Journal of Scientific and Industrial Research
Vol. 41, December 1982, pp. 705-718

346

Vegetable Tannins—A Review

M SANTAPPA & V S SUNDARA RAO
Central Leather Research Institute, Adyar, Madras 600 020

The term 'tannin' denotes the substances which convert the putrefiable hide or skin into imputrescible leather. The tannins are mostly amorphous, astringent in taste and feebly acidic in character. They develop colour with metallic salts and can combine with albumin, gelatin and various alkaloids. Aqueous tannin solutions or infusions known in the trade as 'tan liquors' are colloidal in nature with a wide range of particle size. While Putnam and Gensler[1] and Putnam[2] had erroneously assigned a single structure to the tannin extract, their heterogenic and mixed character was well established by White[3], Kirby *et al.*[4], Hillis[5] and Roux[6] by chromatographic techniques and later corroborated by Hathway[7]. In general, the term 'tannins' includes mixtures of polyphenolic substances that have limited solubility in water and tend to form supersaturated solutions[8]. It is rather difficult to single out any phenolic compound and define it as the respective tannin of the concerned plant material. In view of this complexity in the nature of tannins, the term 'tannin extract' is used. Their molecular weight is in the range 500-3000[7,9-11]. Though Jones *et al.*[12] isolated condensed tannins with molecular weight 7000-8000 and in some exceptional cases even as high as 28000, the molecular weight range of 500-3000[7,9-11] seems to be reasonable, as low molecular weight phenols are too small for effective crosslinking and high molecular weight compounds are either almost insoluble or are too large for crosslinking between suitably oriented polypeptide chains[13].

Tannins occur in most parts of the vegetable kingdom and are more prevalent among the higher plants or angiosperms, specially in certain dicotyledenous families[11,14]. Important tannin-bearing dicotyledenous families are: *Leguminosae*, *Anacardiaceae*, *Rhizophoraceae*, *Myrtaceae*, *Polygonaceae* and *Combretaceae*. Commercially important tanning materials occur in temperate, tropical and sub-tropical countries; tropics, however, produce the major portion[11]. Wattle (*Acacia mearnsii*), quebracho (*Schinopsis lorentzii*), valonea (*Quereus aegilops*, *Q. valonea* and *Q. macrolepsis*), chestnut (*Castanea sativa*), avaram (*Cassia auriculata*), konnam (*C. fistula*), babul (*A. arabica*), myrobalan (*Terminalia chebula*), divi divi (*Caesalpinia coriaria*), etc. are some of the popular tanning materials.

Though tannins are distributed in all parts of the plants, ranging from roots to leaves and fruits, in a particular plant, they are usually concentrated in a specific portion of the plant.

The exact role played by tannins in plants is not yet understood clearly, though it has been suggested that they function as protective agents (due to their astringency), toxic agents (being phenolic) and agents to provide resistance to frost in temperate zones[11]. It is interesting to note that the oldest and largest trees in the world, found in Sequoia National Park, USA, are said to owe their long life to their hardwood and to the protection of their tannin-impregnated bark which is often 3 ft thick. It is estimated that most of these trees are over 3500 years old[15].

It is known that plants synthesize different polyphenolic substances, some of which may contribute to the formation of tannins. Freudenberg's[8] classification of these vegetable tannins, based on their chemical nature and structural characteristics, into (i) hydrolysable tannins and (ii) condensed (or flavonoid) tannins provides a convenient basis for the chemical studies on these vegetable tannins. While the hydrolysable tannins undergo hydrolysis with mineral acids or enzymes, the condensed tannins, which are non-hydrolysable, produce coloured solutions and/or precipitates known as 'phlobaphenes or tannin reds' with these reagents. Besides, tannins based on hydroxystilbenes, e.g. spruce bark (*Picea abies*)[16], are also known. The close metabolic relationship of hydroxystilbenes with the condensed tannins was suggested by Erdtman[17] and Lindstedt and Misiorny[18].

Hydrolysable Tannins

These are based on esters of phenol carboxylic acids (gallic acid and/or hexahydroxydiphenic acid or related acids) with a central carbohydrate core. Depending on the polyphenolic acids that are obtained as products of hydrolysis, these are again sub-divided into: (i) gallotannins and (ii) ellagitannins. Gallotannins yield gallic acid and glucose on hydrolysis, e.g. (a) Chinese gallotannin (plant galls

produced by *Aphis chinensis* on the leaves of *Rhus semialata*, (b) Turkish gallotannin (*Cynips tincotria* galls on twigs of *Q. infectoria*), (c) Sumach gallotannin (leaves of *R. corriaria* and *R. typhina*), (d) Dhava gallotannin (leaves of *Anogeissus latifolia*), (e) Mango gallotannin (seed kernel of *Mangifera indica*), (f) Tara gallotannin (pods of *Caesalpinia spinosa*) and (g) Teri (*Caesalpinia digyna*) pods. The ellagitannins, on the other hand, produce ellagic acid in addition to gallic acid and glucose on hydrolysis, e.g. (a) myrobalan (*Terminalia chebula*) nuts, (b) divi divi (*C. coriaria*) pods, (c) algarobilla (*C. brevifolia*) pods, (d) valonia (*Q. aegilops*) acorn cups, (e) chestnut (*Castanea sativa*) wood, (f) oak (*Q. sessiliflora*) wood, (g) pomegranate (*Punica granatum*) rind, (h) knoppern (*Q. pedunculata*) galls, (i) sal (*Shorea robusta*) seeds, etc.

Gallotannins—Though the chemistry of gallotannins dates back to the eighteenth century, it became the subject of classical researches by a large number of workers, such as Fischer[19], Freudenberg[20] and Karrer[21] on Chinese gallotannin and Turkish gallotannin, Lowe[22] and Karrer[23] on sumach gallotannins and Burton[24] on the tara gallotannin.

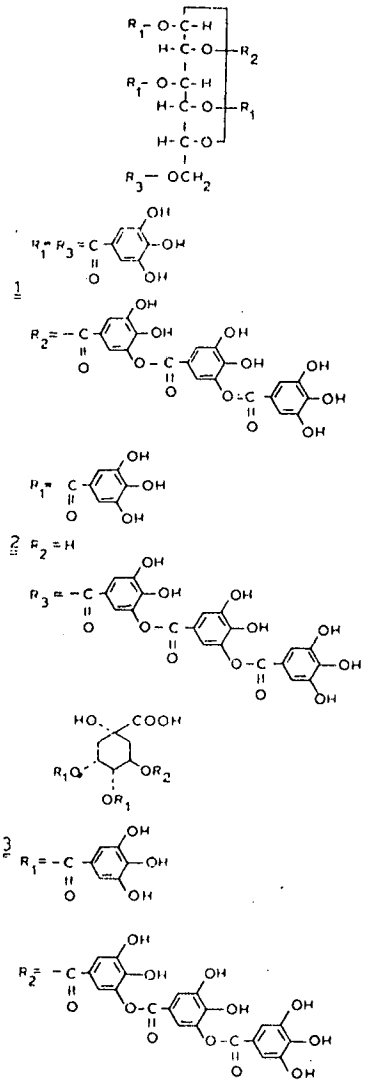
As there were some conflicting ideas about the structures of gallotannins, it was reinvestigated by Haworth and coworkers[25–28]. The methylated gallotannin was hydrolysed and the resulting 3,4-di-O-methyl and 3,4,5-tri-O-methyl gallic acids were analysed for their ratio. The methanolysis of the gallotannin was carried out and the products of methanolysis were analysed at both intermediate and final stages. The amounts of gallic acid and glucose were estimated. Enzymatic degradation studies were carried out on gallotannin using an enzyme (produced by *Aspergillus nigre* on a medium containing tannin) which cleaves the gallotannin into glucose and gallic acid. Based on these studies, together with NMR and mass spectral data, the structure of the Chinese gallotannin was established. The structure of Chinese gallotannin (1) was based on β-penta-O-galloylglucose core to which two gallic acid units are attached depsidically chainwise at position-2 of the glucose.

The structure of Sicilian and Stagshorn sumach gallotannins is similar to that of Chinese gallotannin.

The Turkish gallotannin (2) was shown to be a hexagalloylated glucose based upon a tetra-O-galloyl glucose core in which the second hydroxyl group of the glucose was unesterified; the depsidic linkage in this case was shown to be at C_6 of the glucose.

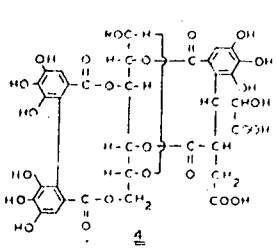
The constitution of the acidic tara gallotannin (3) was found[25,28] to have the average composition of penta-O-galloylquinic acid.

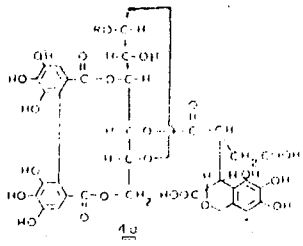
Recently, gallotannins, similar to Chinese gallotannin, have been isolated from dhava leaves[29] and

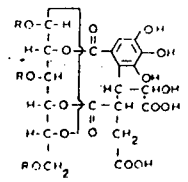


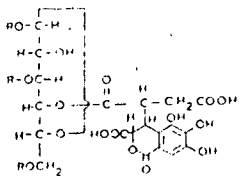
mango seed kernel[30]. However, the dhava gallotanin was found to be an octagalloylated glucose (with one more extra gallic acid compared to the Chinese gallotannin). All the hydroxyl groups in the glucose are esterified by gallic acid and having one depsidic chain containing at least three galloyl groups.

