raw hide/skin that we are trying to maintain and if possible improve upon.

The test samples are the same with the one used for Tensile Strength determination. The % Elongation is therefore always calculated from Tensile Strength as follows:

$$\% \text{ Elongation} = \frac{\text{Actual Chart Extension} \times \text{Cross head Speed} \times 100}{\text{Gauge length} \times \text{Chart Speed}}$$

The results are recorded in table V.

3.8.2 Determination of Stitch Tear Resistance (SLTC 1996)

Leather used for the production of shoes, garment, belts, gloves and upholstry are usually sewn or stitched. Such leather should not tear at the needle point they are stitched hence the used of this test. The aim is to see how resistant the leather is to stitching.

The test is carried out according to the official method of analysis. A piece of leather of 50mm long and 25mm wide is cut from the approved sampling possition and a slot is made in the middle with a press knife according to the approved method before the samples is clamped into the tensile machine with a sharp edge places at the thinnest part of the slot and load applied till the sharp edge tore the leather. The tearing load was recorded from which the Tear Resistance was calculated and recorded in table V.

3.8.3 Determination of Distension and Strength of the grain - The Ball Burst Test (SLTC 1996)

This method may be used with any light leather even though it is intended particularly for use with boot and shoe upper leathers.

The sample was prepared according to the Official Method of Analysis and clamped in the instruments with its "flesh" surface adjacent to the ball and its "grain" surface flat.

The distension was increased at a rate of approximately one-fifth of a millimetre per second and the grain surface watched for any crack. The load that caused the crack was noted while the process continued till the grain burst. The load at burst was also noted. The strength of the grain was calculated and the results recorded as in table V.

3.8.4 Determination of Flexing Endurance (SLTC 1996)

This method is used for light leathers and particularly for shoe upper leathers. The Flexometer is designed to simulate the human walking. The Flexometer is supplied by SATRA, Northants England. The samples were cut from the official sampling positions and were rectangles of 70mm by 45mm and conditioned according to IUP/3 (SLTC 1996).

The specimens were folded according to specification and clamped at each end to maintain it in a folded position in the flexometer. One clamp is fixed and the other moves backwards and forward causing the fold in the speciman to run along it. The specimen was examined periodically to asses any damage that was produced.

The examination was done after 2,4,6,8,12,16,24,32, and 72 hours. The nature of the damaged observed and the cycle at which this occured were recorded. The examination was done

by removing the sample from the machine and viewing same under very good illumination and magnification before the samples were clamped back in the exact possible as best as possible.

The results are recorded in table V.

CHAPTER FOUR

RESULTS AND DISCUSSION

PART I

4.0. CHARACTERISATION AND ANALYSES OF THE TANNINS OF THE EXUDATE

4.1 Solubility of Kino in the various solvents

The solubilities of kino in the various solvents is recorded in Table 4. I below.

Table 4.1 Solubility of kino in the various solvents

	Solvent	Extent of Solubility of Samples in Solvent
1.	Absolute Ethanol	Very soluble (at room temperature)
2.	Acetone (95%)	Very soluble
3.	Ethylacetate	Partially soluble
4.	Butan-ol	Soluble
5.	H_2O	Fairly soluble (turbid)
6.	Pet ether (60-80)	Insoluble
7.	Toluene	Insoluble
8.	Benzene	Insoluble (coagulates)
9.	Chloroform	Insoluble
10.	Carbon tetrachloride	Insoluble
11.	Diethylether	Insoluble
12.	Methanol	Very soluble
13.	NaOH (2M)	Very soluble (gives deep brown solution)
14.	NH_4OH (2M)	Very soluble (gives deep brown solution)

4.2 Characterisation of the Tannins of Kinos

4.2.1 Ferric Chloride Test

Both pyrogallol and catechol react with ferric chloride in a definite way to give specific colours. A blue-black colour was observed as the result of this test. This is an indication that the

4.2.5. Formalin Test

A solution of formalin in hydrochloric acid would precipitate condensed tannins (Gustravson, 1956; Balboa, 1976). A precipitate which was insoluble in hot water and alcohol was formed indicating the presence of condensed tannins in the kinos which support the result of the bromine water test reported above.

4.2.6. Sodium Nitrite Test

Balboa reported that ellagitannins react with sodium nitrite to give a green colour while other tannins would give a brown colour (Balboa, 1976). On the addition of a few crystals of sodium nitrite a brown precipitate was quickly formed which on standing separated into a brown mass on the top and a green precipitate at the bottom. This indicated the presence of ellagitannins and other tannins. The kinos thus contained ellagitannins and other tannins.

4.2.7 Lime Water Test

Lime water reacts with vegetable tannins in a definite way giving precipitates of varying colours depending on the type (Gustavson, 1956; Balboa, 1976). A blue-grey precipitate indicates the presence of gallotannins (Balboa, 1976). The precipitate that was formed in this test was first grey and later on became blue-grey very clearly and sharply. This showed the presence of gallotannins as reported by Balboa.

4.2.8 Test for Anthocyanidin

This test is based on the concept that acid catalysed cleavage of the interflavanoid bond

sprayed with $FeCl_3$ - $K_3Fe(CN)_6$ solution. The chromatogram still showed ill-defined trail of elongated bluish-black streak from the origin to the solvent front with a brownish colored ridge at the solvent front. This agreed with the results and the views in the first trial which Ribereau-Gayon (1972) asserted was due to the polymers of catechins (Flavan-3-ols) The bluish-black elongated spot being due to ellagic acids, gallic or gallotannins as reported by Balboa. (1976).

The suspected predominance of the pyrogallol over catechol tannins suggested the use of known tannin components that are composed largely of pyrogallol derivatives as standards. Tannic acid and gallic acid which were available were used. Figure 3 shows a paper chromatogram of tannic acid, gallic acid and the exudate. A two dimensional chromatography of tannic acid (hydrolysable tannin from galls of Rhus Semialata and Quescus infectoria) had identified pentagalloyl-glucose, gallic acid, m-digallic acid, trigallic acid, gallotannin and an unidentifiable component (Ribereau-Gayon 1972). The spots in figure 3 confirmed the presence of free gallic acid in the exudate. The presence of free ellagic acid was ruled out because it is not present in tannic acid. Literature, particularly from the work of Nduji 1986 showed that it has very low Rf value. In figure 3, the lowest exudate spot had a higher Rf value than the lowest tannic acid spot. Derivatives of ellagic acid may, however, be present. This is suggested by the fact that the product of acid hydrolysis of the exudate gives a deep blue black colouration on spraying at and near the point of origin contracted with the acid hydrolysis product of tannic acid which is not coloured at the origin and only very lightly so close to it. This is also supported by the fact that the test with sodium nitrite for the presence of ellagic tannins was positive.

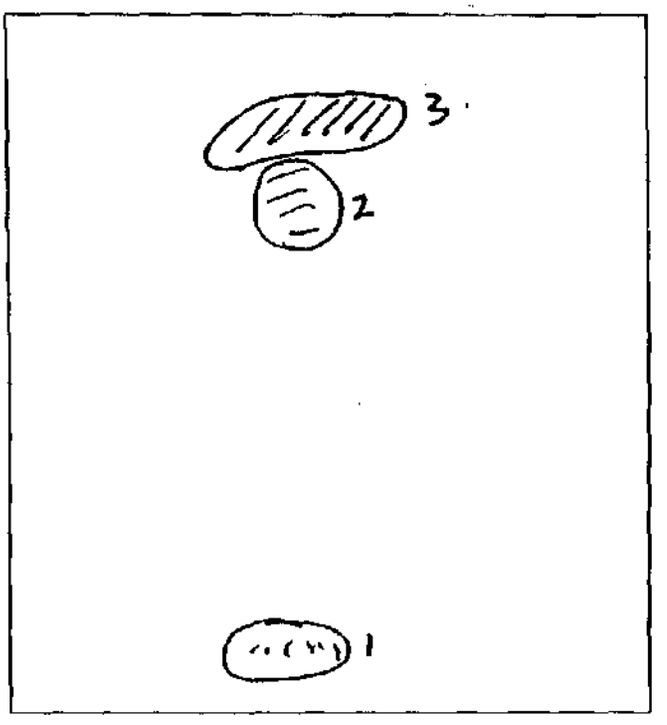


Fig. 1: Paper chromatogram of eucalyptus exudate. Solvent: Acetic acid/n-Butanol/Water (1:4:5)

In trial three, Whatman No. 2 paper was used, it has a large absorptive capacity (Ribereau-Gayon 1972). The extract in the second trial was used and the chromatogram ran in 2-D and sprayed with $FeCl_3$ + NH_3-$AgNO_3$. Bluish - black spot, ill-defined, was still observed as in the previous trials.

Ribereau-Gayon (1972) has reported that extraction of aglycones with immiscible solvents like ether, ethyl acetate and isoamyl alcohol would improve the separation. Consequently the kino was extracted with ether and ran with a mixture of glacial acetic acid/n-butanol/water (1:4:5 v/v/v) as mobile phase overnight. The dried chromatogram was developed with 5% alcoholic $FeCl_3$.

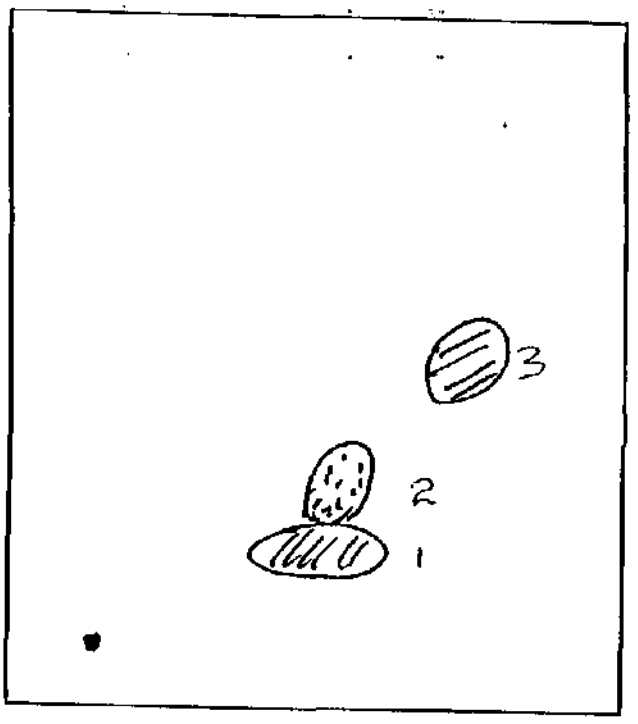


Fig. 2: Two-dimensional paper Chromatogram of eucalyptus exudate. Solvent: Acetic acid/n-Butanol/Water(1:4:5)

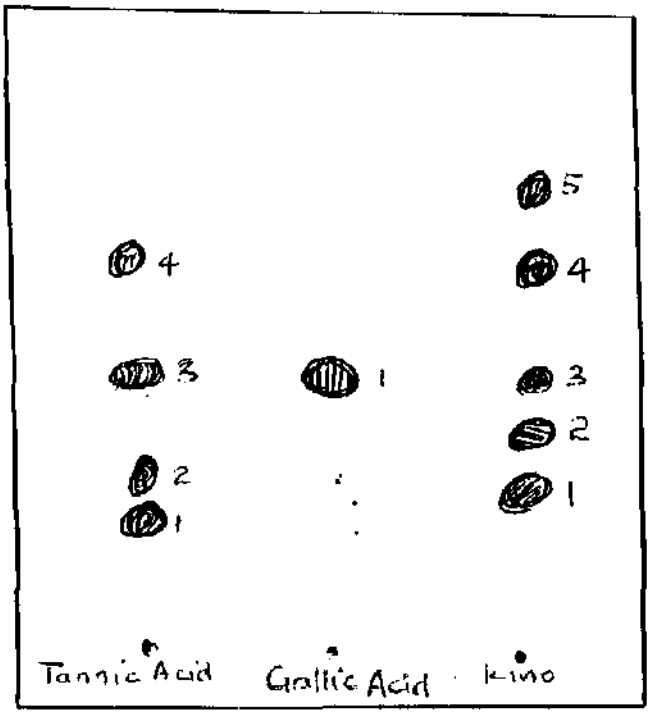


Fig. 3: Paper chromatography of Tannic acid , Gallic acid, and the Eucalyptus exudate: Solvent Acetic acid/n-butanol/water (1:4:5)

The one dimensional chromatography of the exudate (Fig 1) on Whatman No. 1 paper using (glacial 1:4:5 acetic acid/n-butanol/water as mobile phase and run over night showed 3 spots as indicated in figure (1) which on development with 5% alcoholic $FeCl_3$ gave dark brown colouration for spot 3, blue black for spot 2 and bluish green for spot 1. Between sports 1 and 3, however, was a diffuse blue-black colouration which indicated the presence of a large number of pyrogallol constituents. This compares well with the types of constituents obtained from tannic acid (hydrolysable tannin from galls of Rhus semialata and Quercus infectora). (Ribereau- Gayon, 1972). Such constituents include pentagalloyl glucose, gallic acid, m-digallic acid, trigallic acid, gallotannin etc.

