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THE METABOLISM OF GALLIC ACID AND HEXAHYDROXYDIPHENIC ACID IN PLANTS: BIOGENETIC AND MOLECULAR TAXONOMIC CONSIDERATIONS*

ELIZABETH A. HADDOCK, RAJ K. GUPTA, SABAH M. K. AL-SHAFI, KEITH LAYDEN, EDWIN HASLAM and DANIELE MAGNOLATO†

Department of Chemistry, University of Sheffield S3 7HF, U.K.; †Nestlé Technical Research Co. Ltd., CH-1814 La Tour de Peilz, Switzerland

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Key Word Index—Angiosperms; gallic acid metabolism; taxonomy; biogenesis.

Abstract—The distinctive and unique features of gallic acid metabolism in plants are discussed and recent observations on new metabolites are presented. The potential application of these results to taxonomic questions is outlined.

INTRODUCTION

Gallic acid was first isolated from gall nuts almost 200 years ago by the Swedish chemist Scheele[1]. As befits a molecule of such apparent simplicity the first synthesis from wholely aliphatic precursors was not announced until 1975[2]; correspondingly the position of gallic acid in the overall phenolic metabolism of higher plants remains to be fully clarified. Bate-Smith[3] first drew attention to an important anomaly related to gallic acid in his classical review of the occurrence of phenols in dicotyledons in the 1960's. Thus he noted that 3,4,5-trihydroxycinnamic acid (**1**), except as various *O*-methyl ethers, was not encountered in plants but he found that hexahydroxydiphenic acid‡ (**4**), determined as its stable dilactone ellagic acid (**5**) after acid hydrolysis, was widely distributed in plants in ester form (**3**). This evidence persuaded Bate-Smith to suggest that hexahydroxydiphenic acid (**4**) was the taxonomic equivalent of the 'missing' acid, 3,4,5-trihydroxycinnamic acid (**1**). However Schmidt and Mayer[4] in their classical studies of the ellagitannins had earlier postulated that hexahydroxydiphenoyl esters (**3**) are formed *in vivo* by oxidative coupling of appropriately placed galloyl esters (**2**) in a precursor substrate (Fig. 1) and although direct experimental evidence to substantiate this view is still awaited, there is strong circumstantial evidence to support the proposal. It follows that gallic acid (**2**, R = H) is perhaps more correctly considered as the ultimate taxonomic equivalent of the 'missing' 3,4,5-trihydroxycinnamic acid (**1**), although it should be noted in parenthesis that the latter's absence from plant tissues is, in contrast to the early 1960's, now more readily explicable in terms of the established pathways of biosynthesis of the hydroxycinnamyl alcohol precursors of lignin.

The metabolism of gallic acid in higher plants however poses other important questions, notably its distinctive relationship to the patterns and modes of occurrence of other natural phenolic esters (Fig. 2). Thus esters of *o*- and *p*-hydroxybenzoic and protocatechuic acids are relatively rarely encountered in plants[5] and their occurrence is something of a taxonomic speciality (e.g. salicylic acid in the Salicaceae). Esters of the hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic and sinapic) are almost universally distributed[5] but most frequently as mono- and occasionally bis-esters with polyols (e.g. chlorogenic acid and isochlorogenic acid). In contrast to the other hydroxybenzoic acids, gallic acid is metabolized fairly widely by plants and it occurs in ester forms which, in contrast to those of the hydroxycinnamic acids, vary from simple monoesters (e.g. β-D-glucogallin[6] and theogallin[7]) to the complex polyesters with D-glucose whose MWs extend to at least 2000. These latter esters are, moreover, unique to the Plant Kingdom and together with the proanthocyanidins[8] they constitute the so-called vegetable tannins of the earlier chemical and botanical literature.

Bate-Smith has suggested[9] that from the biological point of view the importance of vegetable tannins

*Part 4 in the series "The Metabolism of Gallic Acid and Hexahydroxydiphenic Acid in Plants". For Part 3, see Haddock, E. A. *et al.* (1982) *J. Chem. Soc. Perkin Trans.* 1 (in press).

‡This nomenclature is used as an abbreviation for the 6,6-dicarbonyl-2,2',3,3',4,4'-hexahydroxybiphenyl radical.

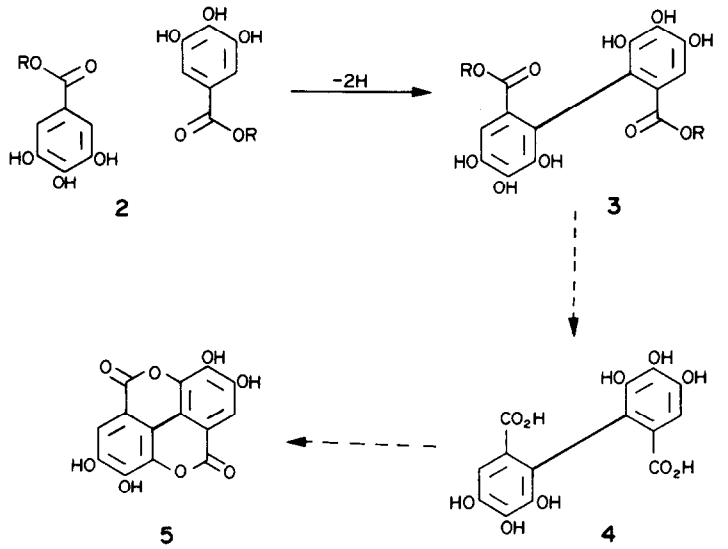


Fig. 1. The biogenesis of hexahydroxydiphenoyl esters [4] and their hydrolysis to give ellagic acid.