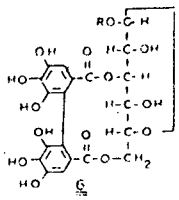
Ellagitannins—The ellagitannins are different from gallotannins in that they deposit on standing/hydrolysis ellagic acid in addition to gallic acid and glucose from their tannin infusions. They show the characteristic phenomenon of the formation of sludge or bloom on leathers. Structure elucidation studies on ellagitannins were carried out by Schmidt and coworkers[31–47]. Constituents present in ellagitannins are: chebulagic acid (4), chebulinic acid (5), corilagin (6), terchebin (7), pentagalloyl glucose (8), 1,3,6-trigalloylglucose (9), 3,6-digalloylglucose (10), β-D-glucogallin (11), chebulic acid (12), isolated from myrobalan nuts and divi divi pods[31–38], brevilagin-1[39] (13), brevilagin-2[40] (14), algarobin[41] (15),

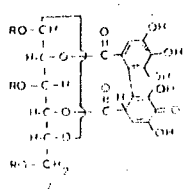

4

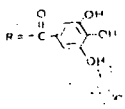

4a

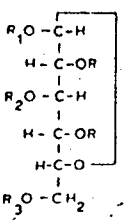

5

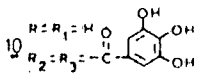

5a

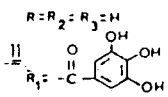

6

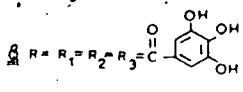


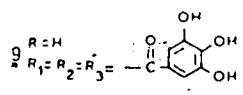


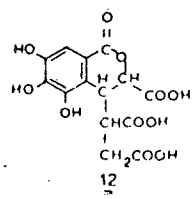


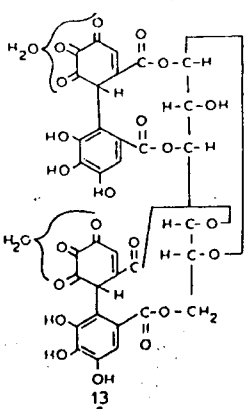

10

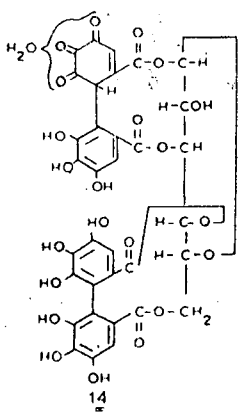

11


8

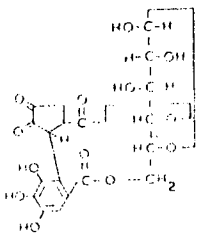

9

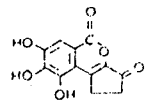

12

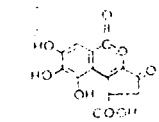

13

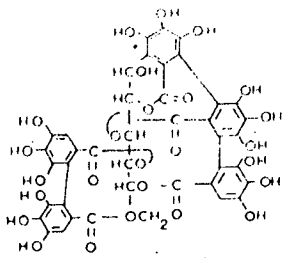

14

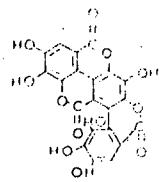
brevifolin (**16**) and brevifolin carboxylic acid[42] (**17**) from algarobilla (*C. brevifolia*), castalagin (**18**), castavaloninic acid (**19**), vescalagin (**20**), vescavaloninic acid (**21**), valolaginic acid (**22**), isovalolaginic acid (**23**), valolinic acid[43] (**24**), valoneaic acid dilactone[44] (**25**) from valonea (*Q. aegilops*, *Q. valonea* and *Q. macrolepsis*), pedunculagin (**26**) from Knoppern galls (*Q. pedunculata*)[45], castalagin (**18**), castalin (**27**), vescalagin (**20**) and vescalin (**28**) from oak wood (*Q. sessiliflora*) and Chestnut wood (*C. sativa*)[46], punicalagin (**29**) and punicalin (**30**) from pomegranate (*Punica granatum*) rind[47].

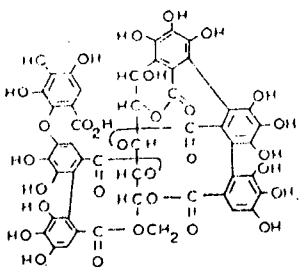

15, Algarobin

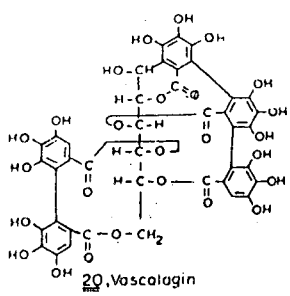

16, Brevifolin

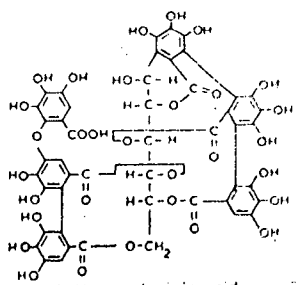

17, Brevifolin carboxylic acid

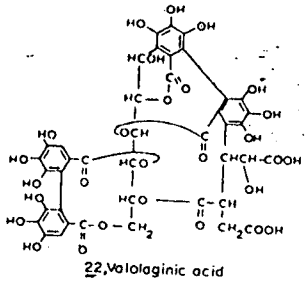

18, Castalagin


18a, Flavogallol

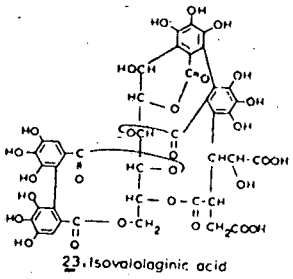

19, Castavaloninic acid


20, Vascalagin

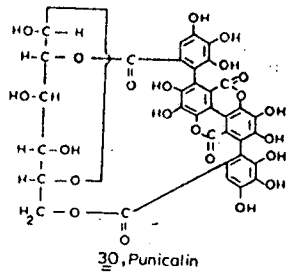

21, Vescavaloninic acid



22, Valolaginic acid

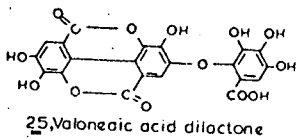


23, Isovalolaginic acid

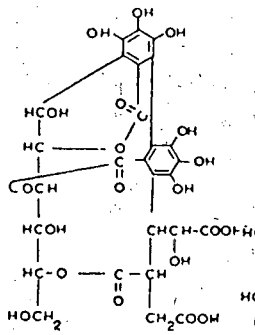


30, Punicalin

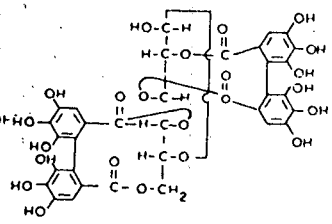
Other members of the hydrolysable tannin group— These include (i) Hamameli tannin, based on α-oxymethyl-D-ribose (31), to which two galloyl groups are attached. This tannin was isolated from witch hazel, *Hamamelis virginiana*[48], chestnut (*C. sativa*) bark[49] and American red oak (*Q. rubra*) bark[50], (ii) acertannin (32), obtained from dried leaves of Korean maple (*Acer tartaricum*) (= *Acer ginnale*)[51,52] and has no value as tannin[53], (iii) gayuba tannin, based on a mixture of penta- and hexagalloyl glucose[54], isolated



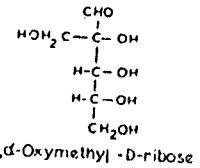
25, Valoneaic acid dilactone



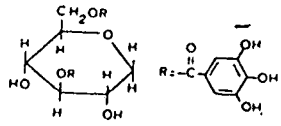
24, Valolinic acid



26, Penduneulagin

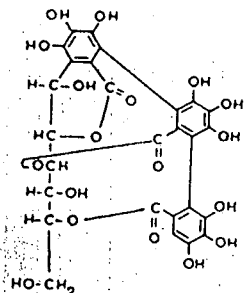


31, α-Oxymethyl -D-ribose

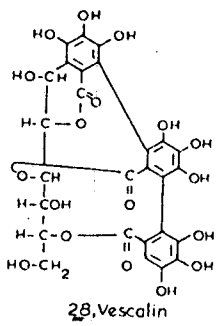


32, Aceritannin

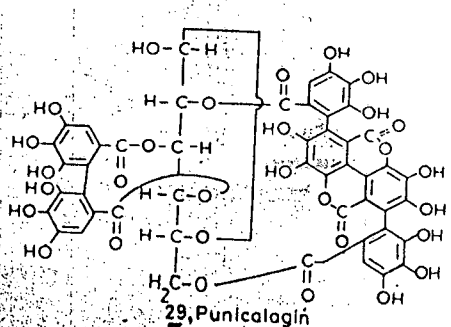
from leaves of *Arctostaphylos uva-ursi*, (iv) geraniin (33), isolated along with corilagin from the bark of *Geranium thembergii*[55], (v) mallotusinic acid (34), which occurs along with geraniin in *Mallotus japonica*[56], (vi) eugeniin (35) isolated from the dried flower buds of *Syzygium aromatium* (syn. *Eugenia*