The two dimensional chromatogramm (Fig 2) of the exudate gave even a clearer picture of the complexity of the composition of the exudate. The chromatogram was covered completely with blue-black colouration on spraying with alcoholic ferric chloride solution. The brown spot could not be definitely identified but it occured in other chromatograms including those of the hydrolysed exudate. Faint patches of green colouration were also observed but unlike hydrolyseable tannins, condensed tannins give streaks rather than discrete spots on chromatograms. The chromatography of condensed tannins are better done with the hydrolysed products acid and base hdrolysis.

Acid hydrolysis was carried out on the exudate and a very dilute red coloured mixture was obtained, confirming the presence of the catechol type tannins, albeit in apparently small proportions. This hydrolysate was chromatographed on paper using various solvent mixtures and the resulting chromatograms observed under UV λ_{max} 366nm. They were then exposed to ammonia and viewed again under UVλ_{max} 366nm. The chromatograms are presented as figures (4), (5) and (6). It has been shown that leucoanthocyanins are the commonest and most typical

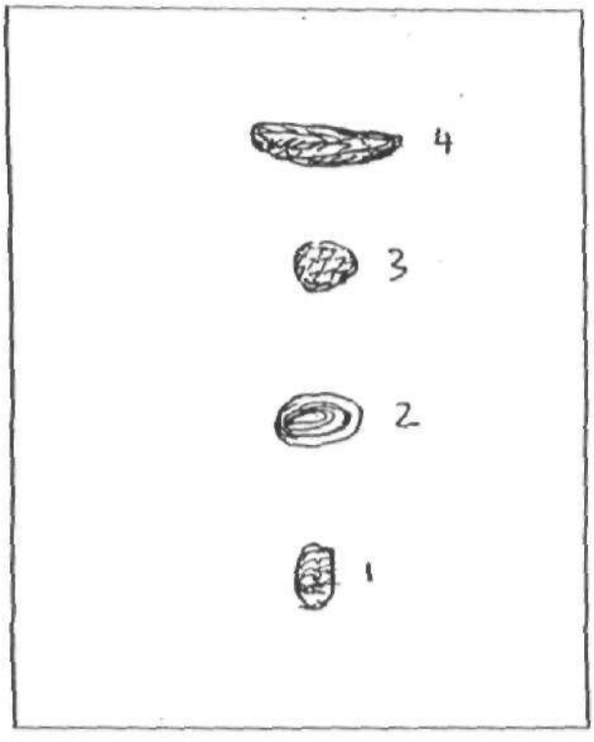


Fig.5 Paper Chromatogram of eucalyptus exudate after acid (2M HCl) hydrolysis. Solvent: Acetic acid/HCl/Water (30:3:10)

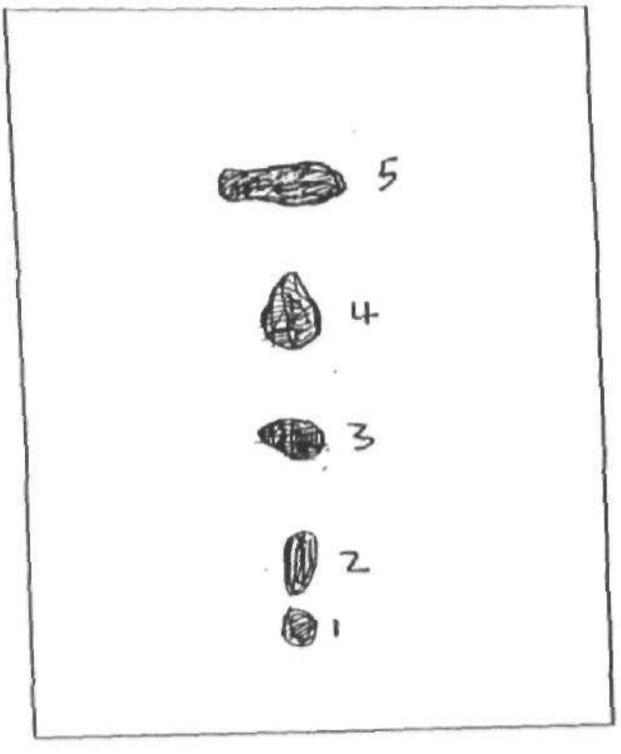


Fig. 6 Paper Chromatogram of eucalyptus exudate after acid (2M HCl) hydrolysis. Solvent: Toluene/Acetic acid/Water (upper layer). (4:1:5)

Table 4:2 Rf Values from Paper Chromatogram of Eucalyptus Exudate After Acid (2M HCl) Hydrolysis

Spot	Rf	U.V
1	0.83	yellow
2	0.89	brown
3	0.95	blue
Solvent: n-Butanol/Acetic acid/H_2O		
1	0.41	yellow
2	0.71	yellow
3	0.86	blue, purple
4	0.97	brown
Solvent: Acetic acid/HCl/H_2O		
1	0.02	yellow
2	0.24	brown
3	0.70	yellow
4	0.80	yellow
5	0.96	brown
Solvent: Tuluene/Acetic acid/H_2O		

The chromatograms of the acid hydrolysed exudate in the three solvent systems used when viewed under UV light showed predominantly yellow colour at the base with Rf values ranging from 0.02-0.80, brown and blue mostly at the solvent front with Rf values ranging from 0.86-0.97. When the blue and the brown colorations at the solvent front were treated with ferric chloride that gave a blue colour with some brownish tinge. The blue coloration is indicative of ellagitannins which are esters. The other blue coloration before the solvent fronts are indicative of gallotannins mostly gallic acid. This is in agreement with similar works done by Ribereau and others (1972). The streak of brownish tinge observed from spraying with ferric chloride is also indicative of catechins which are constituents of condensed tannins.

Basic hydrolysis of condensed tannins yield products that also help to determine the basic structures of the tannins. The hydrolysate was chromatographed in six different solvent systems

in order to observe the behaviours of the constituents in these different solvents mixtures. Figures, (7), (8), (9), (10), (11) and (12) are chromatograms of the hydrolysate in the different solvent mixtures.

Basic hydrolysis

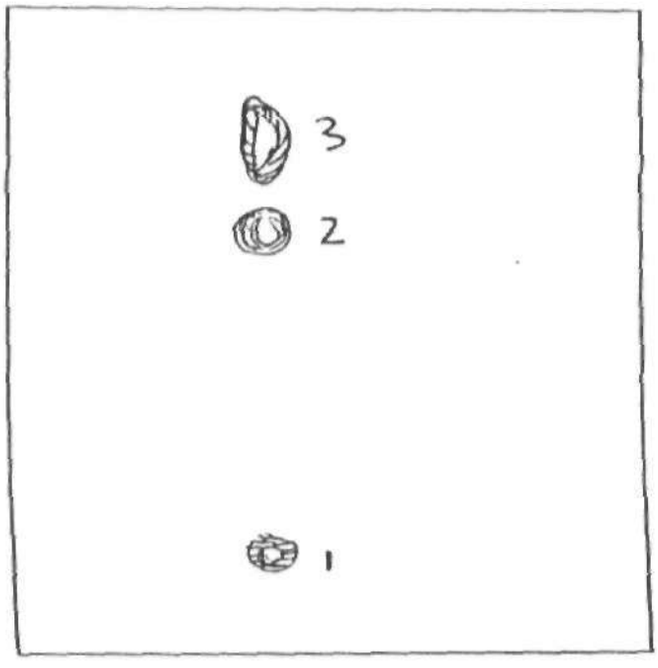


Fig. 7 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis and reacidification. Solvent: Toluene/Acetic acid/Water (upper layer) (4:1:5)

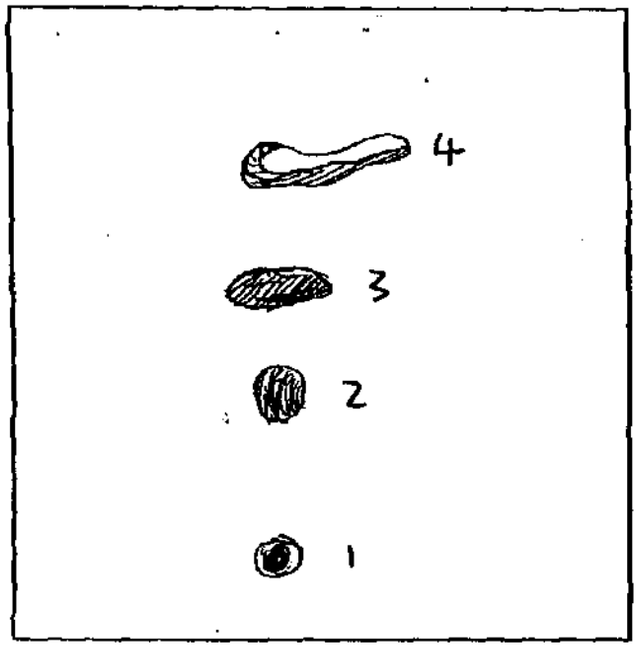


Fig. 8 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis. Solvent: Toluene/Acetic acid/Water (4:1:5)

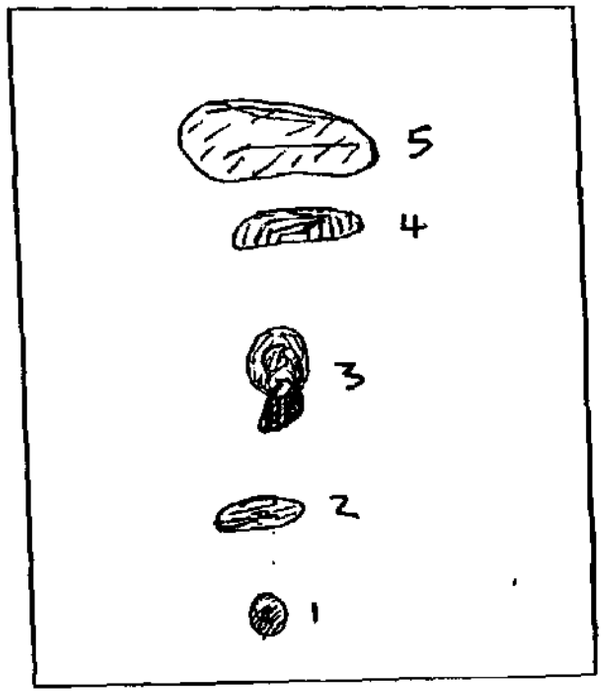


Fig.9 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis. Solvent: Acetic acid/HCl/Water (30:3:10)