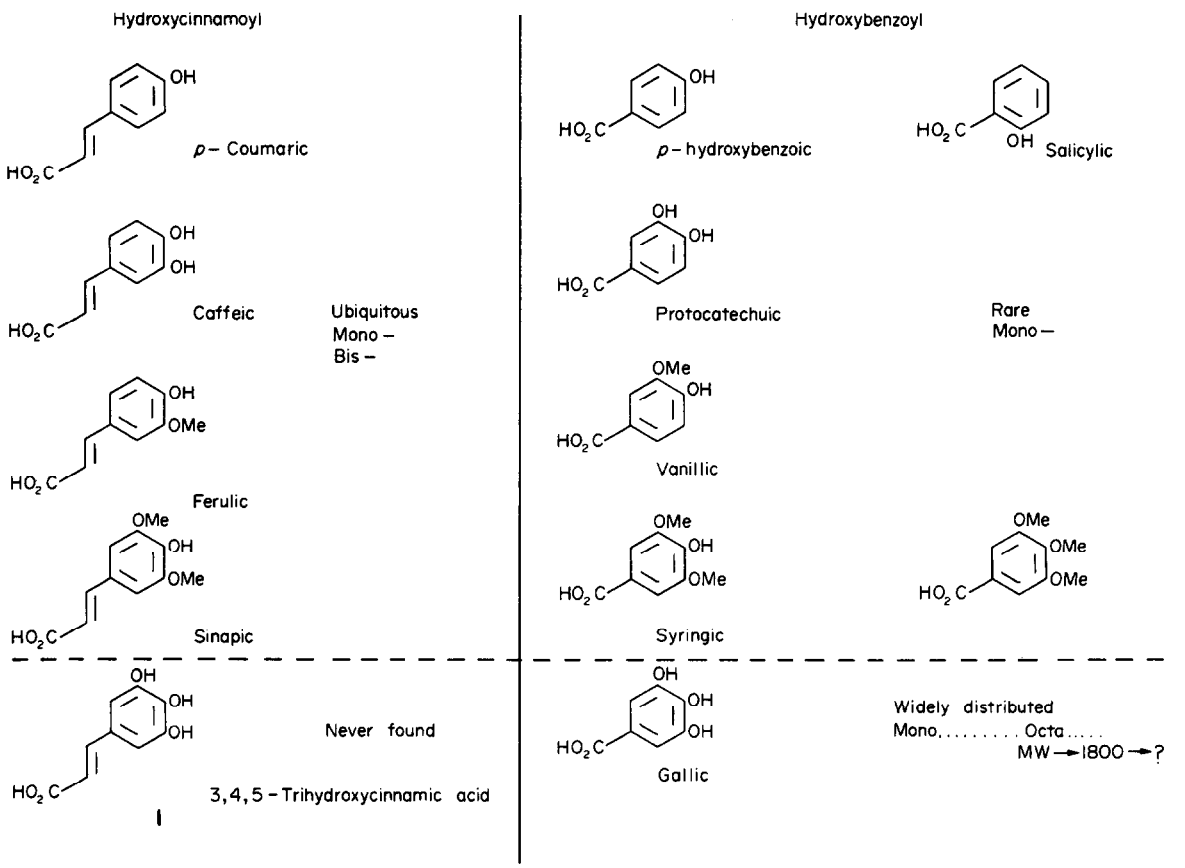


Fig. 2. Occurrence of phenolic esters in plants.

to plants lies in their effectiveness as repellents to both animal and microbial predators. This property derives from their ability to precipitate protein whether this be an extracellular microbial enzyme or the salivary protein of a browsing animal. The capacity of plants to metabolize vegetable tannins is, according to Bate-Smith, a primitive characteristic that tends to become lost with increasing phylogenetic specialization. Our knowledge of the biochemistry and botanical distribution of the proanthocyanidins (syn-condensed tannins) has been substantially improved in the past decade[8, 10–12] but, because of the attention inevitably given to those few plants which metabolize substantial and commercially important quantities of the complex polyesters of gallic acid[13–15] and its derivatives, our knowledge of the wider role played by gallic acid in the overall phenolic metabolism of higher plants has been severely circumscribed.

Gallic acid is almost invariably located in plant tissues in ester form and various esters—with sugars[6, 16, 17, 32], polyols[7], glycosides[18–23] and other phenols[24–26]—have been described. These esters are analogous in most instances to the ubiquitous hydroxycinnamoyl esters[5]. In addition several complex polyesters of gallic acid (gallotannins)[15] and numerous derivatives of hexahydroxydiphenic acid and its derivatives (ellagitannins)[27] have been isolated from leaves, fruit, fruit pods and plant galls[13]. This work has largely been directed towards an understanding of the chemistry of these substances and clearly defined objectives of a biological nature have generally been absent. Darnley-Gibbs[28] has remarked that "it would be helpful to break down tannins into the 'hydrolysable' and 'condensed' groups when making phylogenetic studies." However until very recently, when a series of papers by Bate-Smith outlined its potential[29], little taxonomic use has been made of the distribution of esters and derivatives of gallic acid[30, 31] and this has undoubtedly been due in large measure to the difficulties of isolation and structure determination. Some recent observations upon facets of gallic and hexahydroxydiphenic acid metabolism in plants and their relevance to molecular taxonomy in the Plant Kingdom are recorded here.

RESULTS AND DISCUSSION

Unless otherwise stated the work has been principally concerned with phenolic constituents from the leaves of higher plants. This provides a common basis for comparison of gallic acid metabolism in plants, but it is clear that the absence of gallic acid and its derivatives from the leaves does not imply its absence from other tissues of the same plant. Thus in the Leguminosae the fruit pods of various plants often provide rich sources of gallic acid and hexahydroxydiphenic acid, but these same metabolites cannot be discerned in the leaves of the same plants (Haddock, E. A. *et al.*, unpublished results). This may be interpreted as the local suppression or activation of a synthetic capacity originally possessed by the whole plant. Paper chromatography, augmented where possible by HPLC, have been the principal analytical techniques employed in this survey which has been part of a general chemical study of gallic acid metabolism in plants[38]. Galloyl esters are detected on paper chromatograms by their violet (mono-, bis-esters) to dark blue (tri-, tetra-, etc. esters) absorption in UV light[33] enhanced by fuming with ammonia vapour and by various chromogenic sprays[18, 33]. Specific for galloyl esters, although not highly sensitive, is a saturated solution of potassium iodate[30] which gives a rose pink colour and reacts with free gallic acid to give purpurogallin carboxylic acid (bright orange). Esters of hexahydroxydiphenic acid have a dark blue absorption in UV light and react specifically with a spray of nitrous acid to give a distinctive carmine red colour which rapidly changes through green, brown and purple to yield a moderately stable indigo blue. This reagent is based on the distinctive colour test for ellagic acid originally devised by Procter and Paessler[39] and employed later by Bate-Smith[29].

Using Bate-Smith's original observations[3] on the phenolic constituents of dicotyledons as the principal guide, the leaves of over 150 plants have been screened for galloyl and hexahydroxydiphenoyl esters. Where appropriate compounds were isolated and identified by chemical and spectroscopic means. Table 1 lists the various galloyl-D-glucose esters which have been identified in the leaves of plants. It is interesting to note amongst these esters the apparent preference for the ester groups to be located at positions 1(β), 2 and 6 on the D-glucopyranose ring and this accords with the observations made with other naturally occurring esters of D-glucose. Also particularly noteworthy is the relatively widespread occurrence of β-penta-*O*-galloyl-D-glucose (**6**) and the absence, amongst derivatives so far identified, of any simple α-glucosides. Circumstantial evidence suggests that for a great many plants the metabolism of β-penta-*O*-galloyl-D-glucose (**6**) represents something of a watershed and from this intermediate at least three distinct pathways of further metabolism exist (A, B, C in Table 1 and Fig. 3). These pathways and the associated metabolites furnish a series of chemical 'characters' which may be used to determine relationships between plant families and within a given family.