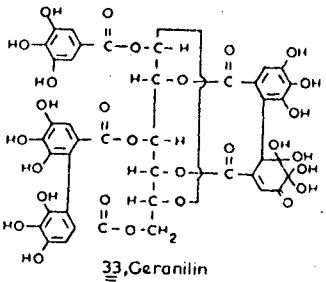
27, Castalin



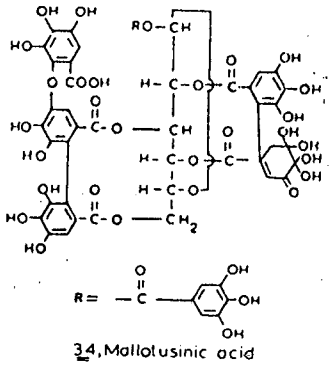
28, Vescalin



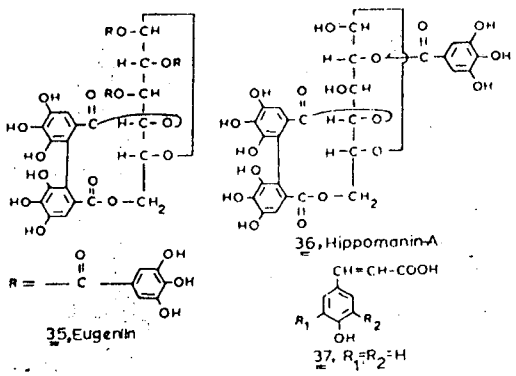
29, Punicalagin



33, Geranilin

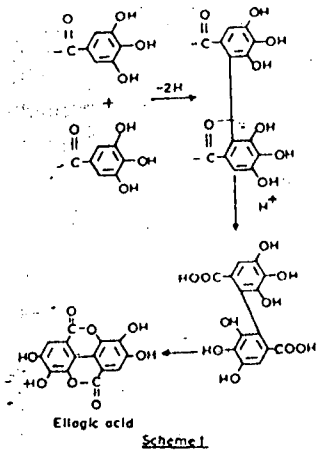


34, Mallotusinic acid



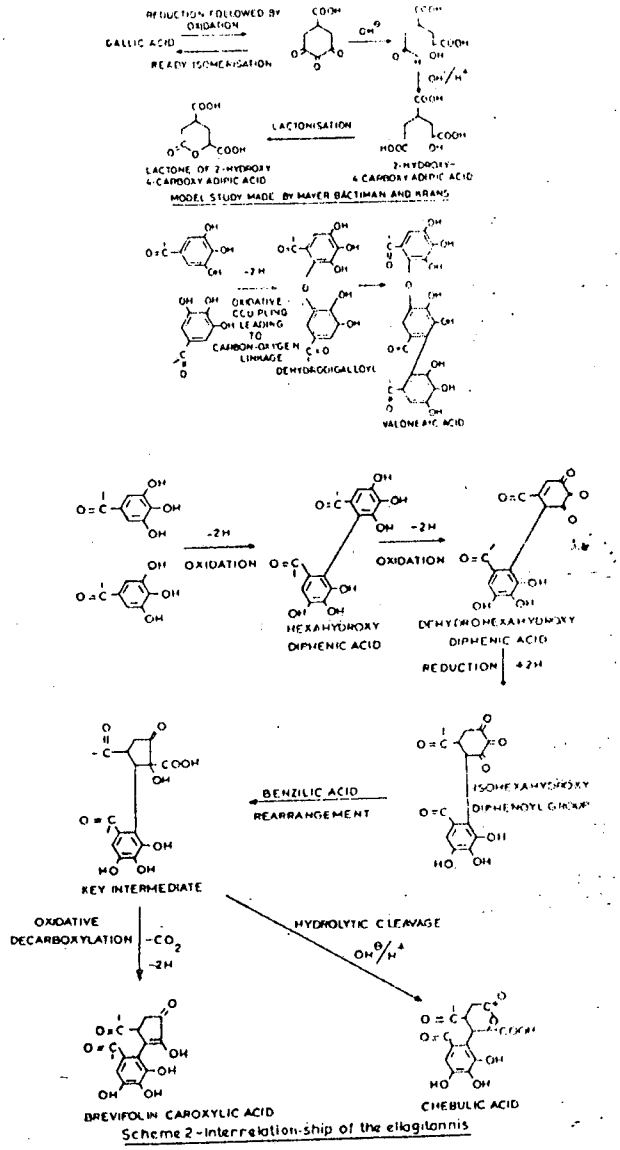
caryophyllata)[57], (vii) jugalanin from walnut skin[58], (viii) hippomanin-A (**36**), isomer of corilagin, isolated from *Hippomano manivella*[59], (ix) derivatives of hydroxycinnamic acids (**37**)[60], etc.

Interrelationship between gallotannins and ellagitannins—The two groups of hydrolysable tannins often occur together in nature and it is very likely that ellagitannins are formed from gallotannins by oxidative coupling of galloyl groups by enzymes, as evidenced from the studies of Hathway[61] and Schmidt[62] in the aerobic oxidation of glucogallin and chebulinic acid at *p*H 7-8, wherein ellagic acid gets formed by the oxidation of galloyl groups (Scheme 1). Raichel and coworkers[63] obtained corilagin by the aerial oxidation of 1,3,6-trigalloyl glucose at *p*H 7.4-8.6.



Scheme 1

Interrelationship between ellagitannin acids—With the elucidation of the structure of chebulic acid (present in chebulinic acid, chebulagic acid, etc. of myrobalan) and brevifolin carboxylic acid in algarobin (one of the tannin constituents of algarobilla), it is now clear that there is a close interrelationship between the ellagitannin acids (Scheme 2).

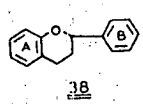


Scheme 2-Interrelation-ship of the ellagitannis

Condensed Tannins

Commercially, condensed tannins are more important from the leather manufacture point of view. Some of the common condensed tanning materials are: Wattle (*Acacia mearnsii*), quebracho (*Schinopsis lorentzii*), gambier (*Uncaria gambir*), spruce (*Picea abies*), cutch (*A. catechu*), hemlock (*Tsuga canadensis*), mangroves (*Rhizophora* species), babul (*A. arabica*), avaram (*Cassia auriculata*), konnam (*C. fistula*), sal (*Shorea robusta*), ghat bor (*Zizyphus xylopyrus*), arjuna (*Terminalia arjuna*) and a host of others.

Structurally related to flavonoids, these tannins are distributed widely in nature and constitute a heterogeneous group. The C_{15} skeleton of the flavonoids is made up of two distinct units, viz. 'A' ring (consisting of a C_6 unit) and 'B' ring (made up of C_6-C_3 unit) (**38**).

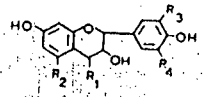


38

Based on the configuration and the state of oxidation of the central C_3 unit in the molecule, the flavonoids are broadly classified into: (i) chalcones and α-hydroxychalcones, (ii) aurones, (iii) flavones and flavonols, (iv) flavanones and flavanonols, (v) flavanols, and (vi) isoflavones. We have also neoflavones, biflavonoids, auronols, lignoflavones, peltogynols, flavonoid sulfates, etc. belonging to this group. In addition, the glycosides of these flavonoids also occur. Of these, the flavanols are the most important compounds from the point of view of vegetable tannins[13,64]. They possess the property of being transformed into the amorphous polymeric tannins by the action of enzymes, mineral acids or even by mere warming in aqueous solutions as well as in the solid state.

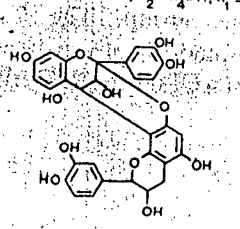
The flavanols are further classified into flavan-3-ols (39), flavan-3,4-diols (40) and proanthocyanidins (41, 42). The term 'proanthocyanidin' was coined by Freudenberg and Weinges[65] to collectively define all the colourless substances isolated from the plants which form anthocyanidin when heated with acid. Weinges *et al.*[66] used the term leucoanthocyanidin (leucoanthocyanidin is the trivial name used to refer to flavan-3, 4-diol) for the monomeric proanthocyanidin and the name "condensed proanthocyanidins" for the various flavan-3-ol dimers and higher oligomers. The proanthocyanidins are supposed to be formed by the oxidative polymerization of flavan-3-ol precursors[67]. The proanthocyanidins, which are dimeric or oligomeric, are further classified into Type A(41), Type B(42), etc., depending upon the type of linkage.