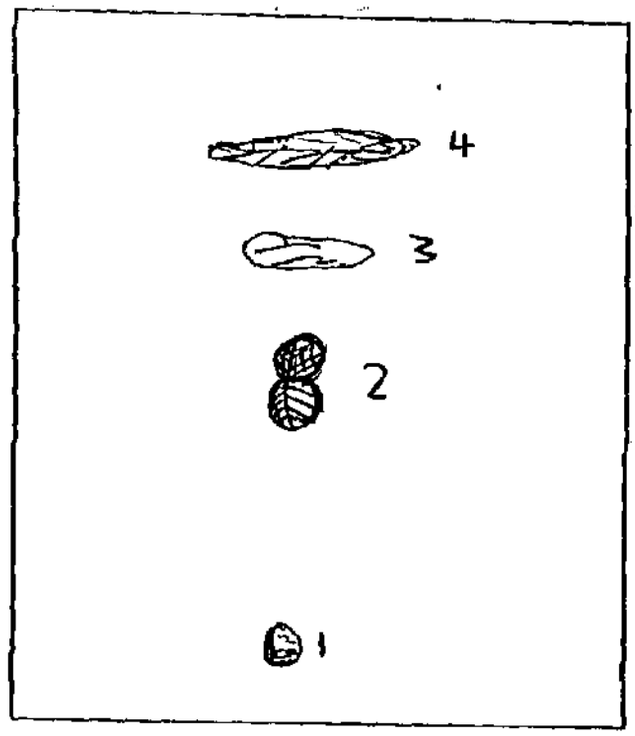


Fig. 10 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis and reacidification. Solvent: Acetic acid/HCl/Water (30:3:10)

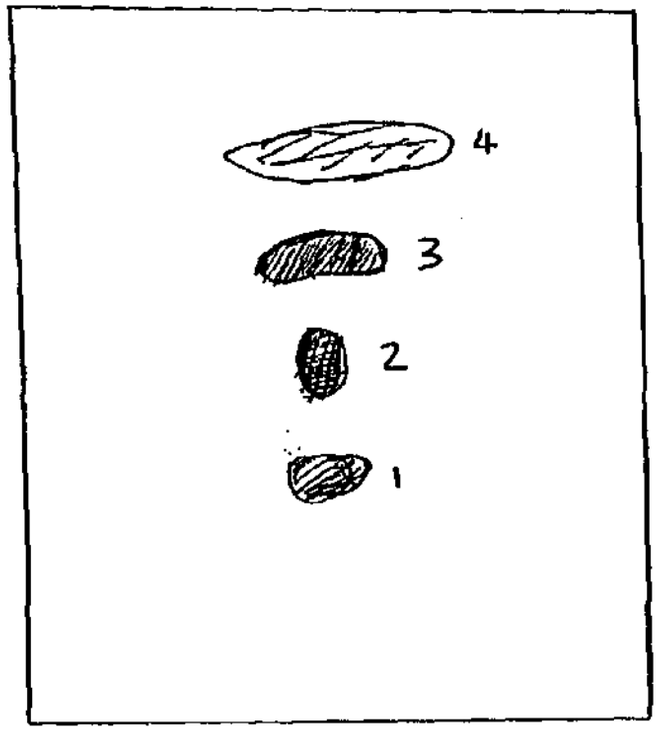


Fig. 11 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis and reacidification. Solvent: n-Butanol/Acetic acid/Water (4:1:5)

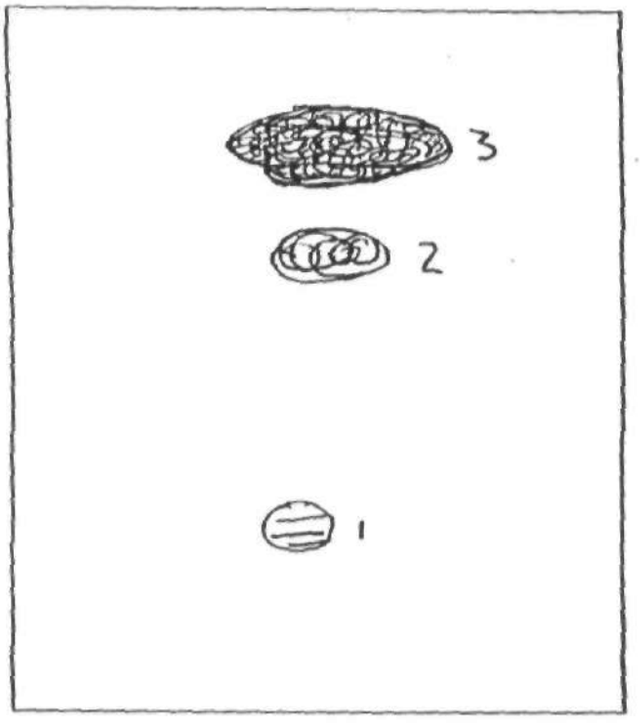


Fig. 12 Paper Chromatogram of eucalyptus exudate after base (4M NaOH) hydrolysis an reacidification. Solvent: n-Butanol/Acetic acid/Water (4:1:5)

The Rf values of the components obtained are hereby given in Table 4.3. Some of t chromatograms were also exposed to NH_3 fumes and viewed under UV light.

The Rf values of the base hydrolysed exudate in various solvent systems are recorded in Table 4.3. The yellow colouration persisted at Rf values between 0.00-0.85 with a few exhibiting dark brown or blue in some of the solvent systems in this range. The flavanols appear in this region and common amongst them that may likely be present in the exudate are kaempferol, quercetin and myricetin (Harborne, 1959b, 1965c). This colour would persist when exposed to ammonia before viewing under U.V (Ribereau 1972). The components that have Rf value at this range with a persistent yellow colour and in this solvent system as reported by Harborne is kaempferol.

In this Rf value range within the same solvent system anthocyanins that may be present are apigeninidin, cyanidin, peonidin, pelargonidin, delphinidin, petunidin, and malvidin (Harborne 1959a). Of these peonidin and cyanidin with Rf values in the range 0.68 - 0.71 which should show red colour under U.V are being proposed because the dark brown colour observed in both when viewed under U.V did not change when exposed to NH_3 before viewing under U.V.

Components with Rf values above 0.85 were observed under U.V. to be mainly blue-black, brown or dark brown most of which after being exposed to NH_3 and viewed under U.V. showed persistent yellow, blue or violet. Some of these particularly the violet or blue could be salicylic acid when it forms an internal ester or they could be said to be product of hydrolysis (Ribereau-Gayon, 1972).

In comparing the Rf values it must be noted however, that we are using a complex mixture, not a compound, unlike Harbone, Ribereau and co-workers who used pure samples. There would be thus differences in our values and apparent inconsistencies in the results.

However, from the results so far discussed it can be summed up that the exudate is mainly

a hydrolysable tannin made up mainly of gallic acids, ellagic and other gallotannins with traces of some flavanols and catechins mostly in the form of anthocyanins and/or anthocyanidins.

4.2.10 Characterisation by HPLC

HPLC, like paper chromatography is a separative and to some extent, identification method. In the case of paper chromatography the Rf value and behaviour of a spot to various reagents are determined in order to identify a compund. In HPLC, however, retention time and peak area, peak height (usually in percentage (s)) are determined as aids in identification. Standard samples of tannic and gallic acid were run separately and together to establish the patterns of the separation and the various parameters mentioned above.

It was not possible to identify conclusively the various components of the chromatogram (coupling with a mass spectrometer would probably assist in identification). It would be expected that there should be a peak with retention time, peak area percentage and height percentage corresponding or close to that of gallic acid since the exudate seemed to be composed mainly of gallotannins but here again it was not easy to reach such a conclusion. This demonstrated once again the problems associated with the identification of tannins.

4.2.11 Characterisation by IR

As has been indicated in Section 3:1, the exudate was collected once a month for 12 months. Chemical tests have confirmed the presence of tannins, both hydrolysable and non-hydrolyseable.

IR spectroscopy was carried out on each of the monthly samples collected. It is significant that the spectra obtained were very similar for all the samples. For example, the broad

peak between 3160 cm^{-1} and 3416cm^{-1} typical of the -OH groups was present. This broad peak showed the stretching -OH group peak of the aromatic ring (phenol) which is to be expected and the aliphatic -OH group peak which would be due to the sugar groups that might be found in the tannin molecules. Absorption peaks at 170 cm^{-1} and 1600 - 1630 cm^{-1} indicate the presence of esters which is expected of hydrolysable tannins, thus confirming their presence. It is not surprising that there are strong peaks at about 1600cm^{-1} indicating the presence of >C = C < of the aromatic rings. At about 1030cm^{-1}, 1200cm^{-1} and 1380cm^{-1} appear peaks which are typical of the >C - O (ester) stretching frequencies. In summary, all the peaks expected of tannins are represented as shown in the relevant spectra.

4.3 TANNIN ANALYSIS

The preliminary investigation on 'kino' exudate of E. citriodora has shown that it is slightly soluble in cold water but completely soluble in warm water and polar solvents like methanol and ethanol (Dashe and Shok, 1993). It was on the basis of this that tannin analyses were conducted on water and other solvent extracts. The results of the analyses using water and ethanol for extraction are recorded in Tables 4.9 and 4.10, while Table 4.11 is a one year analysis using water as the solvent for extraction. For exudate to completely dissolve in water, it was observed that the temperature must not be lower than 40°C.

Table 4.9 TANNIN ANALYSIS OF WATER EXTRACT OF THE EXUDATE OF E. CITRIODORA

	% Soluble	% Non-tans	% Tans	Colour		pH
				Yellow	Red	
Dec. 1989	84.44	22.24	60.20			
Jan. 1990	77.60	15.04	62.56			
Feb. 1990	70.40	12.70	57.70			
March 1990	62.40	12.00	50.40	8.8	1.0	3.01 - 33.38

TABLE 4.10 TANNIN ANALYSIS OF EXUDATE OF E. CITRIODORA EXTRACTED IN ETHANOL (100%)

	% Soluble	% Non-tans	% Tans	Colour of Infusion		pH of Infusion
				Yellow	Red	
Dec. 1989	86.13	20.00	66.13			
Jan. 1990	88.00	23.36	64.64			
Feb. 1990	89.60	9.10	80.50			
March 1990	97.00	20.00	77.00	8.8	1.0	3.00

TABLE 4.11 TANNIN ANALYSES OF MONTHLY COLLECTIONS OF EXUDATE OF E. CITRIODORA EXTRACTED IN WATER

	% Soluble	% Non-tans	% Tans	Colour		pH
				Yellow	Red	
Jan. 1993	71.6	21.0	50.6			
Feb. 1993	73.6	21.6	52.0			
Mar. 1993	68.8	16.8	52.0			
April 1993	76.8	15.8	61.0			
May 1993	68.7	15.1	53.6			
June 1993	64.8	15.4	49.4			
July 1993	62.5	16.2	46.3			
Aug. 1993	58.0	13.0	45.0			
Sept. 1993	58.4	13.0	45.4			
Oct. 1993	57.4	13.2	44.2			
Nov. 1993	57.6	13.3	44.3			
Dec. 1993	68.8	15.0	53.8	8.8	1.0	2.8 3.1

The result showed that the exudate yields between 50 - 63% tannins from the water

extract (average 57.7% tannins) and 64 -80% tannins in ethanol (average 72.1% tannins). This is definitely higher than any known commercial vegetable tannin extract including tannins from the galls (60%) found in China, Japan, Turkey and North Africa however, the tannins from galls do not impart leathery properties to skin but are just a source of tannic acid (Adewoye et al 1986). The % tannins from ethanol extracts are considerably higher than those of the water extracts. This can be explained in two ways. First, the exudate is observed to contain a lot of resin which is poorly soluble in cold water and therefore, traps tannins within the large complex but because resin is soluble in ethanol, the tannins are released which account for the difference. Secondly, it was observed that the exudate polymerised on exposure to air. The larger polymers may not be soluble in water and thereby apparently reducing the quantity of tannins available for extraction. The formation of resin and polymerisation of the exudate giving larger molecules are of advantage in leather manufacture since the large molecules would bridge the skin protein and thereby improving its stability.

