The Metabolism of Gallic Acid and Hexahydroxydiphenic Acid in Plants. Part 1. Introduction. Naturally Occurring Galloyl Esters

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The present state of knowledge of the metabolism of gallic acid in higher plants is reviewed and its unique features are noted. Methods for the structure determination of natural galloyl-D-glucose esters are outlined and applied to the elucidation of the structure of several new galloyl-D-glucose derivatives isolated from the leaves of higher plants. Finally the structure of the ' gallotannins ' based on β-penta-*O*-galloyl-D-glucose is reassessed on the basis of h.p.l.c. analysis and ^{13}C n.m.r. spectroscopic measurements.

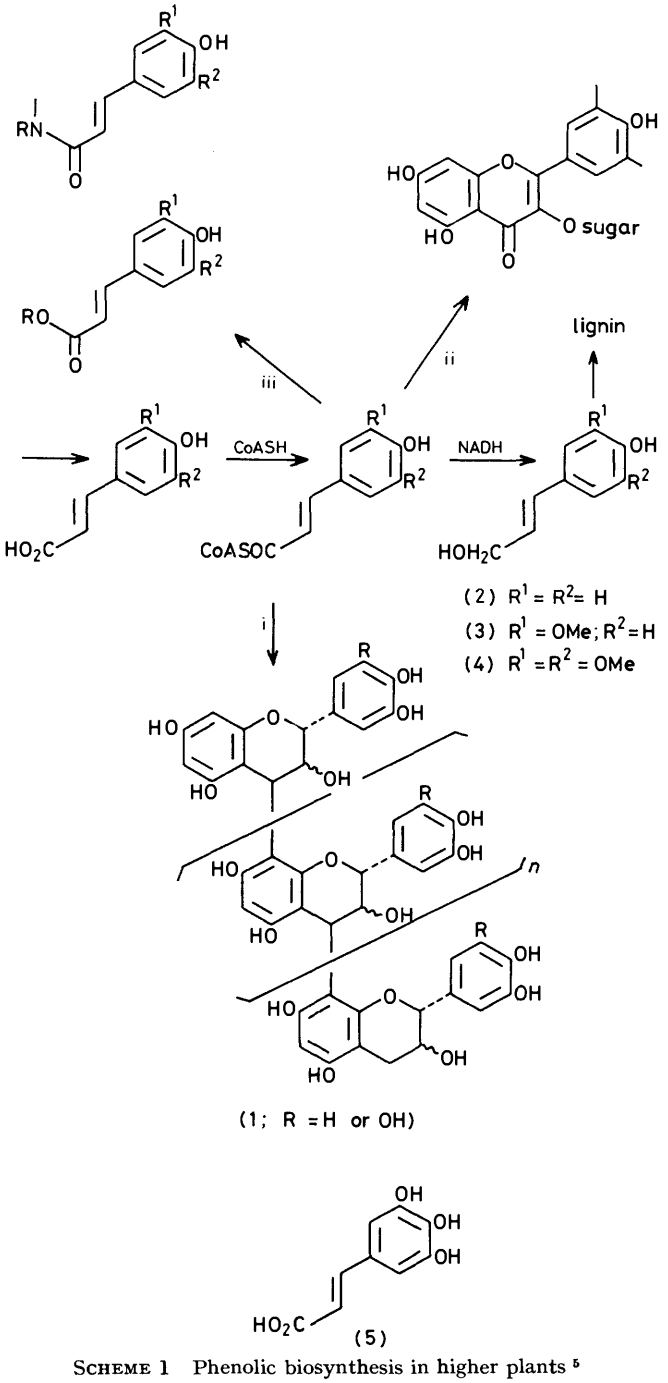
BATE-SMITH,[1] in a classical study, used the patterns of distribution of the three principal classes of phenolic metabolites—(i) proanthocyanidins, (ii) glycosylated flavonols, and (iii) hydroxycinnamoyl esters—which are found in the leaves of plants as a basis for their classification. The biosynthetic origin of these three classes of phenolic metabolite is generally believed to be associated [2] with the development of the capacity of plants to synthesise the structural polymer lignin by the diversion of L-phenylalanine, and in the Gramineae, L-tyrosine from protein synthesis. Vascular plants thus employ (Scheme 1) one or more of the *p*-hydroxycinnamyl alcohols [*p*-coumaryl (2), coniferyl (3), and sinapyl (4)], which are derived by enzymic reduction (NADH) of the coenzyme A esters of the corresponding hydroxycinnamic acids,[3] as precursors to lignin.[4] The same coenzyme A esters also form the points of biosynthetic departure for the three distinctive groups of phenolic secondary metabolites (i, ii, iii; [5] Scheme 1). However, uncertainties arise when the biosynthesis of flavonoids such as myricetin and the prodelphinidins (1; R = OH), which possess a 3,4,5-trihydroxylated ring B, are considered [6] since 3,4,5-trihydroxycinnamic acid (5) is not an intermediate in lignin biosynthesis and, except as its various *O*-methyl ethers, it has not been encountered in nature. In the context of this ambiguity Bate-Smith made a significant observation in his taxonomic work.[1] He found that hexahydroxydiphenic acid † (7; R = H) [Scheme 2; determined after acid hydrolysis as its characteristic dilactone ellagic acid, (8)] was widely distributed in the ester form (7) in plants. Bate-Smith speculated that hexahydroxydiphenic acid (7; R = H) was the taxonomic equivalent of the ' missing ' acid (5). There is, however, strong circumstantial evidence to support the proposition, originally made by Schmidt and Mayer,[7] that the esters (7) are derived in plant tissues by the oxidative coupling of bis-esters of gallic acid (6). Hence it is logical to regard gallic acid (6; R = H) as the systematic taxonomic equivalent of the ' missing ' acid, 3,4,5-trihydroxycinnamic acid (5).