Formation of flavonoid tannins—Bate-Smith's[68,69] studies on the identification of leucoanthocyanidins in a number of dicotyledenous plants using phlobaphene test led to the finding that the red colour produced was

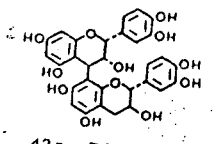


39 FLAVAN-3-OLS (R_1=H)
- a. ROBINETNIDOL, $R_1=R_2=H$; $R_3=R_4=OH$
- b. GALLOCATECHIN, $R_1=H$; $R_2=R_3=R_4=OH$
- c. CATECHIN; $R_1=R_4=H$; $R_2=R_3=OH$

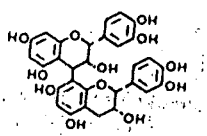
40 FLAVAN-3,4-DIOLS (R_1=OH)
- a. LEUCOROBINETINIDIN; $R_2=H$; $R_1=R_3=R_4=OH$
- b. LEUCOFISETINIDIN, $R_2=R_4=H$; $R_1=R_3=OH$



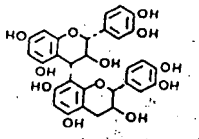
41 Procyanidin A



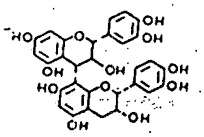
42a B1



42b B2

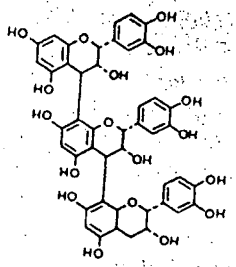


42c B3

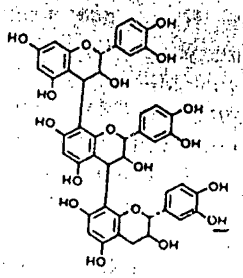


42d B4

Procyanidin Dimers



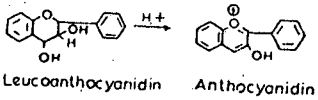
42e C1



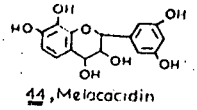
42f C2

Procyanidin Trimers

due to the formation of anthocyanidins from the corresponding leucoanthocyanidins in these extracts (43). This was further indicated by the identification of leucoanthocyanidins in a number of tanniferrous plants like eucalyptus, mangroves, wattle, etc.[70] Hillis[71,72] observed that synthetic flavan-3,4-diol yielded anthocyanidins and, therefore, concluded that eucalyptus tannins are based on flavan-3,4-diols. However, Hillis did not distinguish between the monomeric and polymeric leucoanthocyanidins. King and coworkers[73,74] isolated a novel type of leucoanthocyanidin, melacacidin (44) from wattle heartwood and drew the general conclusion that the 'phlobaphenes' were flavan-3,4-diols. However, their statements were not correct, since the polymeric nature of the condensed tannins (some of which do not yield anthocyanidin upon boiling with acids) was ignored. The difference between monomeric and polymeric tannins was established by Roux and coworkers[75–79]. They isolated and identified, in the low molecular weight fraction, a number of compounds like



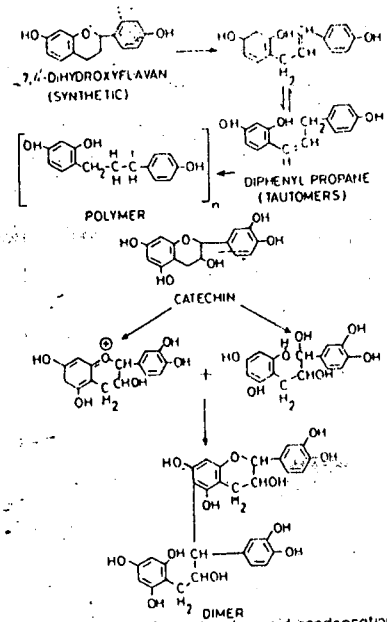
43 Leucoanthocyanidin Anthocyanidin



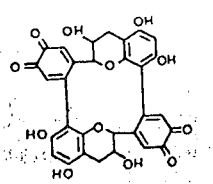
44, Melacacidin

robinetinidol (39a), leucorobinetinidin (40a), mollisacacidin (40b), gallocatechin (39b), catechin (39c), etc. With the isolation and identification of procyanidins[66,80], the views held earlier about flavan-3,4-diols and their oligomers need revision. As mentioned earlier, the capacity to tan depends to a large extent on molecular weight and for maximum tanning capacity, the suitable range is 500-3000[7,9-11]. It is, therefore, relevant to note that the condensed proanthocyanidins, dimers, trimers and tetramers are classified as tannins, since they have molecular weights in this range[13]. Thus, it is clear that the condensed tannins are derived from the polymerization of flavan-3-ols and flavan-3,4-diols, leading to the formation of proanthocyanidins and other polymers. However, the exact mode of linkage is yet to be clearly understood, though the mode of linkage in the case of dimeric and trimeric procyanidins is well established.

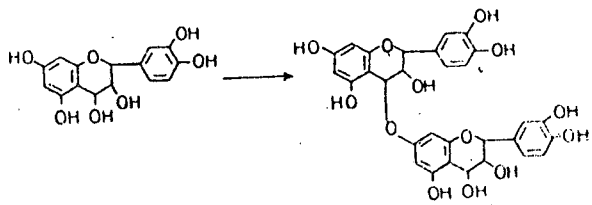
Structure of flavonoid tannins—Three structural theories were proposed for the formation of tannins, viz. (i) condensation by acid, suggested by Heidelberg group[81-88] (Scheme 3), (ii) enzymic condensation through a quinone polymerization, proposed by Hathway[89,90](45) and (iii) an unspecified mechanism to give polymers with ether linkages, which has been advanced on the basis of the work of Kirby and



Scheme 3-Tannin formation by acid condensation



45 Head to tail Dimer of catechin



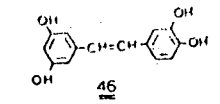
Scheme 4-Unspecified mechanism through ether linkage

White[91], Roux[92] and others (Scheme 4). But these theories are not free from controversy.

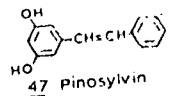
With the isolation and characterization of some of the plant proanthocyanidins (dimers and trimers) in their free phenolic form, the mode of linkage in the polymers is fairly well established, specially in the case of dimers and trimers[66,80]. A number of proanthocyanidin dimers, trimers and oligomers have been isolated from many fruit-bearing plants[66,80]. The structures of dimers and trimers have been fairly well established, but comparatively little is known about the structure of higher oligomeric forms. However, the principles that govern structure formation are thought to be the same as for the simple dimers and trimers.

Stilbenes—Grassmann *et al.*[16] observed that the tannins of spruce bark are made up of the polymerization products of piceatannol (3,4,3',5'-tetrahydroxystilbene) (46) which occurs in the free state or as glycosides. Later, hydroxystilbenes were isolated from the tannin-bearing plants like *Eucalyptus wandoo*[93], *C. fistula*[89,94], *C. marginata*[95], etc.

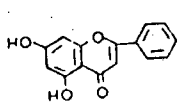
The close metabolic relationship of hydroxystilbenes with the condensed tannins was first suggested by Erdtman[17]. It was also observed that in both groups, one aromatic ring is derived via the acetate pathway and the other from shikimic acid[17,18,96]. For example, pinosylvin (47), chrysin (48) and pinocembrin (49), all having the unsubstituted B ring, occur in the pine species.



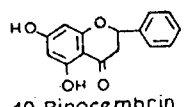
46



47 Pinosylvin



48 Chrysin



49 Pinocembrin

Position of Vegetable Tannages in India

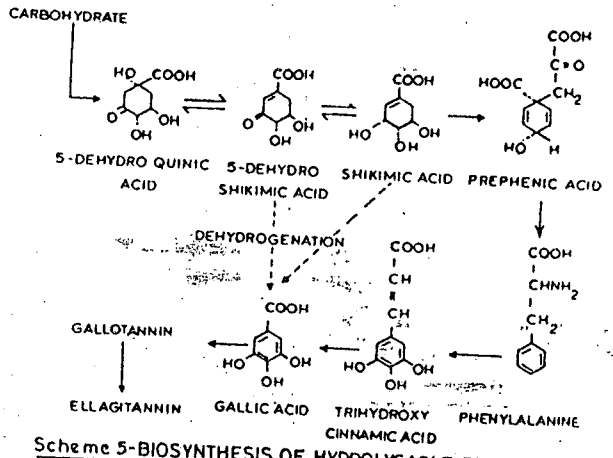
India, with its tropical and sub-tropical climatic conditions, is rich in fauna and flora and there is a good scope for exploring tanning materials, other than wattle, most of which are not useful as self-tanning

materials, due to certain inherent defects associated with them. The hydrolysable tanning materials, such as myrobalan nuts, sal seeds, etc., which are available abundantly, are associated with certain defects like formation of sludge during tanning, hydrolysis of the tannins by acids or enzymes, resulting in the formation of carbohydrates and gallic acid and/or ellagic acid, fermentation, mould growth of the liquors, etc; as a consequence, empty, spongy leathers having a high tendency for water absorption are obtained. The sludge which consists of ellagic acid, chebulinic acid, etc. prevents further penetration of tannins. The condensed tannins from cutch, mangroves, karada, sal bark, etc., on the other hand, undergo progressive polymerization resulting in the formation of phlobaphenes or tannin-reds, and due to their high molecular weights they do not have good penetration. Because of the presence of certain colouring matters like anthocyanidins, quinones, etc., these materials produce darker coloured leathers. It is the association of these defects with the tanning materials that limits proper utilization of these materials. For better utilization, a fundamental knowledge about the chemistry of the tannin as well as non-tannin constituents present in them is essential. With this point in view, studies have been carried out on the chemistry of indigenous tanning materials like avaram (*C. auriculata*)[97], babul (*A. arabica*)[97], konnam (*C. fistula*)[98,99], ghat bor (*Zizyphus zylopyrus*)[100], sal (*Shorea robusta*) bark[101] and seeds[101–105], sain (*Terminalia tomentosa*)[99,106], cashew (*Anacardium occidentale*)[101], vagai (*Cassia marginata*)[100], mango (*Mangifera indica*)[30], dhawa (*Anogeissus latifolia*)[98,99], lyal vagai (*Peltoforum ferrugenium*)[106], amla (*Phyllanthus emblica*)[107] and casuarina (*Casuarina equisetifolia*)[108]. In the light of the results obtained from these studies, the modifications proposed are: (i) blending may be done with other tanning materials[109–114], (ii) treatment may be done with synthetic tannins[115,116] and/or chemicals[117–120] and (iii) other methods like (a) preparation of syntans using vegetable tannins as raw materials in place of phenols[121,122], (b) graft copolymerization of vegetable tannin extracts with acrylic acid[123], etc. These improvements could be achieved by modifying vegetable tanning materials at appropriate stages during the preparation of the extracts. As a result, a broad range of improved products, based on indigenous tanning materials, viz. myrobalan, babul, divi divi, cutch, mangroves, sal seed, cashew testa, tamarind seed testa, karada bark, etc. were developed and successfully translated into commercial production[112]. These products are being marketed under different trade names, such as Wasub, Lycowat, Mortan, Cashtan, Tamlux, etc. Some of these indigenous tanning materials have been modified to minimize the defects associated with them and to bring them nearer to wattle in tanning quality. These modified materials are now used in the production of E.I. leathers, heavy leathers, etc. as full/partial substitutes of wattle. Modifications have also been introduced in the actual tanning processes in respect of E.I. leathers, through full or partial substitution of wattle[124].