The non-tan quantity varies from 12 - 22% and 9 - 23% in water and ethanol extracts, respectively. Similarly, the solubles vary from 62 - 84% and 86 - 97% in water and ethanol extracts, respectively. The variations in the concentration of solubles and non-tans are very important as it is a crucial indicator for the quality and quantity of tannins and hence the level of astringency. Naturally, it is expected that when the quantity of solubles goes up, the non-tan should go down resulting in high concentration of tannins and vice-versa. This is so because the aggregation of non-tans necessarily would give tannins of higher molecular weight while the decomposition of tannins would give substance of smaller molecular weight refers to as non-tans.

If the decrease in the quantity of non-tans is as a result of their conversion to tannins then

this would be reflected by the increase in the quality and quantity of the solubles as suggested by Bate and Smith (1954), and Hills (1955) in their work on the biosynthesis of tannins from leucoanthocyanidins and proanthocyanidins as in the case of E. marginata. This knowledge would serve as a guide to when the optimum quality of tannins would be produced by the Eucalyptus through exudation. If that happens to be the period when maximum exudation occurrs, then it would be advantageous. No work has been done on these precursors in E. citriodora, to be able to establish this fact even though the literature supports the theory.

In Tables 4.9 and 4.10, the amounts of tannins in the month of December 1989 and January 1990, are 60 and 62%, 66 and 64% for water and ethanol extracts, respectively. The results are very close and suggest that the amount of resins available in the extract at this period is low and hence, its fairly good solubility in warm water. The amounts of non-tan and solubles seem to attest to this view also. Since tannage is done in aqueous medium this would mean that the best time for the collection of kino would be at this period when the amount of resin is low.

The ratio of tans to non-tans is averagely between 4:1 and 3:1 for water and ethanol extracts respectively. This confirmed the fact that a large quantity of the solubles are tannins pointing also to the fact that the resin contains a reasonable amount of tannins hence the fairly big difference in tannins content between the water and the ethanol extracts.

This ratio of tans to non-tans is higher than for Bagaruwa 2:1 (Adewoye, 1984) and Parkia Clappertomana 3:1 (Adewoye et al ,1986). It was anticipated that the extract would be astringent and therefore, must be used for tanning under acid condition of pH 3 - 4. This was found to be adequate since the pH of the exudate lies between 2.8 - 3.1. The major fear in this was the possible amount of residual acid in the resultant leather. However, the resultant leather showed

no evidence of acid damage even when kept for over 2 years before physical and chemical analysis. These results are recorded in Tables 4.12 and 4.13. The pH of unfiltered extract, the pH of 10 times dilution and the difference figure are the parameters of signifance in ascertaining the quality of residual acids. These paramaters were found to be within the acceptable standard ranges. It is important to note here also that the rate of penetration at this pH was very good giving very full product with very good leathery properties as seen in Tables 4.12 and 4.13.

Table 4. II recorded a one year screening of the quantity and quality of the tannins of the exudate in water extract. The water extract is selected because tannage is done principally in water medium. The aims of this analysis are;

i) to determine the period of maximum exudation and its influence on the quality and quantity of tannins.

ii) to determine the period of maximum quality and quantity of tannins.

iii) to infer, where possible, what may be the cause of the large quantity and high quality exudation at the period and its various components.

From Table 4.11, it was observed that the highest % tannins in the exudate was in the month of April (61%) followed by May (58%) while the lowest amount of tannins were recorded in the months of October and November, both at 44%. Also, it is important to note that tannin concentrations increased from the month of January (45%) to April (61%) a feature noted earlier on in Tables 4.9 and 4.10. The sharp increase in the amount of tannins in the exudate of December is also worth noting as this agreed with earlier analysis as seen in Table 4.9.

The period from December to February is usually the cold, dry harmattan season while March to April is the dry hot period of the dry season which sometimes may include May in our

particular location. The period lying between June and October is the rainy season which is the growth period for most plants.

It was observed that the period when tannin contents of the exudate was highest coincided with the very dry and hot season preceeding the rains and very dry cold harmattan period. Both of these are very harsh climatic conditions and gumosis or exudation is said to be a response to harsh climatic conditions (Otegbeye, 1990). The period June to October when the tannin contents are lower coincided with good climatic conditions favourable for growth. It was observed that even though the lowest tannin content was obtained at this period the quantity of the exudate was highest. It was noted also that this period was dominated by frequent injuries to the stem of the plant orchestrated by cracks which is said to be as a result of lateral growth of the stem (Otegbeye 1990). Gumosis was necessary therefore, to assist in healing. The large straws or fibre-like resinous materials contain less tannins but better covering properties.

Consequently, it is reasonable to recommend that the best collection period for the exudate of E. citriodora is between the months of December through May which is usually the dry season.

PART II

4.4 Leather Tanning Trials

Preliminary investigations into the use of the kino of E.citriodora reported that good leathery properties can be obtained with as low as 20% of the exudate. It also showed that tanning pH between 3 - 4 would give reasonably good rate of penetration (Dashe and Shok, 1993). Following these results pelts were prepared to pH 2 then depickled to 3.5 before tannage in comparison with those pickled to pH 3.5 before tannage without depickling. It was observed

that those pickled to pH 3.5 and were tanned direct with the exudate showed greater and better rate of penetration without shrunken grain. This decision was arrived at by careful study of the grain of the two sets of leathers being processed. If the uptake of the tannins is very fast at the surface, there would be wrinkles on the grain, if not then the grain would be smooth. This means also that the pH difference between tannins and protein is not wide which was the aim of pickling to adjust the pH of the protein to be as close as possible to that of the tannins so as to reduce the rate of reaction on the surface but encourage gradual penetration of the tannins into the skin. The leathers so produced, were analysed for physical and chemical properties and the results are recorded in Tables 4.2 and 4.13

4.4.1. Leather Analysis-Chemical Properties

The leathers produced from the kino of E. citriodora and the combination tannage are all meant for shoe uppers. The analysis of the leathers showed that both meet minimum requirements of shoe uppers. However, some specific mention must be made of the following:

TABLE 4.12 LEATHER ANALYSIS - CHEMICAL PROPERTIES

	Standard	Full E. citriodora	Semi-chrome
Volatile matter	8 - 12	8.46	7.96
Nitrogen content	10-10.57	10.14	9.94
Hide substance	56 - 65	56.20	55.90
Water Soluble organic substances	6	4.75	8.11
Substance Extractable by Dichloromethane	18 - 26	4.57	4.51
Sulphated Ash	1 - 5	0.56	2.52
Degree of Tannage	50 (Min)	39.50	34.00
pH of unfiltered Extract		3.10	3.00
pH of 10 x dilution	below 4.0	3.70	3.60
Difference Figure	Not above 0.7	0.60	0.60

TABLE V: LEATHER ANALYSIS - PHYSICAL PROPERTIES

	Standard	Full E. citriodora
Tensile strength // To back bone	250 NM^2 (Max)	118.13 NM^2
% Elongation	70 NM^2 (Max)	93.0
Tensile strength ⊥ To back bone	250 NM^2 (Max)	93.9 NM^2
% Elongation	70 NM^2 (Max)	52.9 NM^2
Tear strength	40 NM^2	28.13 NM^2
Lastometer test		
Load at crack	250 NM^2	250.0 NM^2
Distention at crack	6.7 mm	6.7 mm
Water proofness		
Absorption after 2 hrs	35 mm (max)	13.0 mm
Water penetration after 2 hrs	45 (max) gm	42.7 gm
Shrinkage temperature	60 - 70 °C	58.0°C

4.4.1.1 Degree of Tannage

For vegetable tanned shoe upper leather, the minimum degree of tannage is 50% while for lining it is 40% (BASF, 1988). While the result for the kino is approximately 40, the full vegetable and combination tanned are too thick or too full for lining. The physical properties as shown in Table V indicate that the leather can be very good for shoe upper as shown by the tensile strength and elongation at break and supported by the lastometer test. Why the degree of tannage is that low cannot be explained presently since other tests disagree with this result.

4.4.1.2. Sulphated Ash

The results for the full vegetable is very good but for combination tannage the increase can be attributed to the chromium oxide incorporated. The difference is not quite significant since the quantity of salt did not affect the difference figure negatively which is 0.60 for both. The

difference figure gives an indication of the level of acidity in the leather.

4.4.1.3 Substances Extractable by Dichloromethane

Shoe upper leathers are supposed to be springy with a fairly good level of flexibility. They should not possess high quantity of oil therefore, as this would make them too softs and also poor in adhesion as a result of greasy surface. The result of 4.5% for the two leathers showed a good level of fatliquoring as this agrees with the maximum oil 4 - 26% (BASF, 1988, UNIDO, 1986, Adewoye, 1987). The resultant leather is soft and flexible but not greasy and it is quite springy.

4.4.1.4 Water Soluble Organic Substance

These are very important because they serve as buffers and assist the resultant leather to be resistant to atmospheric conditions of various levels of acidity and alkalinity. They are therefore, the neutral salts and organic acids in the tannin extracts either added or natural. The results of 4.75 for full vegetable tanned leather is comparable with the standard of 6.0 (SLTC 1996) but combination tannage was a little higher than the standard of 3.0 (SLTC 1996). Again, this is not quite significant as it did not show much influence on the difference figure. The explanation that can be advanced for this marginal increase is the influence of chromium salt used which can be lowered thereby reducing the quantity of chromium oxide offered or maintain the quality of chrome offered but improve on the level of exhauation with proper washing after tannage and neutralisation.

4.4.1.5. Difference Figure

This is the difference between the pH of solution and that of its ten fold dilution. It is a very critical parameter for the determination of the level of acidity in the resultant leather. It gives an idea of the shelf life of the resultant leather. If it is high then the acidity level would be high and vice versa. Consequently, it means that the leather can be destroyed by the amount of acidity it contains. The result showed that both leathers are quite safe with a difference figure of 0.60. The maximum standard acceptable is 0.70 (SLTC 1996, UNIDO 1986). These leathers have been observed for 3 years and have maintained their good form and nature showing no observable deterioration.

4.4.2. Leather Analysis - Physical Properties

4.4.2.1 Tensile Strength and % Elongation

The result of the tensile strength of $93.9NM^2$ and $118.13M^2$ (// and $\perp$) as recorded in Table 4.13 is satisfactory even though it was observed to be slightly lower than the standards. However, % elongation was quite good as it is close to the standard of 70 . This is important because a leather that is too stretchy would collapse after it has been built into a shoe because of poor shape retention.