In the context of this proposal and supporting the key-position which β-penta-*O*-galloyl-D-glucose (**6**) probably occupies (Fig. 3) it has been observed (with Dr. M. J. Fowler and Mrs. S. Beet, Institute of Biotechnology, University of Sheffield) that callus tissue of *Quercus robur* metabolizes in very small amount, along with gallic acid and pyrogallol, the same ester (**6**) but none of the further derivatives which leaves and other tissues of the fully differentiated plant *Quercus robur* biosynthesize (*vide infra*). This situation appears analogous to that reported in cell suspension cultures of *Baptisia australis* where only those tetracyclic quinolizidine alkaloids which occur early in the biogenetic sequence were found to accumulate[40]. The main alkaloid metabolized by these cultures is lupanine and it was concluded that the other alkaloids found in the plant, such as tinctorine and anagyrine, are derived sequentially from it.

Class A—depside metabolites

The ability to produce metabolites in which ad-

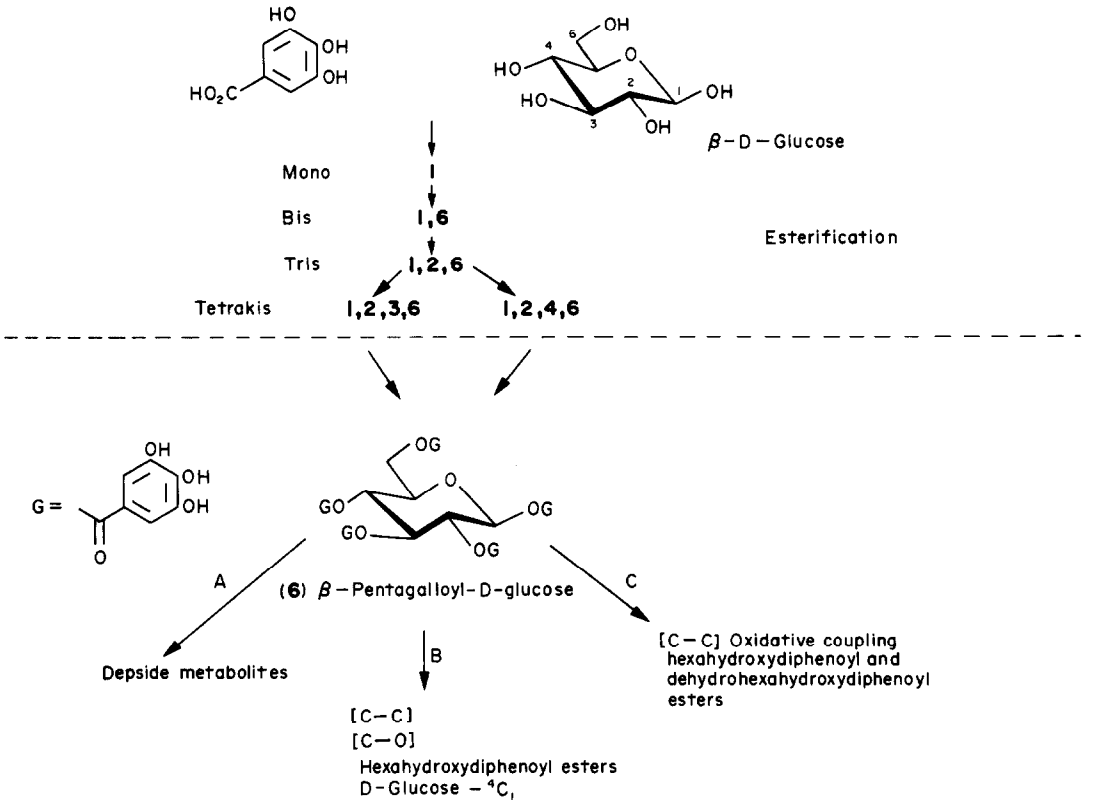


Fig. 3. Gallic acid metabolism in higher plants: the overall pattern.

ditional gallic acid molecules are esterified as *meta*-depsides (**7**) to a pre-existing galloyl ester (usually with D-glucose) is limited to comparatively few plant families (Table 1). In the earlier literature such products were frequently referred to as 'gallotannins' and the most familiar of these is the complex polyester from the twig galls of *Rhus semialata* (Chinese gallotannin or tannic acid)[15, 33] and the leaves of other *Rhus* spp.[33]. The depsidically linked galloyl ester groups (**7**) are specifically cleaved under very mild conditions (methanol, room temp., pH 6.0) leaving other galloyl ester groups unaffected[33]. This reaction, when combined with HPLC and PC (to determine the products, **8** and **9**, both qualitatively and quantitatively) and spectroscopic measurements provides a ready means of identification of this class of metabolite in plant extracts (Haddock, E. A. *et al.*, unpublished results). To date depside metabolites in which further gallic acid molecules are esterified to β-penta-*O*-galloyl-D-glucose (**6**) have been most widely encountered in plants (Table 2). A recent re-examination (Haddock, E. A. *et al.*, unpublished results) of these esters shows that they are usually mixtures of closely related species ranging from β-penta-*O*-galloyl-D-glucose itself to hepta- and octa-galloyl-D-glucose derivatives in which additional galloyl ester groups are linked as *meta*-depsides preferentially to the galloyl ester groups at C-3, C-4 and C-6 (**10**). This supports the view originally expressed by Fisher[41] that these esters are not only mixtures of isomers but also of substances of differing empirical formulae. In plants where these esters are metabolized they frequently dominate the phenolic extract and in many cases to the virtual exclusion of other phenols. The significance of this particular observation however is not clear.

Although the principal form in which gallic acid is found in depside form appears to be that with β-penta-*O*-galloyl-D-glucose (**10**), other esters of this type have been described, in which a tetra-*O*-galloyl-D-glucose[33] and 3,4,5-tri-*O*-galloylquinic acid[37] constitute the basic core of the structure. More recently (Haddock, E. A. *et al.*, unpublished results) a mixture of closely related tri-*O*-galloyl-derivatives of 1,5-anhydro-D-glucitol has been obtained from *Acer ginnale*, *A. tartaricum* and *A. saccharinum*. In this mixture (**11**) there is one additional galloyl ester group, linked as a *meta*-depside, to the galloyl ester group at C-6 or that at C-2 in 2,6-di-*O*-galloyl-1,5-anhydro-D-glucitol ('Acer tannin'[34, 35]). This suggests a partial reassessment of the molecular taxonomy of the Aceraceae[30]. This group of three species is now seen to be related, on the basis of their ability to form *meta*-depsides, to the group *A. platanoides*, *A. rubrum* and *A. campestre*.