In another modification, vegetable tannins are used as raw materials in the manufacture of syntans[121,122]. Using myrobalan tannin, one product, systan MB, a retanning syntan, was developed by Sastry *et al.*[121,122].

Biogenesis and Biosynthesis of Tannins

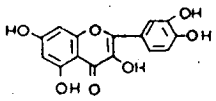
Hydrolysable tannins—Hydrolysable tannins are derived from gallic and hexahydroxydiphenic acids, present in tanniferrous plants as esters with sugar and the biosynthesis of these two acids is likely to give a clear picture of the formation of these two tannins (the hexahydroxydiphenic acid being unstable does not occur in the free state; it is isolated as its dilactone, ellagic acid, which is more stable). Using isotopic feeding experiments, several workers[125] studied the biosynthesis of gallic acid in plants. It was observed that gallic acid could be formed by dehydrogenation of 5-dehydroshikimic acid. Zenk[126], working on the biosynthesis of gallic acid in *Rhus typhina*, concluded that this acid was formed through the metabolism of phenylalanine, as shown in Scheme 5.



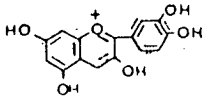
Scheme 5-BIOSYNTHESIS OF HYDROLYSABLE TANNINS

Condensed tannins—Robinson[127] was the first to suggest that flavonoids, which may be considered to have been derived basically from 1,3-diarylpropane, could be formed in plants by the condensation of one C_6 and one C_6-C_3 unit. It was further stated[64] that C_6 unit (ring A) (38) is derived by the acetate pathway and the C_6-C_3 unit (ring B) from the shikimic acid pathway.

This hypothesis was further confirmed by the formation of quercetin (50) in buck wheat (*Fegopyrum tatericum* and *F. esculentum*)[128–130] cyanidin (51) in red cabbage and buck wheat[130–132], etc., using isotopic tracer technique. Hence, the more probable

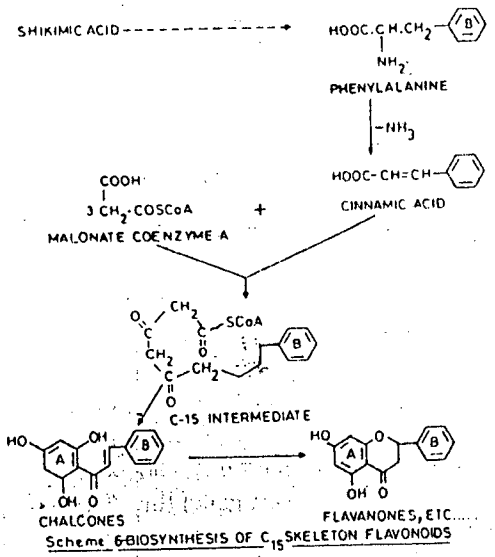


50, Quercetin



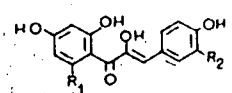
51, Cyanidin

route for the biosynthesis of flavonoids starts from cinnamic acid or related compounds (derived from the shikimic acid pathway), with the addition of three acetate units. The basis C_6-C_3-C_6 compounds are likely to be the chalcones, although other oxidation levels of the C_3 units are not excluded, since evidence in other series shows that a variety of acids can add acetate units (Scheme 6).

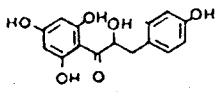


Scheme 6-BIOSYNTHESIS OF C_{15} SKELETON FLAVONOIDS

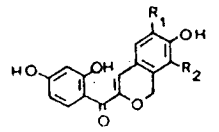
Based on the concomitant occurrence of different closely related flavonoids having similar oxygenation pattern in the rings A and B, many theories have been put forward favouring the sequential formation of flavonoids from a single precursor like chalcone[133,134]. However, with the isolation and identification of α-hydroxychalcones and related compounds[135–141], the theory that chalcone is the intermediate compound in the formation of condensed tannins needs revision. Roux and coworkers isolated α-hydroxychalcone (52-54)[135–138], α-hydroxy-



52, R_1=H, R_2=OH
53, R_1=OH, R_2=H
54, R_1=R_2=OH



55



56, R_1=H, R_2=OH
57, R_1=OH, R_2=H

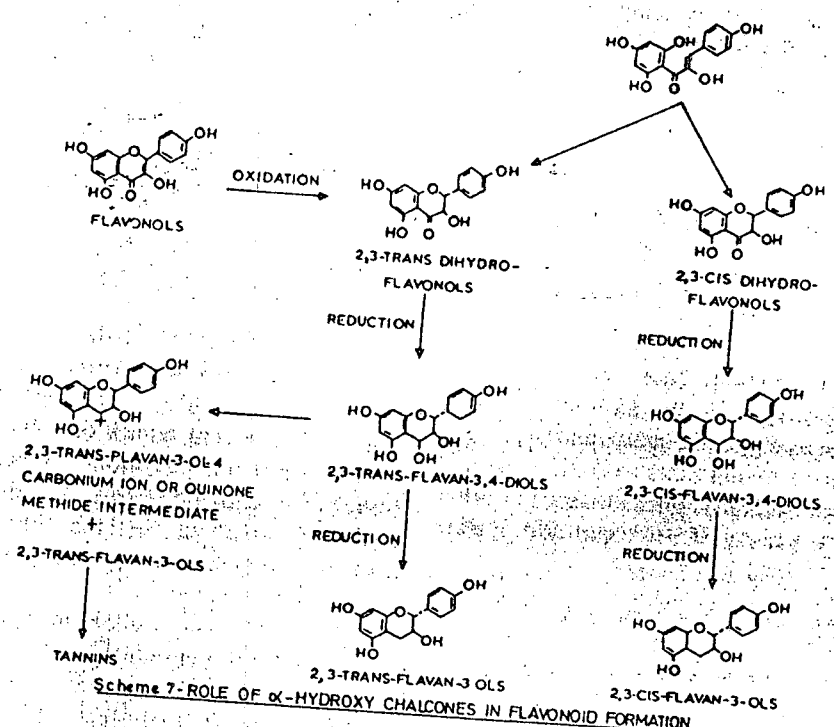
dihydrochalcone (55)[139], the peltogynoid equivalent of α-hydroxychalcone corresponding to mopanol (56)[140] and its isomer, corresponding to peltogynol (57)[141]. In a recent review on α-hydroxychalcones as intermediates in flavonoid biogenesis, Roux and Ferreira[142] concluded that α-hydroxychalcones are probably involved in the biogenesis of 3-hydroxyflavonoids, from which the formation of tannins takes place (Scheme 7).

Thus, there seem to be several opinions on the biogenesis of flavonoids. One point not yet clarified is whether a precursor with a complete flavonoid skeleton is formed first and then further oxidation stages follow or whether the oxidation levels are already fixed at the formation of the flavonoid structure[143]. To understand the exact mode of formation, some more experimental evidence using enzymic, tracer techniques, etc. is necessary.

Mechanism of Vegetable Tannage

The mechanism of vegetable tannage can be viewed as a two-phase system[144]: (i) the liquid phase of tannin solution and (ii) the solid phase of collagen substrate. The interaction between the two is visualized on the reactive groups present in both the phases. Reactive groups available in collagen for interaction with vegetable tannins as listed out by White[145] are: (i) CO and NH group on the backbone, (ii) OH groups of hydroxyproline, serine, etc. for H-bonding, (iii) NH_2 groups for H-bonding and NH_3^+ groups from arginine, lysine and histidine for electrostatic linkages, (iv) COOH groups for H-bonding and $-COO^-$ groups of aspartic and glutamic acids for electrostatic linkages, and (v) those parts of collagen that permit van der Waals forces, including dipoles. In the case of vegetable tannins, the reactive groups available for interaction with collagen are: (i) phenolic hydroxyls, (ii) aliphatic hydroxyls, (iii) ether oxygens, (iv) carbonyl groups of phenolic carboxylic acid, and (v) other sites suitable for H-bonding or van der Waals forces.

In addition to the above, the factors which are also interlinked with reactive groups that govern tannin fixation on collagen are: (i) the astrigency of tannins which depends on the molecular size, (ii) the physico-



Scheme 7-ROLE OF α-HYDROXY CHALCONES IN FLAVONOID FORMATION

chemical properties of tannins, such as molecular weight[7,9–11,144] lying in the range 500-3000, hydrogen ion concentration, concentration of tannins, duration of tanning and temperature of the tanning bath, (iii) *p*H of the collagen substrate and its nature brought out by modification of its reactive groups by esterification, deamination, etc. and the steric factors[146] involving the space and volume between collagen chains and fibres.