4.4.2.2 Stitch Tear Strength or Tear Strength

This was observed to be poor. This cannot be attributed to high acidity in the leather nor to poor hide substance since the Difference Figure showed that the level of acidity in the leather was good while the hide substance was also good as recorded in Table 4.12. What may be responsible

for this poor results is not immediately known. This poor result has some implications in the use of this leather for the production of shoes and other leathergoods. This means that the leather would tear at the stitching point under pressure or when in use. However when a pair of shoes was made out of this it didn't exhibit this poor characteristics nor tear.

4.4.2.3. Grain Distention (Lastometer)

This was found to be approximately 7 mm which agrees with the standard. However, this result does not seem to agree with the Tear Test which was observed to be poor. Both are supposed to give similar indications as they both test the strength of the grain.

4.4.2.4. Water Proofness

This was observed to be slightly higher than the standard of 35 as recorded in Table 4.13. However, this is expected since it is full vegetable tannage. This also gives an indication of how much tannins have been taken up as the tannins absorb water.

4.4.2.5 Shrinkage Temperature (Ts)

For vegetable tanned leathers, the Ts is always around 70°C. The kino of E. citriodora produced a leather of Ts of approximately 60 C. This is not quite good. Ts is directly proportional to the degree of cross-linking in the leather. What was expected because of the seemingly large quantity of the resin in the kino and the correspondingly large quantity of tannins in the resin was that there would be large cross-linking and good filling up resulting into full leather. There appears to be good filling up because the resultant leather was quite full,

unfortunately, this did not result in a good Ts. Generally, it is known that hydrolysable tannins produce leathers with lower Ts than condensed tannins. Thus the low Ts is still in line with the characteristics of hydrolysable tannins of E. citriodora containg a high % of these.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 SUMMARY

The Eucalyptus is a genus of plants of the family Myrtaceae. It is a unique genus because all the members have their origin in Australia and the surrounding islands. It has, however, been possible to grow many members in many temperate and tropical regions including Nigeria.

In Australia where they originate, many species have been used for tanning hides and skins. The bark has been the main source of vegetable tannins, but the heartwood and the leaves also contain tannins. Another interesting source of tannins is the exudate that is formed by some of the plants which is called "Kino". Although this exudate is called gum, it has been shown that it is chemically unlike the true gums which are essentially carbohydrate substances. The kino has also found good use in tanning particularly in Australia.

It is important to note that it is not all the species of the genus that produce "kino" Eucalyptus citriodora, for example, does not produce "kino" in Australia. It is interesting to note, however, that, the same plant, exudes "kino" in Nigeria. Climatic changes have been suggested as the cause of this phenomenon (Otegbeye and Ezenwa, 1990). This exudate was brought to our notice and samples of it used for tanning trials. The initiated tanning trials developed enough interest for further tanning studies and chemical investigations. These investigations have revealed that the exudate is a rich source of hydrolysable tannins (gallotannins and ellagitannins). Qualitative tests with $FeCl_3$, bromine water, gelatin etc helped in establishing these properties. These were further reinforced by the use of IR, paper chromatography and HPLC .

Quantitative analyses of the exudate showed a high percentage of tannins (above 40%)

which compare with all known commercial sources of tannins. Subsequent tanning trials and leather analyses (both physical and chemical) showed that leather of good properties was produced.

The properties being referred to is fullness of leather shrinkage temperature 58°C, load at crack 250 NM^{-2}, distention at crack 6.7, waterproofness 42.7gm all of which compared favourably with the standard. The pH of unfiltered extract, pH of 10 x dilution and difference figure which are very critical to the shelf life of the leather are also comparable with the standard. (Table 4.12 and 4.13). Though the degree of tannage was found to be low, these findings are not in agreement with the physical properties exhibited by the leather, however this may be as a result of the large quantities of gallotannins in the exudate.

The tannins can thus be used for the production of shoe upper, lining, insoles and book-binding leathers, which require high quality leathers that do not oxidise easily a property that has been found to be very good in the exudate. Again this property may be as a result of the large quantity of gallotannins in the exudate. Thus the tannins can be used for full vegetable tannage or combination tannage producing full and flexible leathers with good chemical and physical properties. It has a very good advantage over Acacia nilotica in that it has very stable colour, that is, the resultant leather does not darken on exposure. The tannin extracts also do not darken on exposure nor disintegrate easily on standing. These are very important characteristics observed in E citriodora which are worth noting. The condensed tannins oxidise readily on exposure and this phenomenon is consistantly with the catechin group. The hydrolysable tannins do not oxidise so readily on exposure. The extract from the E. citriodora was stable to atmospheric oxidation characteristic of similar extracts known to contain gallic and ellagic acids

of protons since their major constitutent is gallic or ellagic acid. It is reasonable therefore to think that the exudate of E citriodara would be mostly gallic and/or ellagic acids or other similar components that do not oxidise easily.

5.2 Conclusion:

The exotic plant, Eucalyptus citriodora produces an exudate "kino" throughout the year. The quantities concide with seasonal climatic changes. These are the onset of the harmattan and the onset of the rains. The "kino" has a high percentage of tannins, about 40%. The tannins have been shown to be mainly gallotannins with traces of catechin tannins. Both tannin analyses of the exudate and physical and chemical analyses of the resulting leather support this conclusion.

Finally, the leather produced is of high quality as attested to by physical appearance and physical and chemical tests. All these suggest very strongly that the plant should be cultivated extensively in plantations so that the "kino" may be harvested for the tanning industry. This will serve as an additional economic use of the Eucalyptus plants grown in Nigeria.

5.3 Recommendation:

1. The use of Eucalyptus citriodora "kino" in vegetable tanning should be encouraged.
2. Plantations of the plant should be established where it grows best.
3. The tanning processes should also be done to obtain the optimum parameters for use in the industries.

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		NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
COLLECTION	DATA	27122738	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\	INJECTION	12:27:38	27 JAN 1995
	METHOD	TANI					C:\GOLD\SYS1METH\ABUJAMTH\	ANALYSIS	12:38:22	27 JAN 1995
								REPORT	10:52:57	22 JUN 1995

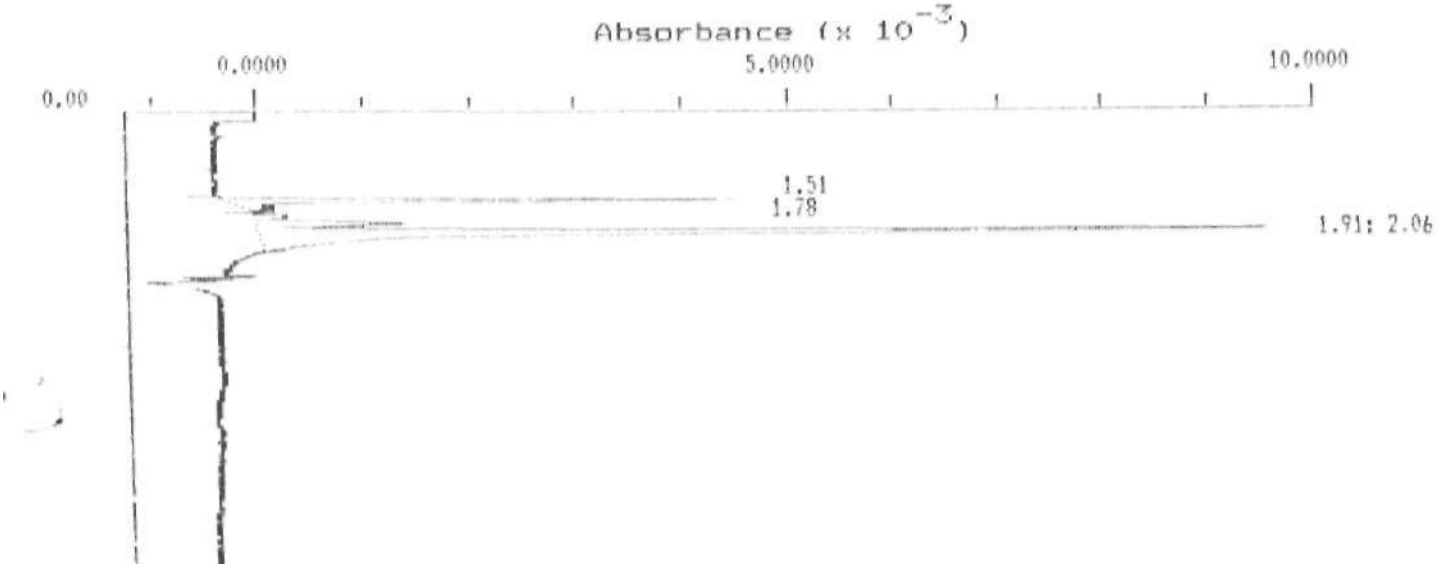
SAMPLE TABLE none
SYSTEM 1: SYSTEM1

Analyst Bola
Comments Analysis of Tannic acid std.
Moble phase:Methanol :Water (20:80).
Flow rate: 1ml/min.
Con. 200um/ml.
Injection: 25ul.

Type	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL	
Unk	27122738			1.00000	1 / 1			(from File)

Peak Number	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	1.508		0.25213	0.00477	BCB	0.0000	0.0000	18.913	29.885
2	1.784		0.02671	0.00031	BCV	0.0000	0.0000	2.004	1.916
3	1.910		0.11750	0.00141	VCV	0.0000	0.0000	8.764	8.820
4	2.058		0.93477	0.00949	VCB	0.0000	0.0000	70.119	59.379
TOTALS			1.33311	0.01598				100.000	100.000

Average Efficiency: 0



APPENDIX II

		NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
COLLECTION	DATA	24105420	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\	INJECTION	10:54:19	24 APR 1995
	METHOD	GALLI					C:\GOLD\SYS1METH\ABUJAMTH\	ANALYSIS	10:59:49	24 APR 1995
								REPORT	10:59:56	24 APR 1995