The observations recorded here indicate a very close association of this form of metabolism with the Rhoideae tribe in the Anacardiaceae. As a chemical character it may well be useful in determining taxonomic features amongst members of the Hamamel-

Table 1. Metabolism of gallic acid in higher plants, a general survey

Order, family and species	A	B	C	Galloyl-β-D-glucopyranose derivatives	Miscellaneous metabolites of gallic acid
Hamamelidales					
(i) Cercidiphyllaceae					
Cercidiphyllum japonicum	−	−	+	+	
(ii) Hamamelidaceae					
Hamamelis mollis	+	−	−	+	(bark) 'hamameli tannin [32]-2',5-digalloyl-D-hamamelose
Parrotia persica	+	−	−	+	
Liquidambar styraciflua	−	+	−	+	
Fagales					
(i) Fagaceae					
Castanea sativa	−	+	−	+	(bark) 'hamameli' tannin [32]
C. crenata (galls)					cretanin, chestanin-derivatives of dehydrodigallic acid [19, 20]
Quercus borealis	−	+	−	(+); 1,2,3,4,6-penta-	
Q. robur	−	+	−	+	
Q. robur (callus tissue)	−	−	−	(+); 1,2,3,4,6-penta-	gallic acid, pyrogallol
Q. hispanica	−	+	−	+	
Q. castaneafolia	−	+	−	+	
Q. aegilops	−	+	−		
Q. petraea	−	+	−	+	
Q. alba	−	+	−	+	
Q. palustris	−	+	−	+	
Q. lusitanica	−	+	−	+	
Q. ilex	−	+	−	+	
Q. cerris	−	+	−	+	
Q. infectoria	−	+	+	+	
Q. infectoria (galls)	−	+	−	(+); 1,6-di-; 1,2,3,6-tetra-; 1,2,3,4,6-penta-	Turkish gallotannin [33]
Q. phellos	−	−	−		
Dilleniales					
(i) Paeoniaceae					
Paeonia officinalis	+	−	−	+	
Theales					
(i) Theaceae					
Camellia sinensis	−	−	−	+	theogallin 5-*O*-galloylquinic acid [7], flavan-3-ol-galloyl esters [24, 25]
C. japonica	−	+	−		
Ericales					
(i) Ericaceae					
Arbutus unedo	−	−	+	+	
Arctostaphylos uva-ursi	+	−	−	+	hydroquinone-β-D-glucoside galloyl esters [18]
Rosales					
(i) Rosaceae					
Rubus fructicosus	−	+	−	(+); 1,2,6-tri-; 1,2,3,4,6-penta-	
Fragaria × *ananasa*	−	+	−		
Potentilla procumbens	−	+	−		
P. anserina	−	+	−		
P. sterilis	−	+	−		
Geum rivale	−	+	−	+	
Rosa canina	−	+	−	(+); 1,2,6-tri-; 1,2,3,4,6-penta-	
Filipendula ulmaria	−	+	−	+	

Table 1—*continued*

Order, family and species	A	B	C	Galloyl-β-D-glucopyranose derivatives	Miscellaneous metabolites of gallic acid
(ii) Saxifragacea					
Bergenia cordifolia	−	+	−	+	roots and rhizomes, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose; (+)-catechin gallate;
B. crassifolia	−	+	−	+	galloyl esters of hydroquinone β-D-glucoside [18, 26]
Tellima grandiflora [31]	−	+	−	+	
Myrtales					
(i) Myrtaceae					
Myrtus communis	−	+	−	−	
Eucalyptus viminalis	−	+	−	+	
E. acaciformis	−	+	−	+	
E. globuli	−	+	−	+	
(ii) Punicaceae					
Punica granatum	−	−	+	+	
(iii) Onagraceae					
Fuchsia spp.	−	+	+	(+); 1,2,6-tri-; 1,2,3,6-tetra-; 1,2,3,4,6-penta-	
Epilobium angustifolium	−	+	−	(+); 1,2,6-tri; 1,2,3,6-tetra-; 1,2,3,4,6-penta-	
(iv) Combretaceae					
Combretum paniculatum	−	+	−		
Quisqualis mussaendiflora	−	+	−	+	
Terminalia chebula [17] (fruit)			+	1,3,6-tri-; 1,2,3,4,6-penta-	See footnote
Cornales					
(i) Nyssaceae					
Davidia involucrata	−	−	+	+	
(ii) Cornaceae					
Cornus mas	−	+	−	+	
C. alba	−	+	−	+	
C. controversa	−	+	−	+	
Proteales					
(i) Elaegnaceae					
Hippophae rhamnoides	−	+	−		
Sapindales					
(i) Aceraceae [30]					
Acer pseudoplatanus	−	−	+	+	
A. monspessulanum	−	−	+	+	
A. griseum	−	−	+	+	
A. spicatum	−	−	+	+	
A. spicatum	−	−	+	+	
A. pensylvanicum	−	−	+	+	
A. rotundilobum	−	−	+	+	
A. saccharum	−	−	+	+	
A. palmatum	−	−	+	+	
A. platanoides	+	−	−	(+); 1,2,3,4,6-*penta*-	
A. campestre					
A. rubrum	+	−	−	+	
A. saccharinum	+	−	−		(+) '*Acer*' tannin [35]; 2,6-di-*O*-galloyl-1,5-anhydro-D-glucitol; 6-*O*-galloyl-1,5-anhydro-D-glucitol
A. tartaricum					
A. ginnale [34]	+	−	−		

Table 1—*continued*

Order, family and species	A	B	C	Galloyl-β-D-glucopyranose derivatives	Miscellaneous metabolites of gallic acid
(ii) Simaroubaceae					
Ailanthus altissima	−	−	−	+	
A. giraldiii	−	−	+	+	
A. vilmoriniana	−	−	+		
(iii) Anacardiaceae					
Rhus typhina	+	−	−		
R. coriaria	+	−	−	(+); 1,2,3,4,6-penta-	tannic acid, Chinese gallotannin[33]
R. semialata (galls)	+	−	−		
Pistacia terebinthus	+	−	−	+	
Schinus lentiscifolius	+	−	−	+	
Cotinus coggygria	+	−	−	(+); 1,2,3,4,6-penta-	
Mangifera indica	+			+	
Geraniales					
(i) Geraniaceae					
Geranium robertanium	−	−	+	(+); 1,2,3,4,6-penta-	
G. pratense	−	−	+	+	
G. lucidum	−	−	+	+	
G. rotundifolium	−	−	+	+	
Pelargonium spp.	+	−	−	(+); 1,2,3,4,6-penta-	
Juglandales					
(i) Juglandaceae					
Juglans nigra	−	+	−	+	
J. regia	−	+	−	+	

Class A, depside metabolites, gallotannins.

Class B, (*S*)-hexahydroxydiphenoyl metabolites, D-glucopyranose ($^4C_1$).

Class C, hexahydroxydiphenoyl and dehydrohexahydroxydiphenoyl metabolites, D-glucopyranose ($^1C_4$).