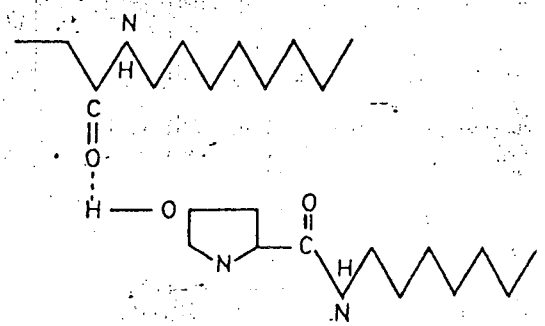
Hence, in view of the complex nature of vegetable annins and collagen and their ramifications, a number f mechanisms have been proposed, which are broadly lassified into: (a) deposition theory, (b) electrovalent heory, and (c) chemical theory, comprising H-bonding nd dipole-dipole interaction. Many wor- ers,[144,147–149] considered tannin fixation as physical eposition and mutual solubilization by peptization nd deposition in collagen lattice.

Proctor and Wilson[150] considered the mechanism as ectrical neutralization of charges of tannins and otein groups. However, this concept was not voured by Thomas and Kelly[151,152] and Vogl[153]. The chemical theory, which takes into account the rticipation of ionic and non-ionic groups of collagen the fixation of tannins, is able to explain to a large ent the complicated reactions involved in vegetable nage. The *p*H of the system plays a vital role in getable tannage[153–156], as it affects the swelling of lagen, accessibility of its reactive groups and the lity of reactive groups of tannins as regards their rge, ionization, degree of aggregation and secondary chemical reactions and the fixation of tannins. The specific reactive groups of proteins and their effect on tannin fixation were investigated extensively by Thomas *et al.*[157], Bowes and Kenten[158], Lollar *et al.*[159] and Gustavson[160] who revealed that basic amino groups take part in the fixation of tannins more pronouncedly in hydrolysable type of tannins, while in the case of condensed tannins, it is attributed to greater availability of coordination sites caused by deamination. Gustavson[161–163] and Batzer *et al.*[164] also investigated the effect of carboxyl and non-ionic groups on normal collagen, denaturated collagen[165–166], treated under heat and lyotropic salts, esterified protein and polyamide as to the fixation of tannins and concluded that the non-ionic protein groups (peptide bonds) contribute as sites for H-bonds, linking vegetable tannins.

Studies on the shrinkage temperature of vegetable tanned leathers indicated that hydrolysable tannins are fixed mainly by non-ionic groups, resulting in slight increase in shrinkage temperature, while condensed tannins fix with basic groups multipointly and help increase the shrinkage temperature considerably over the tanned collagen[146,167–173]. These findings were corroborated by the investigations on the stripping action of urea[174] and organic solvents[175,176] on vegetable tanned leathers. Shuttleworth[176] and others[161,177–181] further considered that protein acceptor oxygen atoms and hydroxyl groups of tannin may react by hydrogen bonding through peptide, charged amino, unionised carboxyl groups and

hydroxyl groups of collagen, ultimately involving the ionic and non-ionic groups of collagen.

Another mechanism suggested on the basis of dipolar groups involves interaction with hydroxyl and amino groups. In dipolar groups, such as $\geqslant CH$, there are two active electron pairs, σ-electrons and π-electrons, between the carbon atoms. While σ-electrons are firmly bound to positively charged nuclei of carbon, the π-electrons are mobile and can participate in asymmetric distribution in the presence of a substituent group, such as hydroxyl and amines on one of the carbon atoms, resulting in a negative charge on the carbon atom. Thus, a molecule with weak dipoles is formed and the dipoles bind the tannins with dipole groups of the protein[182-184]. It can, therefore, be concluded that the mechanism of vegetable tannage involves the coordination of the peptide CONH, basic NH_2 and NH_3^+ and $-COOH$ through H of the phenolic hydroxyls of tannins by H-bonding. Similarly, oxygen atoms of phenolic hydroxyl of tannin may act as coordinating acceptor with any hydrogen atoms on various groups of collagen as per the illustration below:



Conclusion and Suggestion for Future Work

(1) In tanniferrous plants, the tannins are usually concentrated in a particular part of the plant, though they are present in small quantities in other parts also. Tannins present in each part belong to either hydrolysable type or condensed type, e.g. the fruits may contain hydrolysable type and the bark may contain condensed type. However, the presence of hydrolysable tannins (for example, chebulinic acid, chebulagic acid, etc.) and condensed tannins (like proanthocyanidins, dimers, trimers, etc.) together in one and the same part of the tanniferrous plant is not noticed yet, though the presence of hydrolysable tannin constituents along with monomers or precursors of the condensed type or vice versa is a common phenomenon. The so-called mixed type of tannins, e.g. babul (*Acacia arabica*) bark, contain essentially only one type along with the precursors or monomers of the other type. The significance of such a phenomenon must be looked into.

Recently, a new ellagitannin based on leucodelphinidin, gallic acid, *m*-digallic acid and hexahydroxydiphenic acid, esterified with glucose, was reported from young stem bark of *Caesalpinia pulcherrima*[185,186]. However, the exact attachment of the galloyl, *m*-digalloyl and hexahydroxydiphenoyl units on the glucose moiety is not established clearly.

(2) Plants synthesize different polyphenolics, but tannins isolated so far are based essentially on gallic acid, hexahydroxydiphenic acid or related acids and C-15 flavonoid compounds. It may be that there are tannins based on other types of phenolics synthesized by plants, e.g. stilbenes in *Picea abies*[16], phlorotannins present in *Halidrys siliquosa*[187], *Cystoseira baccata*, *Laminaria ochrolenca*[188], etc.

(3) Several routes were suggested for the biosynthesis and biogenesis of hydrolysable and condensed tannins. Although in principle it is possible that more than one route operate for the formation of natural products, the exact pathway is yet to be understood clearly, particularly making use of enzymic, tracer techniques, etc.

(4) It is not clear whether a precursor with a complete flavonoid skeleton is formed first and then oxidation steps occur or the oxidation levels are already fixed at the formation of the flavonoid structure[143].

(5) As regards the material balance and mechanism of tannage, further work is necessary to understand as to how such a large quantity of tannin, almost equal to the quantity of the hide substance, is fixed and what amount of it is entering into reaction by H-bonding or dipole-dipole activity and causing crosslinking of protein structure.

Summary

The present day knowledge on the chemistry and biogenesis of vegetable tannins and their reactions with hide protein is reviewed. The position of vegetable tannage in India with regard to the availability of vegetable tannins, the defects associated with them and the suitable modifications leading to their better utilization are discussed.

References

1 Putnam R C & Gensler W, *J Am Leath Chem Ass*, 46 (1951) 613.
2 Putnam R C, *J Am Leath Chem Ass*, 47 (1952) 148.
3 White T, *J Soc Leath Trades Chem*, 33 (1949) 39.
4 Kirby K S, Knowles E & White T, *J Soc Leath Trades Chem*, 35 (1951) 338.
5 Hillis W E, *Aust J appl Sci*, 2 (1951) 85.
6 Roux D G, *J Soc Leath Trades Chem*, 36 (1952) 274.
7 Hathway D E, in *Chromatographic and electrophoretic techniques*, Vol 1, edited by I Smith (William Heineman, London), 1960, 308.
8 Freudenberg K, *Die chemie der naturlichen gerbustoffe*, Vol 2 (Springers Verlag, Berlin), 1920, 22.

9 White T, in *The chemistry and technology of leather*, Vol 2, edited by Fred O'Flaharty, W T Roddy and R M Lollar (Reinhold Publishing Corp, New York), 1958, 98.

10 Gustavson K H, *J Polym Sci*, 12 (1954) 317.

11 Sastry K N S & Nayudamma Y, *J Proc Instn Chem India*, 38 (1966) 181.

12 Jones W T, Broadhurst R B & Littleton J W, *Phytochemistry*, 15 (1976) 1407.

13 Haslam E, in *The flavonoids*, edited by J B Harborne, T J Mabry & Mabry H (Chapman and Hall, London), 1975, 528.

14 Metcalfe C R & Chalk T, *Anatomy of dicotyledons* (Oxford University Press, Oxford), 1950.

15 *Knowledge*, 12 (1963) 2165.

16 Grassmann W E & Endres H, *Leder*, 10 (1959) 237.

17 Erdtman H, in *Progress in organic chemistry*, Vol I, edited by J W Cook [Butterworth & Co (Publishers) Ltd, London] 1952, 27.

18 Lindstedt G & Misiorny A, *Acta chem scand*, 5 (1951) 121.

19 Fischer E, *Berichte der deutchen chemichen Gessellschaft*, 46 (1913) 3253; 52 (1919) 809.

20 Freudenberg K, *Naturlische gerbstoffe* (Verlag Chemie, Berlin) (1920) 101; *Tannin, cellulose and lignin* (Verlag Chemie, Berlin) (1933) 1938.

21 Karrer P, Saloman & Peyer, *Helv chim Acta*, 6 (1923) 17.

22 Lowe J, *Analyt Chem*, 12 (1873) 128.

23 Karrer P, Widmer R & Staub M, *Justus Liebigs Annln Chem*, 433 (1923) 288.

24 Burton D & Nurston H E, *Proceedings, symposium on the chemistry of vegetable tannins* (Society Leather Trades Chemists, Croydon), 1956, 61.