SAMPLE TABLE none
SYSTEM 1: SYSTEM1

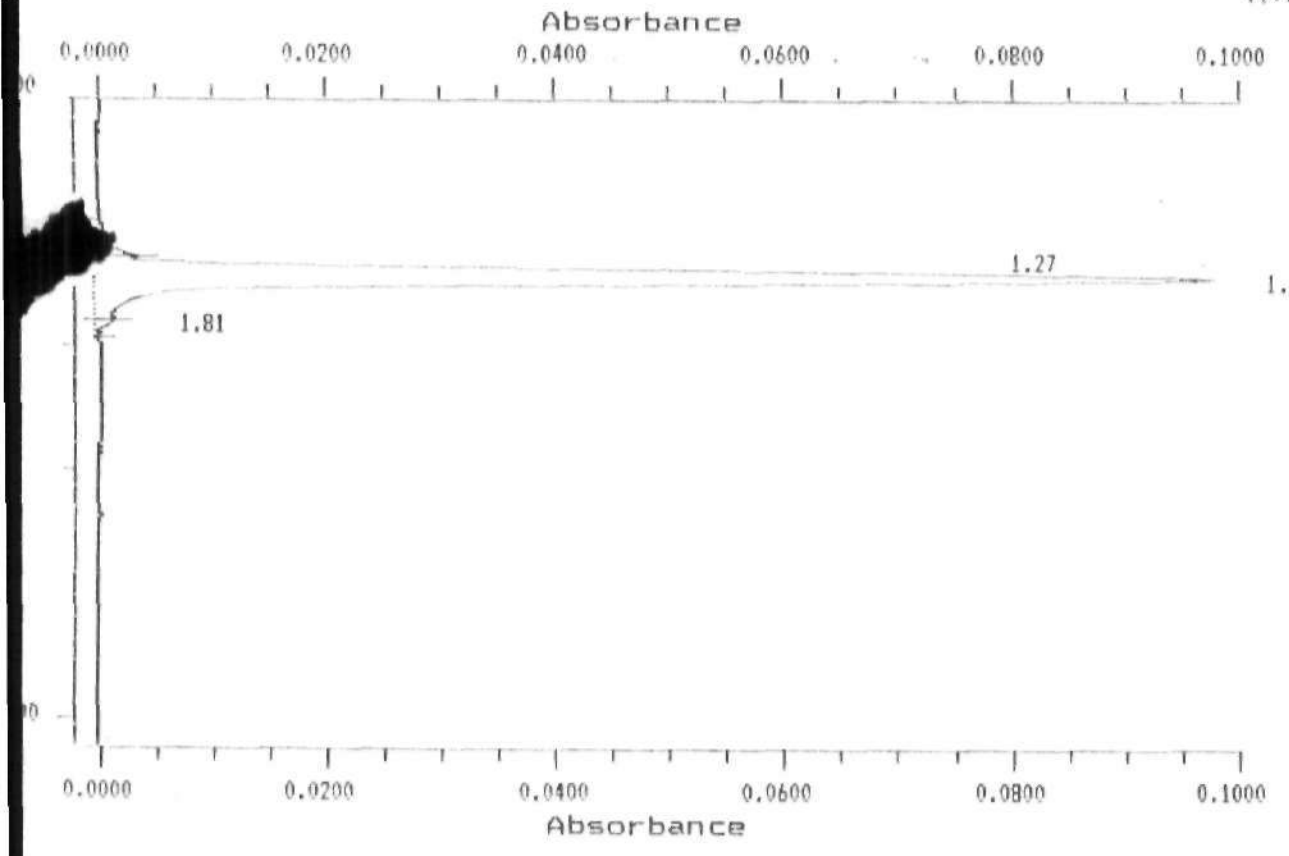
Analyst Bola
Comments Analysis of Gallic acid std.
Moble phase:Methanol :Water (20:80).
Flow Rate: 1ml/min.
200ug/ml.
25ul.

Type	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL	
Unk	24105420			1.00000	1 / 1			(from File)

Peak Number	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	1.275		0.33924	0.00362	BNS	0.0000	0.0000	2.368	3.512
2	1.450		13.89558	0.09817	SCS	0.0000	0.0000	96.975	95.150
3	1.808		0.09411	0.00138	SNB	0.0000	0.0000	0.657	1.338
TOTALS			14.32893	0.10317				100.000	100.000

Average Efficiency: 0

APPENDIX III



	NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
ECTION DATA	24110212	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\	INJECTION	11:02:12	24 APR 1995
METHOD	GALLI					C:\GOLD\SYS1METH\ABUJAMTH\	ANALYSIS	11:08:07	24 APR 1995
							REPORT	11:08:14	24 APR 1995

.ABLE none
1 1: SYSTEM1

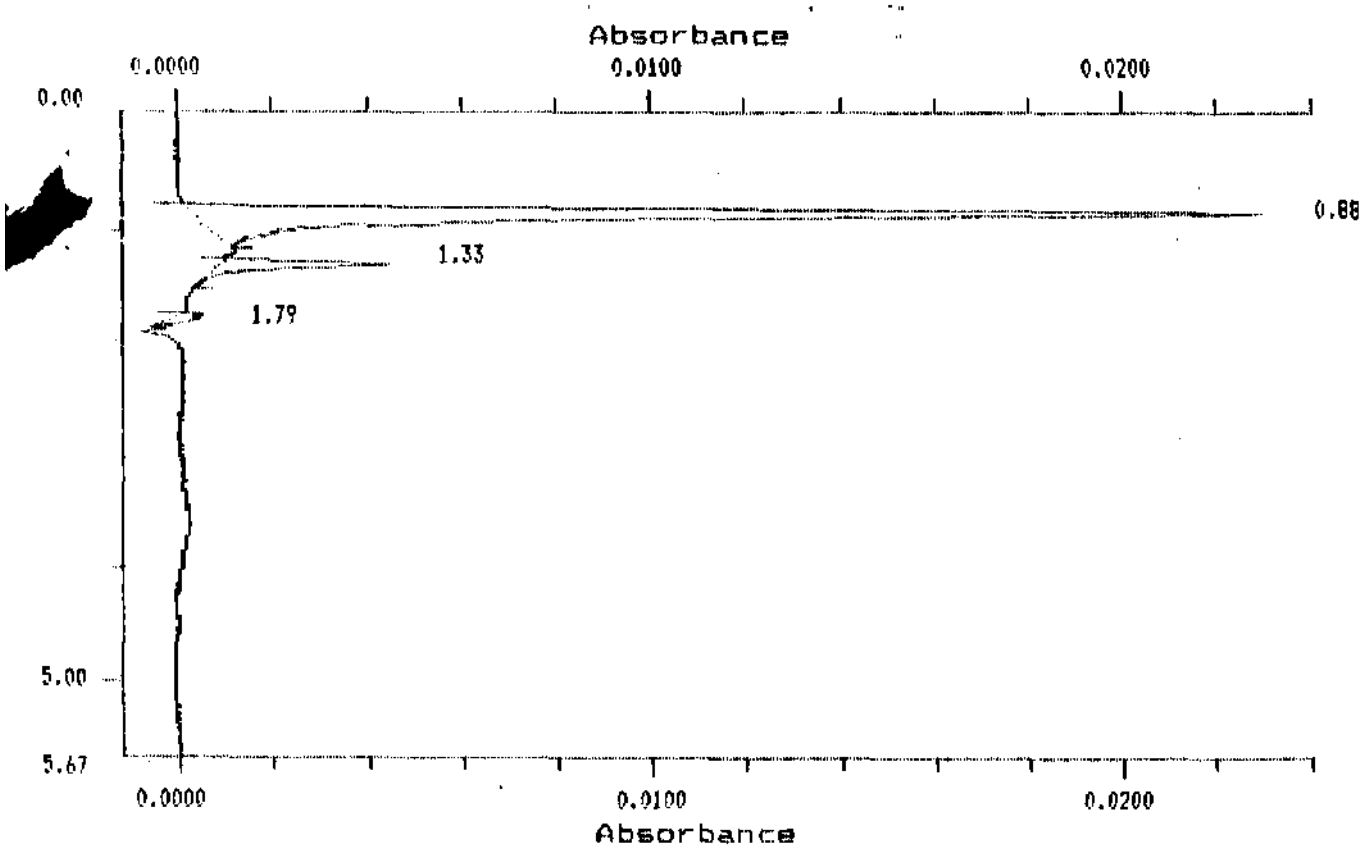
yst Bola
ents Analysis of Tannic acid sample 1
Mobile phase:Methanol :Water (20:80).
Flow Rate: 1ml/min.
200ug/ml.
25ul.

e	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL
	24110212			1.00000	1 / 1		(from File)

k ber	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	0.884		2.07775	0.02260	BCB	0.0000	0.0000	86.006	84.551
2	1.327		0.28700	0.00358	BCB	0.0000	0.0000	11.880	13.401
3	1.793		0.05106	0.00055	BCB	0.0000	0.0000	2.114	2.048
ALS			2.41580	0.02673				100.000	100.000

rage Efficiency: 0

APPENDIX IV



	NAME	CHAN	LEV	REP	TYPE	DIRECTORY
ECTION DATA	24111011	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\
METHOD	GALL1					C:\GOLD\SYS1METH\ABUJAMTH\

	TIME	DATE
INJECTION	11:10:11	24 APR 1995
ANALYSIS	11:16:03	24 APR 1995
REPORT	11:16:10	24 APR 1995

SAMPLE TABLE none
SYSTEM 1: SYSTEM1

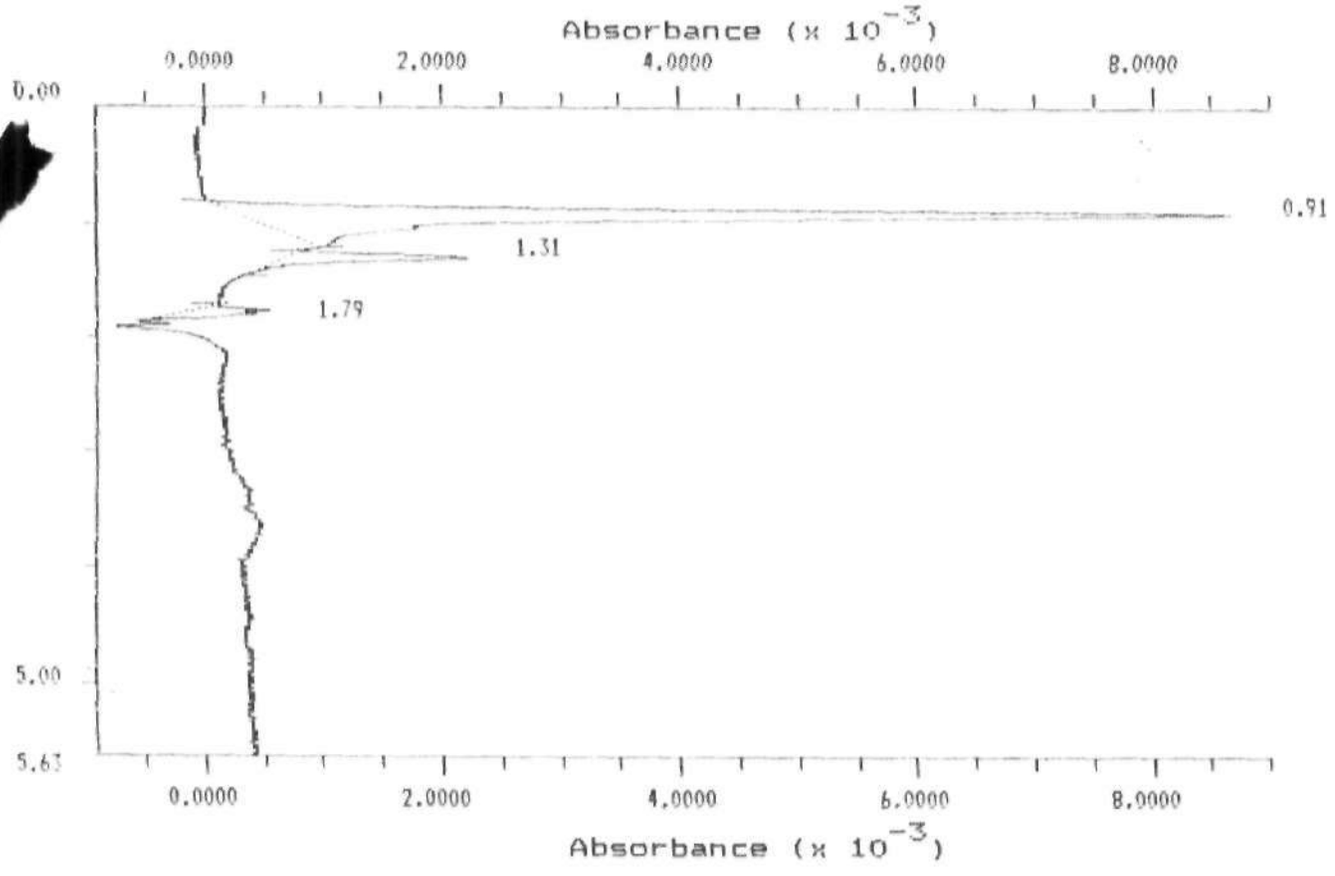
Analyst Bola
Comments Analysis of Tannic acid sample 2
Mobile phase:Methanol :Water (20:80).
Flow Rate: 1ml/min.
200ug/ml.
25ul.