Except where otherwise stated the analysis is based on the phenolic constituents of plant leaf and stem. Definitions of 'presence' (+) and 'absence' (−) are as defined by Hegnauer[36] for taxonomic purposes. Many of the traditional sources of the ellagitannins[13] are not included here since they do not clearly conform to one of the patterns delineated. These are those from the Leguminosae where the fruit pods are rich sources of phenolic compounds: *Caesalpinia spinosa* (Tara), depside[37]; *Caesalpinia brevifolia* (Algarobilla)[27], ellagitannin; *Caesalpinia coriaria* (Divi-divi)[27], ellagitannin; *Acacia arabica* and *Ceratonia siliqua*, galloyl-D-glucoses and flavan-3-ol-gallates.

idaceae, Ericaceae, Aceraceae[30] and Geraniaceae since its occurrence in these families is limited to certain members.

Class B—hexahydroxydiphenoyl metabolites of D-glucopyranose ($^4C_1$)

A widely distributed metabolic fingerprint is that in which (it is assumed on the biogenetic premise outlined[4]) β-penta-*O*-galloyl-D-glucose (**6**) is further transformed by oxidative coupling of pairs of adjacent galloyl ester groups (2, 3 and 4, 6) on the D-glucopyranose ring. Some facets of this pattern of metabolism were alluded to in earlier work[42]. Thus Wilkins and Bohm[31] and Nonaka *et al.*[43] have isolated β-1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose (**12**) from *Tellima grandiflora* and *Eugenia caryophyllata* respectively. Similarly Jurd[44] and Schmidt *et al.*[45] obtained 2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose (**16**, juglanin or pedunculagin) from walnut pellicles and oak galls respectively.

Plants whose phenolic metabolism places them within this category furnish, frequently along with β-penta-*O*-galloyl-D-glucose (**6**), one or more of the five hexahydroxydiphenoyl esters (**12–16**) as key phenolic metabolites. The presence of one or both of the isomeric α- and β-glucosides (**14** and **15**) is difficult to distinguish except by isolation. The occurrence of the α-isomer indeed presents something of a biosynthetic enigma since it seems reasonable to assume that all the esters (**12–16**) are derived by oxidative coupling of adjacent pairs of galloyl ester groups in the putative precursor (**6**). Tentatively it is suggested that the α-glucoside (**15**) is formed as indicated in the biogenetic scheme (Fig. 4). The ratio of the metabolites (**12–16**) varies markedly from plant to

Table 2. Class A, depside metabolites

Family and species	Number of depside galloyl groups (average value)
Metabolites based on β-penta-*O*-galloyl-D-glucose (**6**)	
Hamamelidaceae	
Hamamelis mollis	1–2
Parrotia persica	1–2
Paeoniaceae	
Paeonia officinalis	1–2
Ericaceae	
Arctostaphylos uva-ursi	1
Aceraceae	
Acer platanoides	1
A. campestre	1
A. rubrum	1
Anacardiaceae	
Rhus typhina	2
R. coriaria	2
R. semialata (galls)	2
Pistacia terebinthus	—
Schinus lentiscifolius	—
Cotinus coggygria	1–2
Mangifera indica	—
Geraniaceae	
Pelargonium spp.	1–2
Metabolites based on 2,6-digalloyl-1,5-anhydro-D-glucitol	
Aceraceae	
Acer saccharinum	1
A. tartaricum	1
A. ginnale	1

plant and has distinct possibilities for molecular taxonomy within a given plant family (Table 3). Concomitant with these variations there are parallel variations in the occurrence of the substances denoted on the composite paper chromatogram as T_1–T_4 (Fig. 5). Thus in the plant family Rosaceae, leaves of *Rubus idaeus*, *R. fruticosus*, *Geum rivale* and various *Potentilla* spp. predominantly metabolize esters **14–16** and these occur alongside the principal phenolic metabolite T_1. In the older terminology this compound may well be described as the 'tannin' of these plant species. Amongst other members of the Rosaceae different patterns are found (Table 3) which suggest a biogenetic relationship between esters **12–16** and the metabolites T_1–T_4. As yet only the ester T_1 has been examined in detail and the structure proposed fully bears out this proposition. The ester T_1 has a 'dimeric' structure formed, presumably by oxidative coupling, of one molecule of the β-glucoside(**14**) with one of the α-glucoside (**15**). All the features of this structure (**17**) have been confirmed (Haddock, E. A. *et al.*, unpublished results) except the position of the linkage from the galloyl ester group of the β-glucosidic fragment (**14**) to the 4,6-hexahydroxydiphenoyl ester group of the α-glucoside (**15**). Hexahydroxydiphenic acid as it bridges the 2,3 and 4,6-positions of the D-glucopyranose ring has the *S*-configuration ([46] and Scopes, P. M. and Haslam, E., unpublished results). This chirality follows from theoretical considerations and it does not appear to have biosynthetic or taxonomic implications [38].

In the plants surveyed (Table 1) there appears to be little overlap in individual species with group A (*vide supra*) and group C (*vide infra*) metabolites. However in this context it is interesting to note the cases of various *Fuchsia* spp., where group C metabolities are also detected, and *Quercus infectoria*. In this latter instance although the leaves of this plant only metabolize esters **12–16** the familiar galls produced by this plant (Aleppo galls) also produce substantial amounts of a gallotannin (class A) [33].

Assuming the validity of the Schmidt–Mayer hypothesis [4] it is clear that, for class B metabolites, oxidative coupling of galloyl groups takes place with the precursor (**6**) adopting the energetically preferred ($^4C_1$) conformation of the sugar ring [47]. Coupled with the observation (Table 1) that this is the predominant mode of further oxidative metabolism of galloyl esters in plants it seems reasonable to conclude that for plants it is the energetically preferred pathway.

Class C—hexahydroxydiphenol, dehydrohexahydroxydiphenoyl ester metabolites-D-glucopyranose ($^1C_4$)

Although investigations are still at a relatively early stage and the parameters which define the limits of this third class of metabolites may ultimately need