Gallic acid thus occupies a distinctive position in phenol metabolism in plants; other features of its metabolism are similarly unique. Almost always it occurs in ester form but in contrast to other natural phenolic acids,[8] which are found invariably as mono- and occasionally bis-esters with polyols, gallic acid is encountered in a wide range of esterified forms ranging from simple mono-esters to the complex polyesters with D-glucose whose molecular weights extend to at least 2 000 daltons. Together with the proanthocyanidins (1) (Scheme 1) these complex esters indeed constitute the vegetable tannins [9] of the earlier botanical and chemical literature. Because of their earlier importance to commerce the tissues of those few plants which metabolise substantial quantities of these polyesters of gallic acid (and hexahydroxydiphenic acid) have been the subject of the most intensive chemical scrutiny.[10,11] This understandable attention has necessarily circumscribed our knowledge of the wider role of gallic acid metabolism over the whole plant kingdom and the purpose of this and subsequent papers is to seek to delineate this role more fully. Preliminary details of some of this work have been published.[12]

The accumulation of phenolic constituents in different organs and tissues of a plant may differ quite markedly in both a quantitative and a qualitative sense. However the capacity for synthesis of phenolic metabolites in the leaf is often a reliable guide to the general capacity of the plant as a whole. It also provides the most convenient basis for the comparison of phenolic biosynthesis in different plants [1] and it is used as such in this work. Various simple esters of gallic acid isolated from plants have been described earlier, *e.g.* esters with flavan-3-ols,[13,14] phenolic glycosides,[15–18] quinic acid [19] and flavonoid glycosides.[20,21] Most often, however, gallic acid occurs in association with sugars, in particular D-glucose, although apart from the elligitannins [10] and gallotannins,[11] (*vide infra*), only four naturally occurring galloyl-D-glucose derivatives have been previously described from natural sources—namely, β-D-glucogallin,[22] β-1,3,6-tri-*O*-galloyl-D-glucose,[23] β-1,3,4,6-tetra-*O*-galloyl-D-glucose,[24] and β-penta-*O*-galloyl-D-glucose [23]—as natural products in their own right.

Using Bate-Smith's original taxonomic observations [1] as the principal guide the phenolic metabolites from the

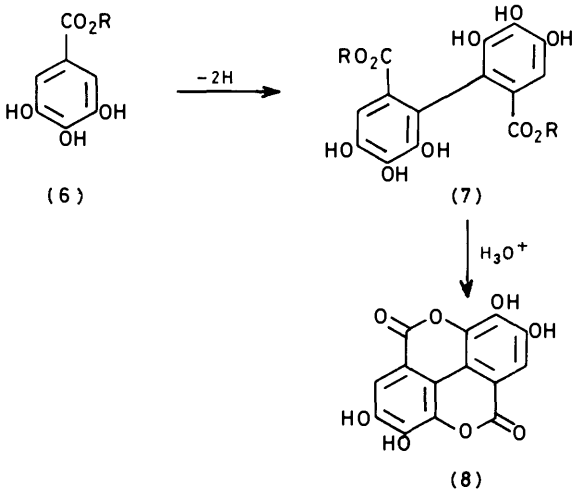
† This nomenclature is utilised as a convenient abbreviation for the 6,6-dicarbonyl-2,2′,3,3′,4,4′-hexahydroxybiphenyl radical.



SCHEME 1 Phenolic biosynthesis in higher plants [5]

leaves of over 150 plants have been examined. The preliminary analysis and screening of phenolic extracts was facilitated by h.p.l.c. and by paper chromatography which gave ' fingerprints ' of a plant's phenolic metabolism.[25] Galloyl esters were isolated by chromatography on Sephadex LH-20. Some esters crystallise from aqueous media, others separate as granular solids (*e.g.* β-penta-*O*-galloyl-D-glucose) and the remainder were isolated as amorphous solids by freeze-drying or by evaporation from acetone. In all cases homogeneity was established by h.p.l.c. and paper chromatography.