25 Armitage R, Bayless G S, Grimshaw J, Haslam E, Hawroth R D, Jones K, Rogers H J & Searle T, *J chem Soc*, (1961) 1842.

26 Haslam E, Haworth R D, Jones K & Rogers H J, *J chem Soc*, (1961) 1829.

27 Armitage R, Haslam E, Haworth R D & Searle T, *J chem Soc*, (1962) 3808.

28 Haslam E, Haworth R D & Keen P G, *J chem Soc*, (1962) 3814.

29 Reddy K K, Rajadurai S, Sastry K N S & Nayudamma Y, *Aust J Chem*, 17 (1964) 239.

30 Ansari M A El, *Contributions to the chemistry of vegetable tannins*, Ph D thesis, Cairo University, 1969.

31 Schmidt O T & Mayer W, *Justus Liebigs Annln Chem*, 571 (1951) 1.

32 Schmidt O T, Lademan R & Himmele W, *Chem Ber*, 85 (1952) 408.

33 Schmidt O T & Lademan R, *Justus Leibig Annln Chem*, 569 (1950) 149; 571 (1952) 232.

34 Schmidt O T & Schmidt, *Justus Liebig Annln Chem*, 578 (1952) 31.

35 Schmidt O T & Nieswandt W, *Justus Liebig Annln Chem*, 568 (1950) 31.

36 Schmidt O T, *Proceedings, symposium on the chemistry of vegetable tannins* (Society of Leather Trades Chemists, Cryodon), 1956, 68.

37 Schmidt O T, *Fortschr Chem org NatStoffe*, 14 (1956) 78.

38 Schmidt O T, *Proceedings, seminar on vegetable tannins*, 1962, edited by S Rajadurai and K U Bhanu (Central Leather Research Institute, Madras), 1962, 1.

39 Schmidt O T, Schanz R, Eckert R & Wurmb R, *Justus Liebigs Annln Chem*, 706 (1967) 154.

40 Schmidt O T, Schanz R, Wurmb R & Groebke W, *Justus Liebigs Annln Chem*, 706 (1967) 154.

41 Schmidt O T, Eckert R, Gunther E & Fiesser H, *Justus Liebigs Annln Chem*, 706 (1967) 204.

42 Schmidt O T & Bernauer K, *Justus Liebigs Annln Chem*, 588 (1954) 211.

43 Mayer W, *Das Leder*, 28 (1977) 17.

44 Schmidt O T & Komarke E, *Justus Liebigs Annln Chem*, 591 (1955) 156.

45 Schmidt O T, Wurtele L & Harreus A, *Justus Liebigs Annln Chem*, 690 (1965) 150.

46 Mayer W, *Das Leder*, 22 (1971) 277.

47 Mayer W, Gorner A & Andra K, *Justus Liebigs Annln Chem*, (1977) 1976.

48 Gruttner F, *Arch Pharm, Berl*, 236 (1898) 278.

49 Mayer W & Kunz W, *Naturwissenschaften*, 46 (1959) 206.

50 Mayer W, Kunz W & Loebich F, *Justus Liebigs Annln Chem*, 688 (1965) 232.

51 Perkin A G & Uyeda Y, *J chem Soc*, (1922) 66.

52 Kutani N, *Chem pharm Bull, Tokyo*, 8 (1960) 72.

53 Grimshaw J, in *Rodd's chemistry of carbon compounds*, Vol III-D, edited by S Coffey (Elsevier Scientific Publishing Co, Amsterdam), 1976, 237.