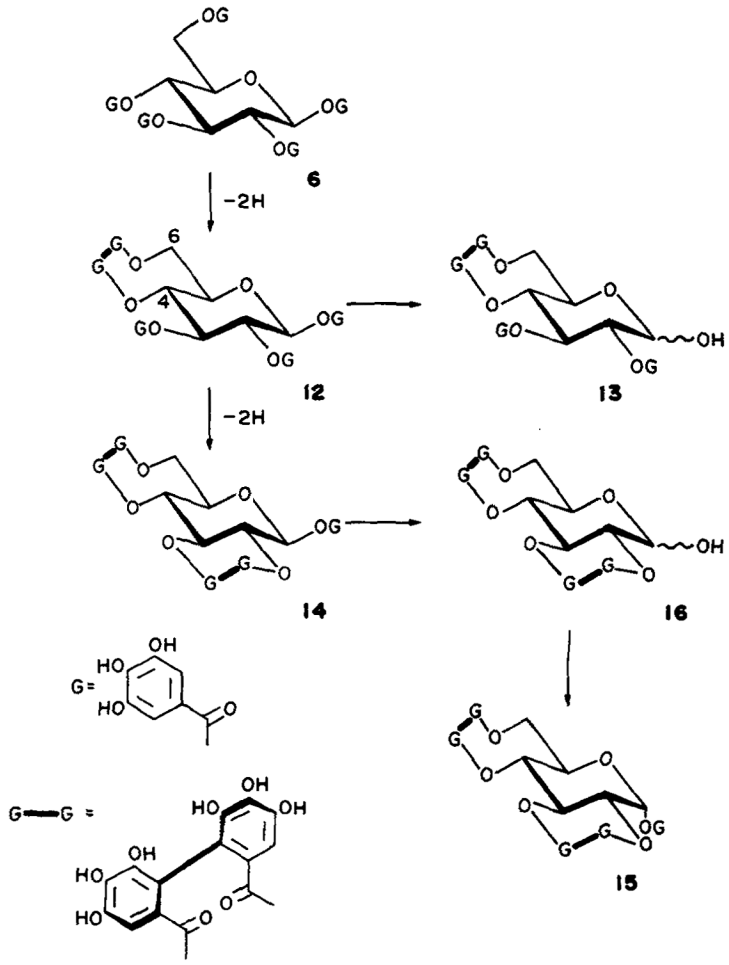
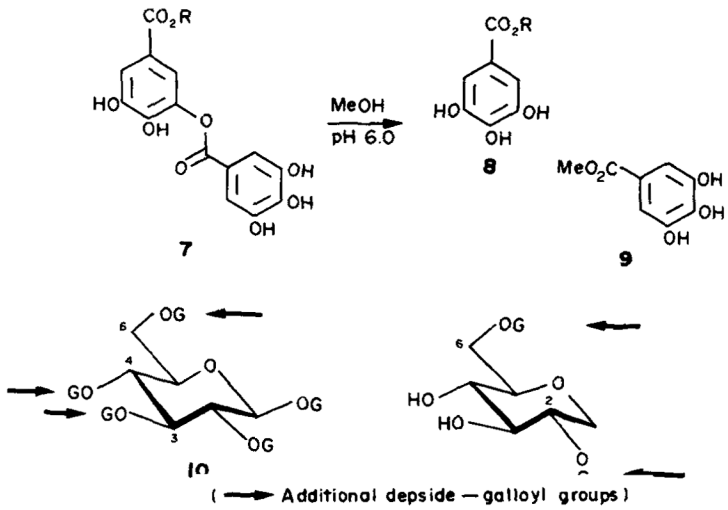


Fig. 4. Class B metabolites: biogenetic relationships.



adjustment and refinement, preliminary indications suggest that a much smaller group of plants operate a different mode of further oxidative metabolism of galloyl esters to that noted above. Work in this laboratory suggests that plants within this third category furnish products of oxidative coupling via energetically less favourable conformations of the precursor (6). In some cases this may be the all axial $^1C_4$

Table 3. Class B metabolites, (*S*)-hexahydroxydiphenoyl esters, D-glucopyranose – $^4C_1$

Family and species	Metabolites								
	12	**13**	**14/15**	**16**	T_1	T_{2A}	T_{2B}	T_3	T_4
Hamamelidaceae									
Liquidambar styraciflua	++++	++	+	+	–	–	–	–	–
Fagaceae									
Castanea sativa	+++	++	++	++		+	+		
Quercus borealis	+++	++++	–	–		+	+	+++++	
Q. robur	+++	tr	+++	++			tr		
Q. petraea	–	–	+	+++	+		+		
Q. hispanica	+++	tr	+++	++		+	+		
Q. castaneafolia	+	–	+	+++		+	tr		
Q. aegilops	–	–	–	++	–	+	+		
Q. alba	+++	+	+++	++		tr	tr		
Q. palustris	++	++	–	tr		+	+		
Q. lusitanica	+++	tr	++	+		+	+		
Q. ilex	+++	+	++	++		+			
Q. infectoria	–	–	–	+++					
Q. infectoria (galls)	+++	tr	++	+					
Q. cerris	+++	–	++	++		+			
Q. macracantha	tr		tr	tr		tr	tr		
Theaceae									
Camellia japonica	–	–	–	++					
Rosaceae									
Rosa canina	+++	++	++	+		++	++		
Filipendula ulmaria	+++	++	–	–		++	++		
Rubus idaeus	+	tr	+++++	+	+++++				
R. fructicosus	tr	tr	+++	+	+++++				
Fragaria × ananasa	–	–	tr	+	+++				
Potentila procumbens	–	–	+++	++	+++				
P. anserina	–	–	+	++	+++				
P. sterilis	–	–	tr	tr	tr				
Geum rivale	+	–	+++	+	+++++				
Saxifragaceae									
Bergenia crassifolia	+	–	+	–		+			
B. cordifolia	+	–	+	–		+			
Tellima grandiflora	+++	++	–	–		++	+++		
Myrtus communis									
Myrtus communis	+	+	+	–		++	++		
Eucalyptus viminalis	+++	++	tr	+		++	++		
E. acaciformis	+	+	+	+			+		
E. globuli	–	–	–	+++					
Onagraceae									
Fuchsia spp.	++	++	–	–					
Epilobium angustifolium	++	++	–	–					
Combretaceae									
Combretum paniculatum	–	–	–	+			+		
Quisqualis mussaendiflora	+	+	+	++	+		+++++		
Cornaceae									
Cornus mas	++	+++	–	–		++	++		
C. alba	++	+++	–	–		++	++		
C. controversa	++	+++	–	–		++	++		
Elaegnaceae									
Hippophae rhamnoides	+++	tr	–	–		+	++		
Juglandaceae									
Juglans nigra	–	tr	+	+				++	
J. regia		–	tr	+	++				++

Relative amounts (generally by visual examination of chromatograms) are denoted by a series of '+' signs; tr, indicates traces.

12, β-1,2,3,-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose.
13, 2,3-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose.
14, β-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose.
15, α-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose.
16, 2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose.