Type	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL	
Unk	24111011			1.00000	1 / 1			(from File)

Peak Number	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	0.911		0.91130	0.00837	BCB	0.0000	0.0000	83.815	78.878
2	1.309		0.11539	0.00157	BCB	0.0000	0.0000	10.612	14.754
3	1.785		0.06059	0.00068	BCB	0.0000	0.0000	5.573	6.368
TOTALS			1.08728	0.01061				100.000	100.000

Average Efficiency: 9

APPENDIX V



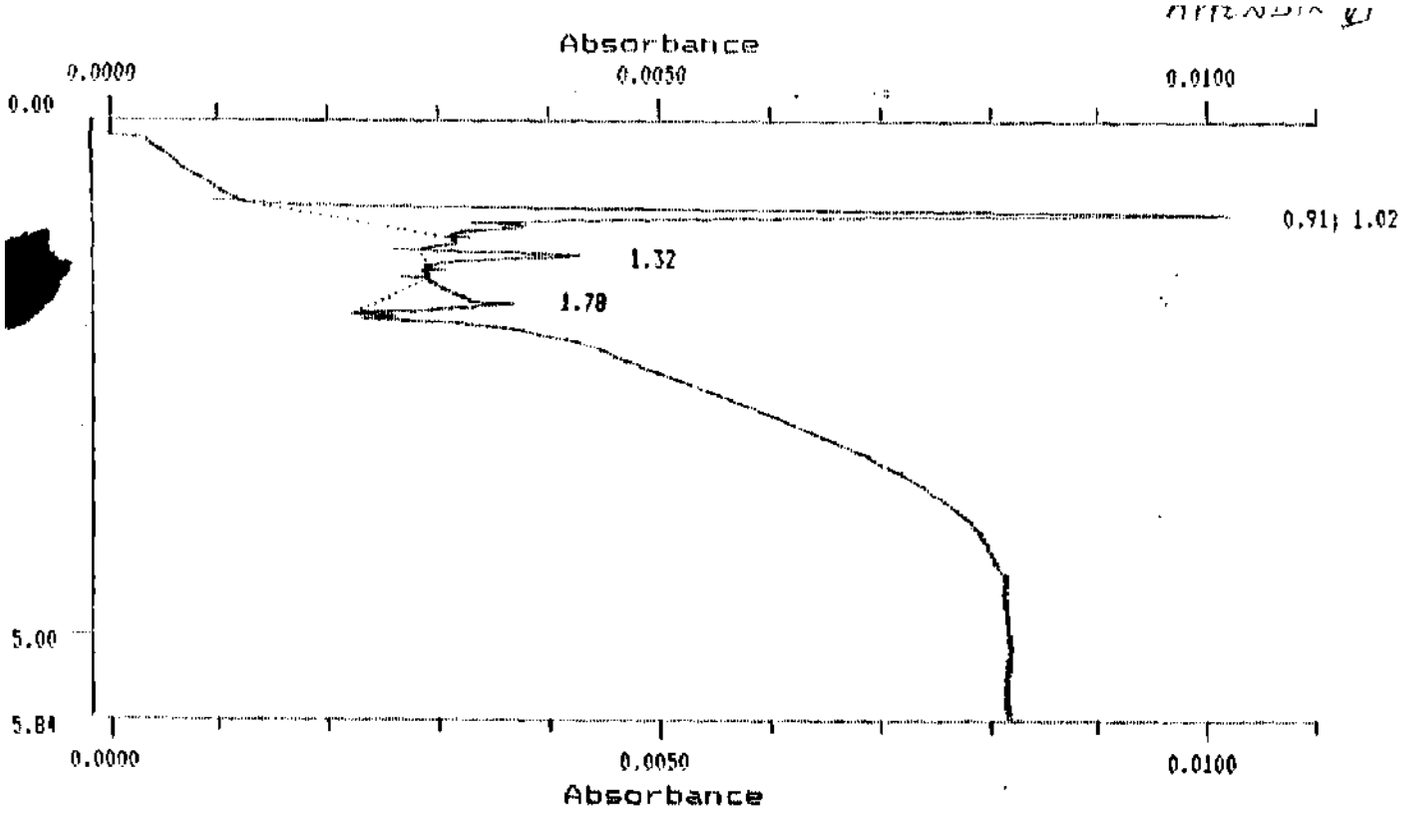
	NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
LECTION DATA	24111720	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\	INJECTION	11:17:20	24 APR 19
METHOD	GALLI					C:\GOLD\SYS1METH\ABUJAMTH\	ANALYSIS	11:22:13	24 APR 19
							REPORT	11:22:20	24 APR 19

SAMPLE TABLE *none*
SYSTEM 1: SYSTEM1

Analyst Bola
Comments Analysis of Tannic acid sample 3
Moble phase:Methanol :Water (20:80).
Flow Rate: 1ml/min.
200ug/ml.
25ul.

Type	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL	
Unk	24111720			1.00000	1 / 1			(from File)

Peak Number	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	0.892		1.14613	0.00947	BCS	0.0000	0.0000	70.950	66.115
2	1.075		0.15227	0.00140	SNV	0.0000	0.0000	9.426	9.765
3	1.300		0.25650	0.00274	VCB	0.0000	0.0000	15.878	19.155
4	1.775		0.06051	0.00071	BCB	0.0000	0.0000	3.746	4.965
TOTALS			1.61540	0.01432				100.000	100.000



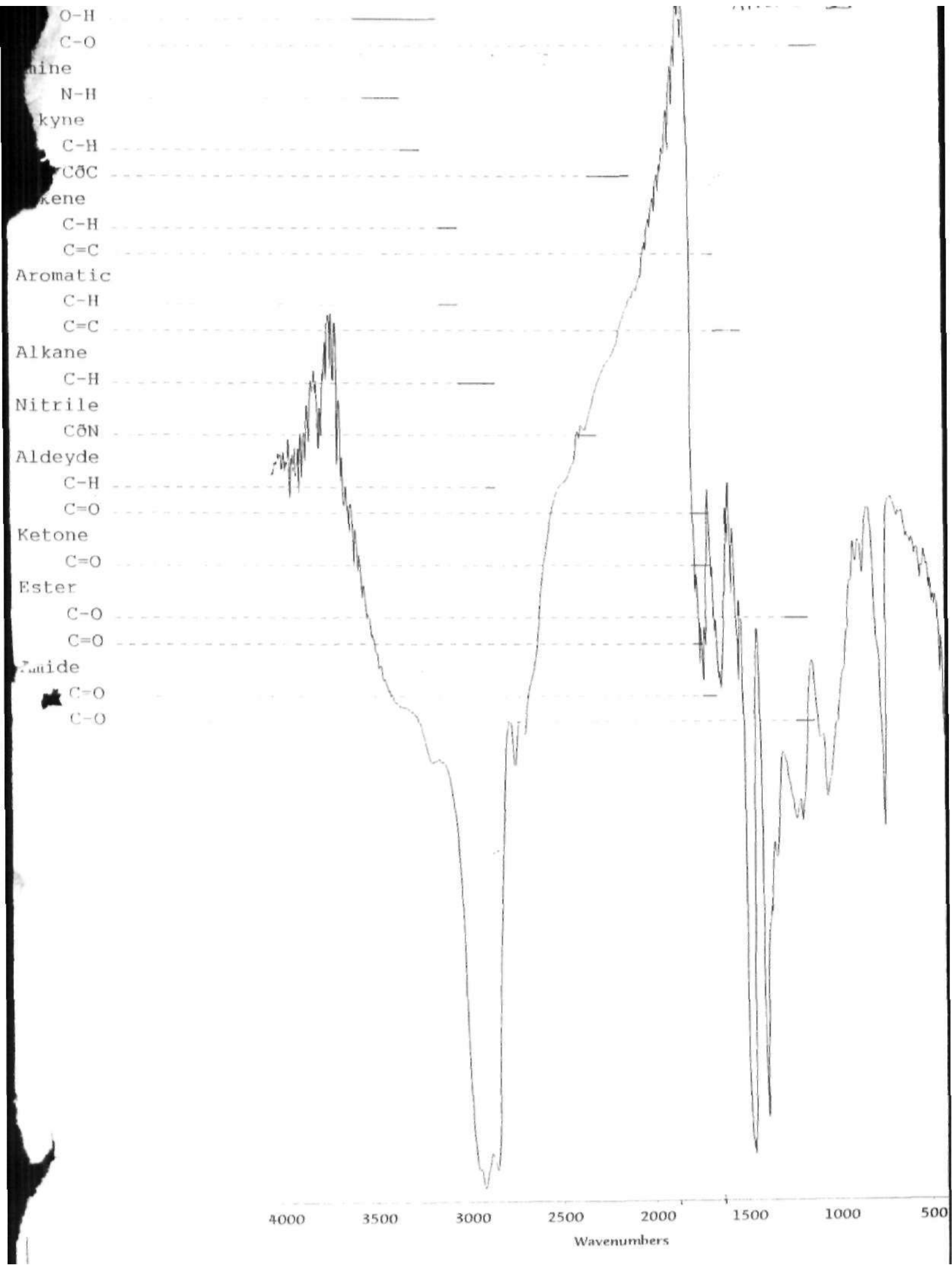
	NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
CTION DATA	24113644	A	1	1	Orig	C:\GOLD\SYS1DATA\ABUJADAT\	INJECTION	11:36:44	24 APR 1995
METHOD	GALLI					C:\GOLD\SYS1METH\ABUJAMTH\	ANALYSIS	11:52:21	24 APR 1995
							REPORT	11:52:29	24 APR 1995

SAMPLE TABLE none
SYSTEM 1: SYSTEM1

Analyst Bola
Comments Analysis of Tannic acid sample 4.
Mobile phase:Methanol :Water (20:80).
Flow Rate: 1ml/min.
200ug/ml.
25ul.