54 Britton G & Haslam E, *J chem Soc*, (1965) 7312.

55 Okuda T, Nayeshiro H & Seno K, *Tetrahedron Lett*, (1977) 4421.

56 Okuda T & Seno K, *Tetrahedron Lett*, (1978) 139.

57 Nonaka G I, Harada M & Nishioka I, *Chem pharm Bull, Tokyo*, 28 (1980) 685.

58 Jurd L, *J Am chem Soc*, 80 (1958) 2249.

59 Rao K V, *Lloydia*, 40 (1977) 169.

60 Harborne J B & Corner J J, *Biochem J*, 81 (1961) 242.

61 Hathway D E, *Biochem J*, 67 (1957) 445.

62 Schmidt O T, *Fortschr Chem org NatStoffe*, 14 (1956) 71.

63 Reichel L, Hensler, Palsuska G & Schlug M, *Naturwissenschaften*, 44 (1957) 89.

64 Haslam E, *Chemistry of vegetable tannins*, (Academic Press Ltd, London), 1966, 158.

65 Freudenberg K & Weinges K, *Tetrahedron*, 8 (1960) 336.

66 Weinges K, Bahr W, Ebert K, Goritz K & Marx H D, *Fortschr Chem org NatStoffe*, 27 (1969) 158.

67 Thompson R S, Jacques D, Haslam E & Tanner R J N, *J chem Soc, Perkin I*, (1972) 1387.

68 Batesmith E C, *J exp Bot*, 4 (1953) 1.

69 Batesmith E C & Lerner N H, *Biochem J*, 53 (1954) 126.

70 Hillis W E, *J Soc Leath Trades Chem*, 38 (1954) 91.

71 Hillis W E, *Nature, Lond*, 175 (1955) 597; 182 (1958) 137.

72 Hillis W E & Urbach G, *Nature, Lond*, 182 (1958) 657; *Aust J biol Soc*, 9 (1956) 263.

73 King F E & Bottomley W, *Chemy Ind*, (1953) 1368; *J chem Soc*, (1954) 1399.

74 King F E & Clark Lewis J W, *J chem Soc*, (1955) 3384.

75 Roux D G, *Nature, Lond*, 180 (1957) 973; 181 (1958) 1454.

76 Roux D G & Maihs A E, *Nature, Lond*, 182 (1958) 1798.

77 Roux D G & Bill M C, *Nature, Lond*, 183 (1959) 42.

78 Roux D G and Evelyn S R, *Biochem J*, 69 (1958) 530; 70 (1958) 344.

79 Roux D G & Paulus E, *Biochem J*, 77 (1960) 316; 78 (1961) 120, 785; 80 (1961) 62.

80 Haslam E, *Phytochemistry*, 16 (1977) 1625.

81 Freudenberg K, *Scient Proc Dubl Soc*, 27 (1956) 153; *J pol Sci*, 29 (1958) 433.

82 Freudenberg K, Alanse D & Lama J M, *Justus Liebigs Annln Chem*, 612 (1958) 78.

83 Freudenberg K & Maitland P, *Collegium* (1934) 776, 656; *Justus Liebigs Annln Chem*, 510 (1934) 193.

84 Freudenberg K, Stocker J H & Porter J, *Berdtsch chem Ges*, 90 (1957) 957.

85 Freudenberg K & Weinges K, *Prog org Chem Nat Prod*, 16 (1958) 1.

86 Mayer W & Bauni G, *Das Leder*, 7 (1956) 33; *Justus Liebigs Annln Chem*, 611 (1958) 264.

87 Mayer W & Merger F, *Chemy Ind*, (1959) 485.
88 Schmidt O T & Mayer W, *Angew Chem*, **68** (1950) 103.
89 Hathway D E & Seakins J W T, *J chem Soc*, (1957) 1562; *Biochem J*, **67** (1957) 239.
90 Hathway D E, *J chem Soc*, (1958) 520; *Biochem J*, **70** (1958) 34; **71** (1959) 533.
91 Kirby K S & White T, *J Soc Leath Trades Chem*, **38** (1954) 215.
92 Roux D G, *J Am Leath Chem Ass*, **54** (1959) 614.
93 Hathway D E & Seakins J W T, *Biochem J*, **72** (1959) 369.
94 Rayudu G V N, *Studies on tannins and related compounds in indigenous tanniferous plants*, Ph D thesis, Madras University, 1970.
95 Sundara Rao V S & Rajadurai S, *Aust J Chem*, **21** (1968) 1921.
96 Birch A J, *Fortschr Chem org NatStoffe*, **14** (1957) 189.
97 Bhanu K U, *Studies on vegetable tannins*, Ph D thesis, Madras University, 1963.
98 Reddy K K, *Chemistry of vegetable tannins*, Ph D thesis, Madras University, 1963.
99 *Annual Report*, National Chemical Laboratory, Poona 1977-78, 17.
100 Sundara Rao V S, *Studies on the chemistry of vegetable tannin and certain aspects of synthetic tannins*, Ph D thesis, Madras University, 1970.
101 Ramayya T V, *Studies on vegetable tannins*, Ph D thesis, Madras University, 1978.
102 Reddy K K, Vijayaramayya T, Sundara Rao V S, Sastry K N S & Santappa M, *Leath Sci*, **23** (1976) 403.
103 Reddy K K, Vijayaramayya T, Sundara Rao V S, Sastry K N S & Santappa M, *Leath Sci*, **24** (1977) 243.
104 Vijayaramayya T, Reddy K K, Sundara Rao V S, Sastry K N S, Ganesan A & Santappa M, *Sal seeds - A new source of tanning material*, Paper presented at the Symposium on the Production and Utilisation of Forest Products, Regional Research Laboratory, Jammu, 5 - 7 March 1979.
105 Sambasiva Rao P, Margaret Theresa Y, Koteswara Rao C & Santappa M, *Leath Sci*, **24** (1977) 24.
106 Sulochana V, *Chemistry of vegetable tannins*, Ph D thesis, Madras University, 1972.
107 Theresa Y M, *Studies on vegetable tannins*, Ph D thesis, Madras University, 1966.
108 Madhusudanamma W, Sastry K N S, Sundara Rao V S, Reddy K K & Santappa M, *Leath Sci*, **25** (1978) 369.
109 Koteswara Rao C & Nayudamma Y, *Indigenous vegetable tannin extracts and their use in leather industry*, Paper presented at the 5th Tanners' Get Together, Central Leather Research Institute, Madras, 31 January - 6 February 1970.
110 Koteswara Rao C, *Tanner*, **28** (1973) 255.
111 Koteswara Rao C, *Transfer of technology in respect of vegetable tanning materials*, Paper presented at the 8th Tanners' Get-Together, Central Leather Research Institute, Madras, 31 January - 6 February 1973.
112 Koteswara Rao C, Santappa M & Nayudamma Y, *Vegetable tanning materials - A minor forest produce in India*, Paper presented at the 8th World Foresty Congress, Jakartha, October 1978.
113 Wali B K, Razdan R K & Krishnaswamy S P, *Tanner*, **24** (1969) 53.
114 Wali B K & Razdan R K, *J Indian Leath Technol Ass*, **18** (1970) 439.
115 Venkataboopathy K, *Studies on vegetable tanning agents*, Ph D thesis, Madras University, 1977.
116 Sastry K N S, Vijayaramayya T, Adesh Kumar, Sundara Rao V S, Reddy K K & Santappa M, *Divi divi - A versatile tanning material*, Paper presented at the 16th Tanners' Get Together on 'Challenges of the 80's, Central Leather Research Institute, Madras, 31 January - 6 February 1981.
117 Bangaruswamy S, Selvarangan R & Nayudamma Y, *Bull cent Leath Res Inst*, Madras 9 (1962) 1.
118 Selvarangan R & Nayudamma Y, *Tanner*, **20** (1966) 303.
119 Thampuran K R V & Ghosh D, *Leath Sci*, **20** (1973) 352.
120 Kedlaya K J, Selvarangan R & Nayudamma Y, *Leath Sci*, **13** (1966) 71.
121 Sastry K N S, Sundara Rao V S, Shanmugasundaram K A, Vijayaramayya T, Reddy K K & Santappa M, *Syntan M B - A good retanning agent*, Paper presented at the 13th Tanners' Get Together 1978, Central Leather Research Institute, Madras, 31st January - 6 February 1978.
122 Madhusudanamma W, Sundara Rao V S, Sastry K N S, Reddy K K & Santappa M, *Leath Sci*, **28** (1981) 274.
123 Trakhtenberg S I & Savchuk E A, *Izv vyssh ucheb zaved Tekhnol Legk Promsti*, (1979) 55-60; (1980) 55.
124 Ghosh D, Process released through NRDC, 1975.
125 Haslam E, *The chemistry of vegetable tannins* (Academic Press Ltd, London), 1966, 162.
126 Zenk M H, *Z Naturf*, **19B** (1964) 83.
127 Robinson R, *Nature, Lond*, **137** (1936) 132.
128 Neish A C, Underill E W & Watkin J E, *Can J Biochem Physiol*, **35** (1957) 219.
129 Neish A C, Watkin J E, *Can J Biochem Physiol*, **38** (1960) 559.
130 Grisebach H & Bopp M, *Z Naturf*, **14B** (1959) 485.
131 Grisebach H & Patschke L, *Chem Ber*, **93** (1960) 2326.
132 Bopp M & Matthis B, *Z Naturf*, **17B** (1962) 811.
133 Seshadri T R, *Tetrahedron*, **6** (1959) 169.
134 Grisebach H & Ollis W D, *Experientia*, **17** (1961) 4.
135 Van Der Merwe J P, Ferreire D, Brandt E V & Roux D G, *Chem Commun*, (1972) 521.
136 Malan E & Roux D G, *Phytochemistry*, **13** (1974) 1575.
137 Volsteedt F, Du R, Rall G J H & Roux D G, *Tetrahedron Lett*, (1973) 1001.
138 Volsteedt F, Du R, Rall G J H, Ferreira D & Roux D G, Unpublished results.
139 Bhakuni D, Bittner M, Silva M & Sammics P G, *Phytochemistry*, **12** (1973) 2777.
140 Gottlieb O R & Rego de Sousa J, *Phytochemistry*, **11** (1972) 2841.
141 Brandt E V, Ferreira D & Roux D G, Unpublished results.
142 Roux D G & Ferreira D, *Phytochemistry*, **13** (1974) 2039.
143 Freudenberg K & Weinges K, *Proceedings of the seminar on vegetable tannins*, edited by S Rajadurai & K U Bhanu, (Central Leather Research Institute, Madras) 1962, 51.
144 Balfe M P, *Progress in leather science*, Vol 3 (British Leather Manufacturers' Association, London SE1) 1948, 564-622.
145 White T, *J Soc Leath Trades Chem*, **40** (1956) 78.
146 Page R O, *J Soc Leath Trades Chem*, **28** (1944) 156.
147 Moeller H, *Collegium* (1916) 311.
148 Cheshire A, *J Soc Leath Trades Chem*, **27** (1943) 145.
149 White T, Kirby K S & Knowles E, *J Soc Leath Trades Chem*, **36** (1952) 148.
150 Proctor N R & Wilson J A, *Trans chem Soc*, **109** (1916) 1327.
151 Thomas A W & Kelley M W, *Ind Engng Chem*, **18** (1926) 625.
152 Thomas A W & Kelley M W, *Ind Engng Chem*, **17** (1925) 41.
153 Vogl L, *Dissertation*, Darmstadt, 1926.
154 Thomas A W & Kelley M W, *Ind Engng Chem*, **15** (1923) 1148.
155 Thomas A W & Kelley M W, *Ind Engng Chem*, **16** (1924) 800.
156 Braybrooks W E, Mc Condlish D & Atkin W R, *J Soc Leath Trades Chem*, **23** (1939) 111.
157 Thomas A W & Kelley M W, *J Am chem Soc*, **48** (1926) 1312.
158 Bowes J H & Kenten R H, *Biochem J*, **44** (1949) 142.
159 Kreman S S & Lollar R M, *J Am Leath Chem Ass*, **43** (1948) 542.
160 Gustavson K H & Larsson A, *Hagglund Festschrift Stockholm*, 1948, 99.

161 Gustavson K H, *J Soc Leath Trades Chem*, 38 (1954) 162.
162 Gustavson K H & Holm B, *J Am Leath Chem Ass*, 47 (1952) 700.
163 Gustavson K H, *J Polym Sci*, 12 (1954) 317.
164 Batzer H & Weissenberger G, *Makromolek Chem*, 7 (1951) 320.
165 Gustavson K H, *Colloid Symp Monogr*, 4 (1926) 79.
166 Gustavson K H, *J Am Leath Chem Ass*, 40 (1946) 47.
167 Chater W, *J Soc Leath Trades Chem*, 14 (1930) 28.
168 Gustavson K H, *J Am Leath Chem Ass*, 42 (1947) 313.
169 Balfe M P, *Progress in leather science*, Vol 3 (British Leather Manufacturers' Research Association, London), 1948, 580.
170 Gustavson K H & Nestvold M, *J Soc Leath Trades Chem*, 36 (1952) 105.
171 Kedlaya K J, Ramanathan N & Nayudamma Y, *J Am Leath Chem Ass*, 66 (1971) 256.
172 Sugano E, *Kikaku Kagaku*, 19 (1974) 201.
173 Scaria K J & Barat S K, *Proceedings of the seminar on vegetable tannings*, (Central Leather Research Institute, Madras), 1962, 127.
174 Simoncini E, *Cuoio, Pelli, mat concianti*, 29 (1953) 204.
175 Shuttleworth S G & Cunningham G E, *J Soc Leath Trades Chem*, 32 (1948) 183.
176 Shuttleworth S G, *J Am Leath Chem Ass*, 47 (1952) 603.
177 Tu S T & Lollar R M, *J Am Leath Chem Ass*, 45 (1950) 324.
178 Roux D G & Sykes R L, *J Soc Leath Trades Chem*, 41 (1957) 14.
179 Santhanam P S & Nayudamma Y, *Leath Sci*, 15 (1968) 1.
180 Vijayalakshmi K & Santhanam P S, *Leath Sci*, 17 (1970) 391.
181 Rao J B & Nayudamma Y, *Proceedings of the seminar on vegetable tannins* (Central Leather Research Institute, Madras) 1962, 149.
182 Kuntzel A & Schwank M, *Collegium*, (1940) 455, 497, 500.
183 Otto G, *Das Leder*, 4 (1953) 1, 193.
184 Russel A E, Shuttleworth S G & Williams-Wynn D A, *J Soc Leath Trades Chem*, 51 (1967) 349.
185 Aswathi K K & Misra K, *A novel ellagitannin from Caesalpinia pulcherrima bark*, Paper presented at the 11th IUPAC Int Symp Chem Nat Prod, 2 (1978) 207.
186 Aswathi K K, Anoopkumar & Misra K, *Curr Sci*, 49 (1980) 769.
187 Sattler E, Glombitza K W, Wehrli F E & Eckhardt G, *Tetrahedron*, 33 (1977) 1239.
188 Glombitza K W, Widenfeld G & Koch M, *Phlorotannins from various Phaeophyceae*, Paper presented at the 11th IUPAC Int Sym: Chem Nat Prod, 2 (1978) 206.