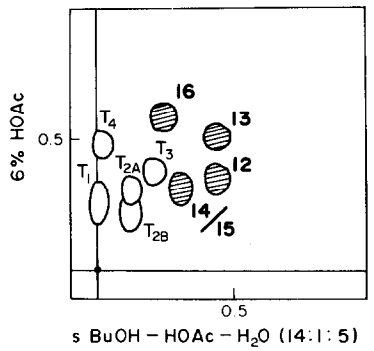
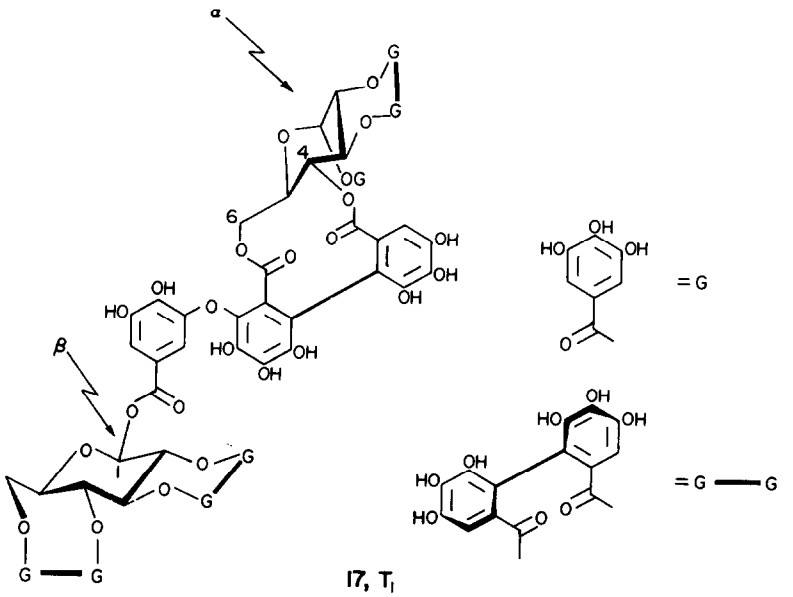


Fig. 5. Class B metabolites: paper chromatographic fingerprint formulae (**17**, T_1).

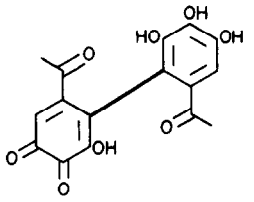
conformation and in others skew boat variations[48]. The products of oxidative metabolism include hexahydroxydiphenoyl esters and derivatives of the dehydrohexahydroxydiphenoyl ester group (**18**) whose apparent stability in plant products may at first seem somewhat surprising in view of its *ortho*-quinonoid structure. The presence of the dehydrohexahydroxydiphenoyl ester group in plant extracts is confirmed by its distinctive spectroscopic features[38, 46], by the smooth formation of a phenazine condensation product with *o*-phenylene diamine[49, 50] and by its reduction products (platinum/hydrogen)[38]. Isomerization of the group (**18**) occurs in solution and leads to an equilibrium mixture of isomers. Recently Okuda *et al.* [46] proposed that the isomerization involved the formation of the two hemi-acetal structures (**18a**, **18b**). In a screening process the presence of the dehydrohexahydroxydiphenoyl ester group is most satisfactorily determined by a combination of the nitrous acid spray (*vide supra*), by PC before and after catalytic reduction and by the change in UV absorption concomitant upon formation of a phenazine in acetonitrile solution.

Geraniin (**19**) is one of the key metabolites of this metabolic pathway and its presence dominates the phenolic extracts of plants where it has been found. In addition to the plants listed in Table 4 Okuda *et al.*[51] have recently discussed its distribution in members of the Geraniaceae and Euphorbiaceae. Co-occurring with geraniin (**19**) in the plants listed is a substance isomeric with it and into which it is transformed by refluxing in water. This substance is named *iso*-geraniin (Haddock, E. A. *et al.*, unpublished results). A further isomer has been obtained both in this laboratory and by Okuda *et al.*[52] from *Punica granatum* who has assigned to this metabolite (granatin B) a structure identical to geraniin but in which the dehydrohexahydroxydiphenoyl group is in the enantiomeric configuration. Further work is in progress to establish the relationship of these two substances to geraniin itself. From the leaves of *Davidia involucrata* β-1,6-(*S*)-hexahydroxydiphenoyl-2,3,4-tri-*O*-galloyl-D-glucose and a substance provisionally formulated as β-1,6-(*S*)-hexahydroxydiphenoyl - 2,4 - dehydrohexahydroxydiphenoyl - D - glucose have been isolated and the main burden of the evidence at this stage tends to suggest that the occurrence of the dehydrohexahydroxydiphenoyl ester group (**18**) in these metabolites is associated with oxidative metabolism via the relatively unfavourable $^1C_4$ conformation (**20**, and its variants) of the precursor (**6**). Only in these conformations can the oxidative coupling between adjacent pairs of galloyl ester groups (1,6; 3,6; 2,4) occur. Not surprisingly therefore this is probably a higher energy metabolic pathway and its occurrence in the Plant Kingdom appears to be much more sporadic (Tables 1 and 4). It should be noted, however, that the structures proposed by Schmidt *et al.*[49, 53] for the brevilagins (from Algarobilla) and by Okuda *et al.*[52] for granatin A (from pomegranate) contain the dehydrohexahydroxydiphenoyl ester group linked to D-glucopryanose in its energetically favourable $^4C_1$ conformation. This may indicate the necessity at some stage to revise the premises on which class C has been defined.

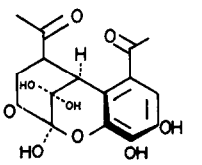
Geraniin (**19**) acts as a clear taxonomic marker in the Aceraceae and its distribution confirms earlier



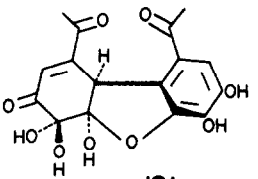
17, T_1



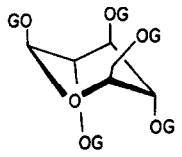
18



18a



18b



$20_1\,{}^1C_4$

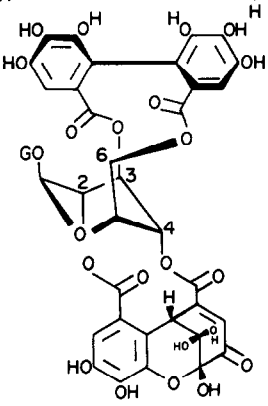


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Table 4. Class C metabolites, hexahydroxydiphenoyl and dehydrohexahydroxydiphenoyl esters, D-glucopyranose-($^1C_4$)

Family and species	Geraniin	Isogeraniin	Miscellaneous metabolites
Cercidiphyllaceae			
Cercidiphyllum japonicum	++++	+	
Ericaceae			
Arbutus unedo	+		
Onagraceae			
Fuchsia spp.	++		
Combretaceae			
Terminalia chebula (fruit)	–	–	corilagin[54], chebulinic acid[55, 56], chebulagic acid[55, 56], terchebin[56, 57]
Nyssaceae			
Davidia involucrata	–	–	β-1,6-(*S*)-hexahydroxydiphenoyl-2,4-dehydro-hexahydroxydiphenoyl-D-glucose, β-1,6-(*S*)-hexahydroxydiphenoyl-2,3,4-trigalloyl-D-glucose (Haddock, E. A. *et al.*, unpublished results)
Aceraceae			
Acer pseudoplatanus	++++	+	
A. monspessulanum	++++	+	
A. griseum	+++	+	
A. pensylvanicum	+++	+	
A. saccharum	+++	+	
A. spicatum	++++	+	
Punicaceae			
Punica granatum [58]	–	–	granatin B ([52] and Haddock, E. A. *et al.*, unpublished results)
Simaroubaceae			
Ailanthus altissima	+++	++	
A. giraldii	+++	++	
A. vilmoriana	+++	+	
Geraniaceae			
Geranium robertanium	++++	+	
G. pratense	++++	+	
G. lucidum	++++	+	
G. rotundifolium	+++	+	

predictions[30]. Finally the occurrence of this metabolite in *Fuchsia* spp. may also be noted. This plant genus remains, to date, the sole example noted of an overlap between the two forms of oxidative metabolism (classes B and C).