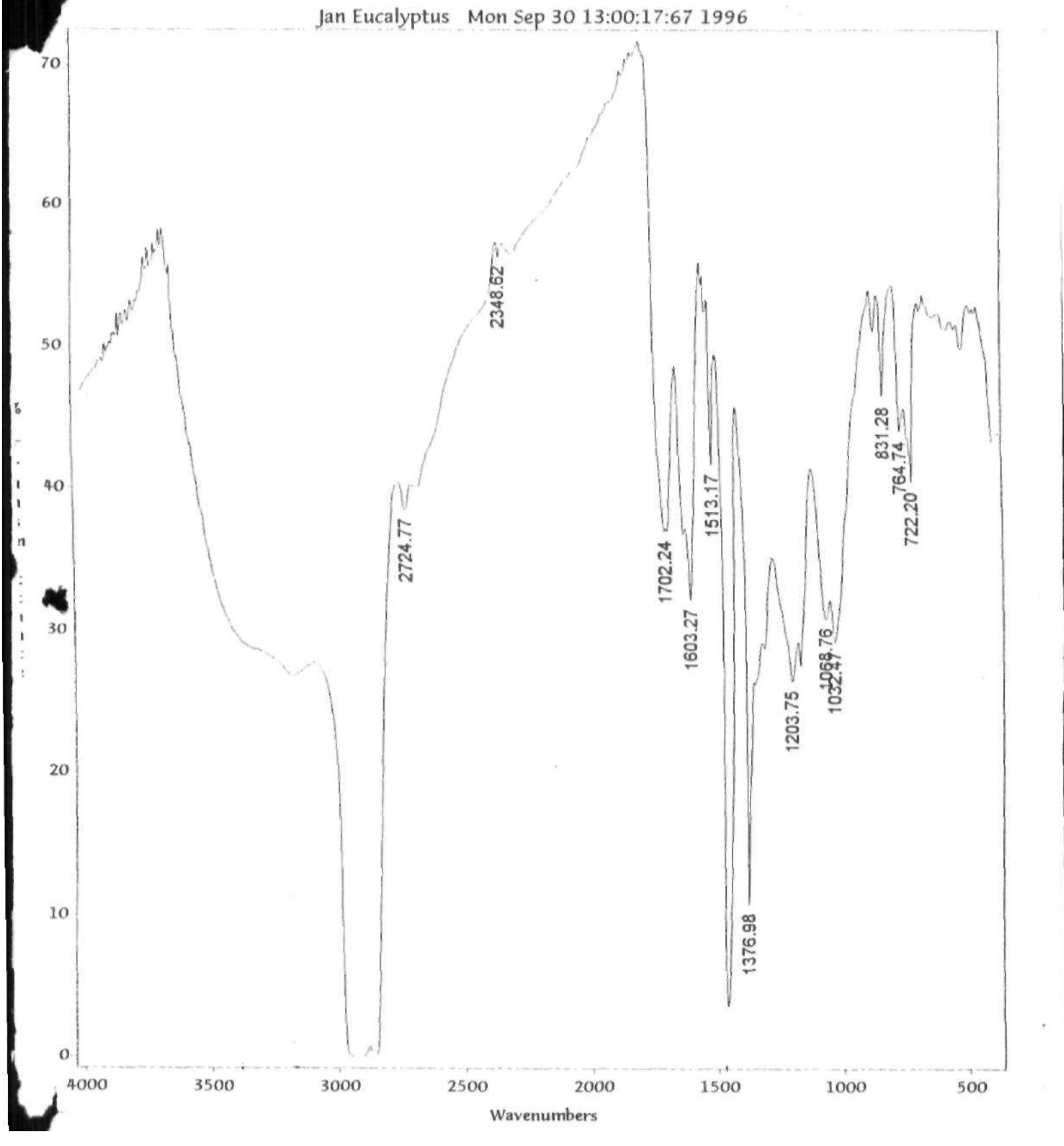
Type	Sample Name	Sample Amount	Int Std Amount	Scale Factor	Nr. Inj	Vial Nr.	Inject Vol uL	
Unk	24113644			1.00000	1 / 1			(from File)

Peak Number	Retention Time	Component Name	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	0.927		0.20940	0.00167	BCV	0.0000	0.0000	31.174	29.087
2	1.051		0.29737	0.00233	VCV	0.0000	0.0000	44.271	40.595
3	1.285		0.16494	0.00174	VCB	0.0000	0.0000	24.555	30.318
TOTALS			0.67171	0.00575				100.000	100.000


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Aromatic
C-H
C=C
Alkane
C-H
Nitrile
CδN
Aldeyde
C-H
C=O
Ketone
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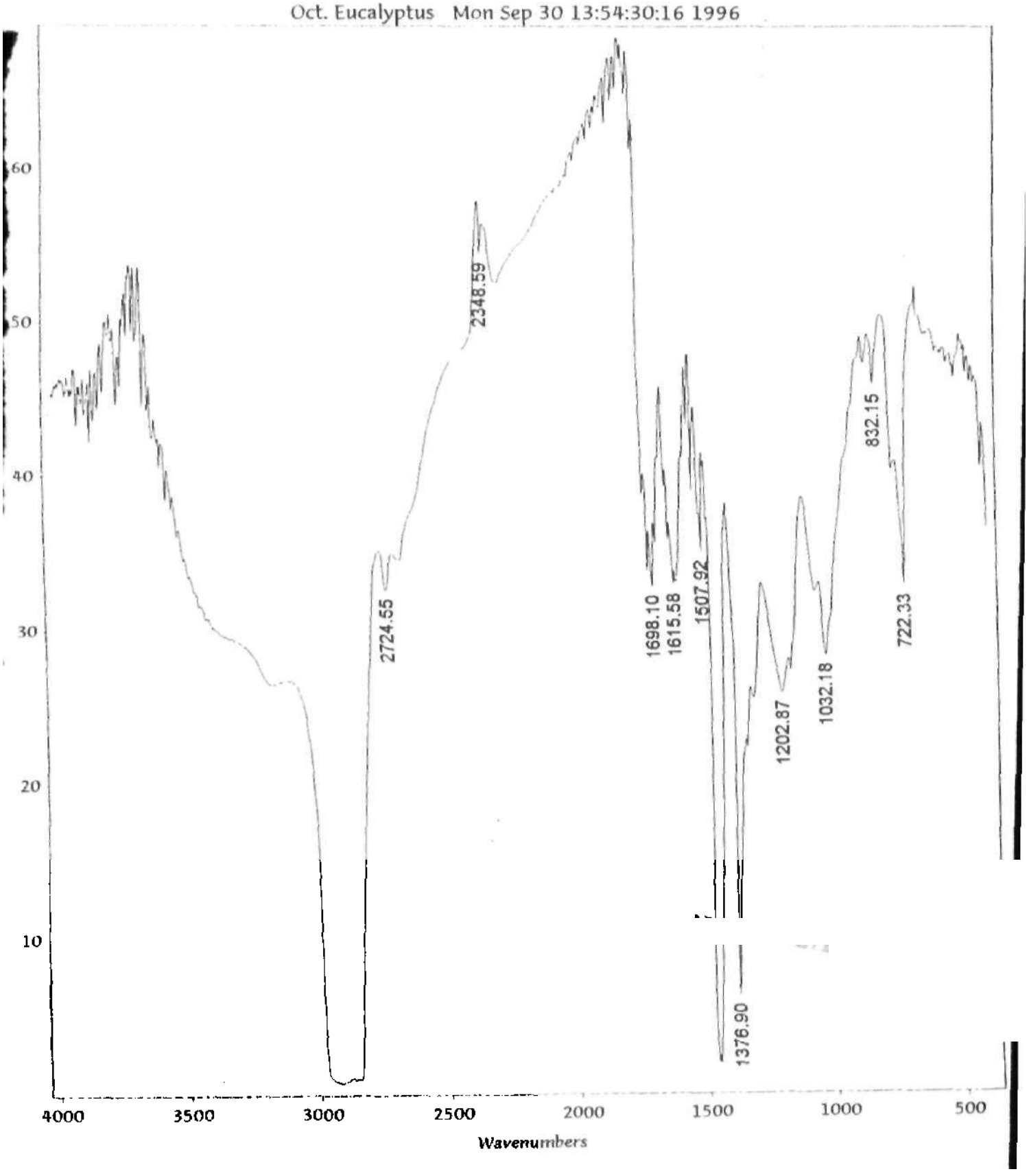
APPENDIX VIII

me: B. Dashe
te: 30/09/96
mple: Jan. Eucalyptus
mments: Nujol



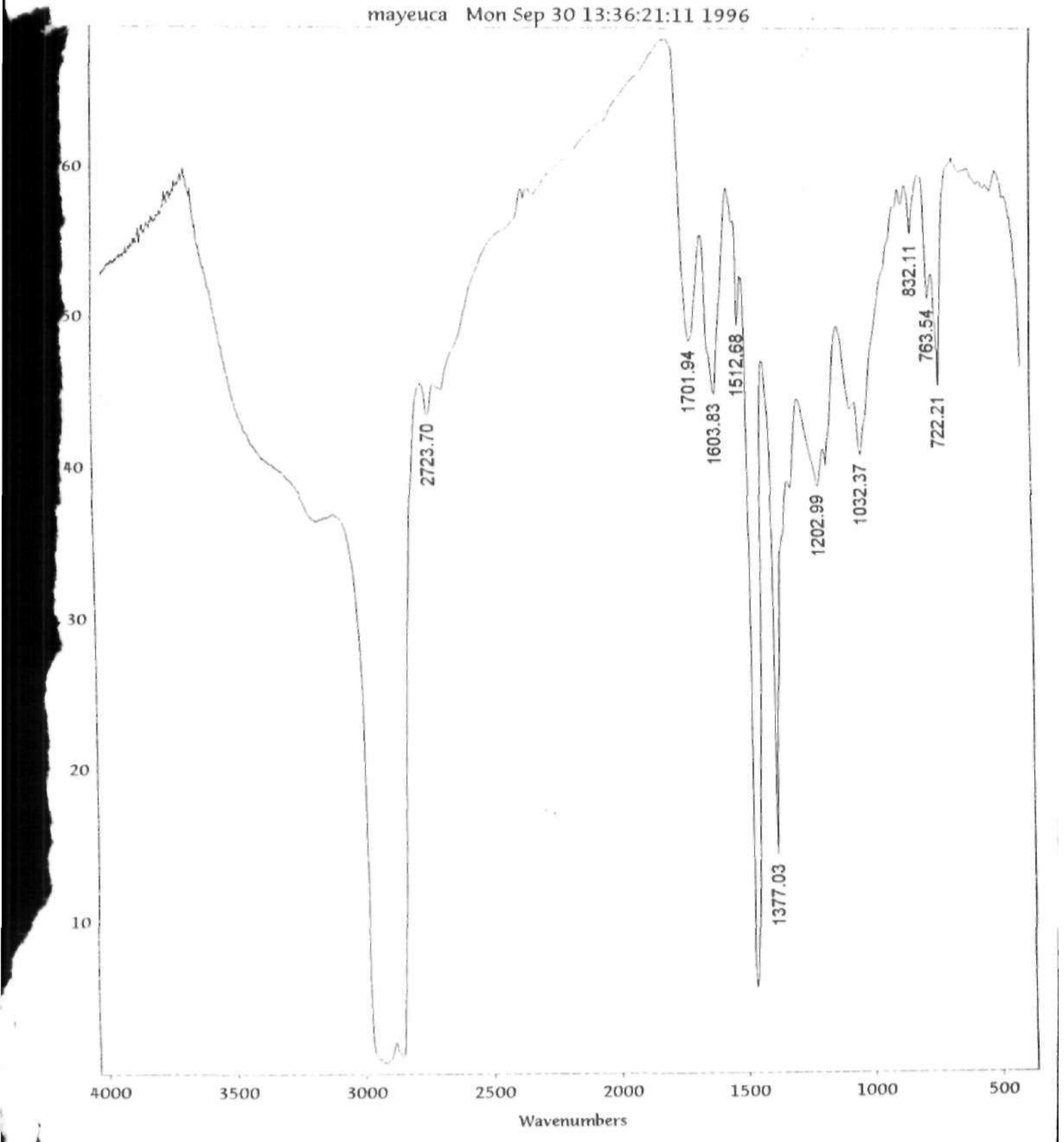
Name: B, Dashe
Date: 30/09/96
Sample: October Eucalyptus
Comments: in Nujol

APPENDIX 81



Name: B. Dashe
Date: 30/09/96
Sample: May Eucalyptus
Comments: In Nujol

APPENDIX X



Name: B. Dashe
Date: 27/09/96
Sample: April Eucalyptus
Comments: Nujol

APPENDIX IX