The structural patterns distinguished amongst gallic acid metabolites in plants are highly reminiscent of those found in other variants of secondary metabolism in other organisms, for example polyketide metabolism in moulds and fungi. A plausible explanation of the phenomenon in these organisms is that an accumulation of intermediates is built up in some primary pathway and that enzymes are induced for the synthesis of secondary products from one or more of these primary metabolites. Usually one key secondary compound is visualized as being formed and this then undergoes a wide range of chemical modifications leading to the production of a variety of secondary products only slightly different from each other. What is important from the organisms' point of view is to dispose of the primary accumulation product. Circumstantial evidence suggests that for many plants the key secondary metabolite in gallic acid metabolism in plants is β-penta-*O*-galloyl-D-glucose (**6**). The problem which must now be approached is to identify the primary accumulation product(s) responsible for the initiation of this form of secondary metabolism.

EXPERIMENTAL

General methods. Chromatographic procedures were as previously described[10, 33]. PC was carried out using Whatman No. 2 paper (27.5 cm^2) in solvent systems A, 6% HOAc and B, *iso*-BuOH–HOAc–H_2O (14:1:5) at 20 ± 3°. Galloyl esters were detected by sprays of ferric chloride–potassium ferricyanide[10, 33], 2,6 - dibromoquinone - *N* - chlorimide (Gibbs reagent)[10], diazotized benzidine and satd potassium iodate soln[30] and by their absorption or violet fluorescence (enhanced by fuming with NH_3) in UV light. Hexahydroxydiphenoyl esters were detected by the above spray reagents and by a nitrous acid spray (ice cool 10% $NaNO_2$ soln to which HOAc had been added). Geraniin was detected in plant extracts by its absorption in UV light, the formation of a phenazine derivative[49, 53] and the concomitant change in UV spectrum, and by catalytic reduction (Pt/H_2, 1 atm). A major product of this reduction is β - 1 - *O* - galloyl - 2,4:3,6 - bis - hexahydroxydiphenoyl - D - glucose; [R_f(A) 0.44; R_f(B) 0.25]. Methanolyses of depside galloyl esters were conducted by refluxing in MeOH (7 days) or by stirring in deoxygenated 0.5 N (pH 6.0) acetate buffer–MeOH (1:10) at 37° for 7 days[33].

R_f values in solvent A, followed by R_fs in solvent B for individual metabolites were recorded as follows: gallic acid 0.50, 0.62; β - penta - *O* - galloyl - D - glucose (**6**) 0.06, 0.50; β - 1,2,3,6 - tetra - *O* - galloyl - D - glucose 0.08, 0.48; β - 1,2,4,6 - tetra - *O* - galloyl - D - glucose 0.11, 0.45; β - 1,2,6 - tri - *O* - galloyl - D - glucose 0.30, 0.35; β - 1,6 - di - *O* - galloyl - D - glucose 0.43, 0.43; 2,6 - di - *O* - galloyl - 1,5 - anhydro - D - glucitol 0.26, 0.55; trigalloyl - 1,5 - anhydro - D - glucitol 0.13, 0.59; hexa - octagalloyl - β - D - glucose 0.0–0.35, 0.35–0.50; β - 1,2,3 - tri - *O* - galloyl - 4,6 - (*S*) - hexahydroxydiphenoyl - D - glucose 0.35, 0.37; 2,3 - di - *O* - galloyl - 4,6 - (*S*) - hexahydroxydiphenoyl - D - glucose 0.48, 0.40; α- or β - 1 - *O* - galloyl - 2,3:4,6 - bis - (*S*) - hexahydroxydiphenoyl - D - glucose 0.28, 0.25; 2,3:4,6 - bis - (*S*) - hexahydroxydiphenoyl - D - glucose 0.56, 0.15; complex ester T_1 0.29, 0.02; geraniin 0.17, 0.21; isogeraniin 0.17, 0.24; granatin B 0.16, 0.14; β - 1,6 - (*S*) - hexahydroxydiphenoyl - 2,4 - dehydrohexahydroxydiphenoyl - D - glucose 0.42, 0.17; β - 1,6 - (*S*) - hexahydroxydiphenoyl - 2,3,4 - tri - *O* - galloyl - D - glucose 0.15, 0.21.

HPLC. Isochratic (reverse phase) separations were carried out with a Du Pont 860 machine using a Zorbax-NH_2 column (250 × 4.6 mm) fitted with a guard column. Samples (~1 mg/ml) were filtered before injection (5 μl). The solvent composition (acetonitrile–H_2O–phosphoric acid) was varied from 4:1 to 20:1 (acetonitrile–H_2O) and contained 0.1% phosphoric acid. Compounds were determined against known standards.

Gradient elution utilized a Brownlee RP 18 Lichrosorb 10 μm (250 × 4.6 mm) column. Samples were eluted with a gradient of increasing acetonitrile concn (0–100%) in H_2O containing 0.05% phosphoric acid. Using a flow-rate of 1.4 ml/min and a solvent gradient profile of: (a) 1–5 min, 0% acetonitrile; (b) 5–50 min, 0–30% acetonitrile and (c) 50–60 min, 30–100% acetonitrile, components were eluted with the following retention times: gallic acid, 15.6 min; methyl gallate, 26.8 min; β - 1,3,6 - tri - *O* - galloyl - D - glucose, 37.1 min; β - 1,2,4,6 - tetra - *O* - galloyl - D - glucose, 43.4 min and β - penta - *O* - galloyl - D - glucose, 45.8 min. In the analysis of the 'gallotannins' peaks at 45.8–46.0 min were assigned to β - penta - *O* - galloyl - D - glucose, twin peaks at 48.6–48.8 and 49.6–49.8 min to hexagalloyl - D - glucose species, and a peak at 51.2–51.4 min to heptagalloyl - D - glucose species.

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