Structural information was generally most readily obtained using the free phenolic form of the ester. Hydrolysis with the adaptive enzyme tannase [26,27] is a specific means of cleavage of natural galloyl esters to give gallic acid and the associated polyol—usually D-glucose—which is identified by paper chromatography and in the



SCHEME 2 Biogenesis of hexahydroxydiphenoyl esters [7]

case of D-glucose determined quantitatively using the anthrone procedure.[26] High-resolution (220 and 400 MHz) ^{1}H n.m.r. spectroscopy combined with spin decoupling and the knowledge [28] that acylation of a hydroxy-group of the parent polyol produces a substantial downfield shift of the associated methine or methylene protons has provided a complete structural analysis for the natural galloyl-D-glucose derivatives which have been isolated (Table 1).* When the anomeric centre is acylated the galloyl ester group, in the cases encountered to date, invariably adopts the β-configuration and the D-glucopyranose ring the C-1 or $^4C_1$ conformation.[29] Immediate recognition of these two features is given by the low-field doublet for the anomeric proton ([$^2H_6$]acetone, δ 6.39—5.7, J_{12} 9.5 Hz). The position of this signal is remarkably sensitive to the presence of other galloyl ester groups in the molecule and is a convenient index of the extent of esterification of the D-glucopyranose residue (Table 1). The galloyl ester groups themselves are readily distinguished by a series of two-proton singlets ([$^2H_6$]acetone, δ 6.95—7.25) and by the ^{13}C resonances due to the ester carbonyl groups, Table 2. The position of these resonances is solvent sensitive. Thus in prototropic media ([$^2H_4$]methanol) they are displaced downfield from $SiMe_4$ relative to the signals observed in [$^2H_6$]acetone and this is probably due to hydrogen bonding of solvent to the carbonyl oxygen in methanolic media. The relative intensities of these signals and their multiplicity provides an additional confirmation of the extent of esterification of the D-glucopryanose ring. The assignment of the individual ^{13}C resonance signals to the

* Throughout δ values are quoted in p.p.m. from $SiMe_4$.

TABLE 1

^{1}H N.m.r. characteristics of some galloyl-D-glucose derivatives in [$^2H_6$]acetone: chemical shift (δ values, $SiMe_4$): coupling constants J (Hz)

β-D-Glucopyranose Derivative	β-D-Glucopyranose protons							Aryl protons
	1-H	2-H	3-H	4-H	5-H	6-H	6-H	
1. 1,2,3,4,6-Pentagalloyl	6.39 (d, J 9.5)	5.66 (t, J 9.5)	6.06 (t, J 9.5)	5.70 (t, J 9.5)	4.6 (m)	4.45 (dd, J 12, J 3)	4.6 (m)	7.02, 7.08, 7.10, 7.17, 7.23
2. 1,2,3,6-Tetragalloyl	6.17 (d, J 9.5)	5.49 (t, J 9.5)	5.71 (t, J 9.5)	4.10, 4.20 (bs)	4.10, 4.20	4.63 (m)	4.63 (m)	7.02, 7.10, 7.12, 7.20
3. 1,2,4,6-Tetragalloyl	6.10 (d, J 9.5)	5.45 (t, J 9.5)	4.40 (t, J 9.5)	5.42 (t, J 9.5)	4.3 (m)	4.3 (m)	4.53 (bd, J 13)	7.12, 7.14, 7.18, 7.18
4. 1,3,6-Trigalloyl [23]	5.89 (d, J 9.5)	3.98 (m)	5.35 (t, J 9.5)	3.98 (m)	4.50 (m)	4.26 (dd, J 13, J 5)	4.51 (dd, J 13, J 2)	7.14, 7.17, 7.17
5. 1,2,6-Trigalloyl	5.95 (d, J 9.5)	5.25 (t, J 9.5)	4.0 (t, J 9.5)	3.76 (t, J 9.5)	3.95 (dd, J 9.5, J 4.5)	4.49 (dd, J 12.0, J 4.5)	4.56 (d, J 12.0)	7.06, 7.09, 7.14
6. 1,6-Digalloyl	5.77 (d, J 9.5)	3.6–3.85 (m)	3.6–3.85 (m)	3.6–3.85 (m)	3.6–3.85 (m)	4.42 (dd, J 12.0, J 5.0)	4.57 (d, J 12.0)	7.05, 7.08

carbon atoms of the D-glucopyranose ring were made in collaboration with Dr. B. F. Talyor [30] and the values for β-penta-*O*-galloyl-D-glucose accord with the relative positions of the ^{13}C signals for D-glucopyranose and its derivatives recorded elsewhere.[31,32] In all cases the anomeric carbon atom occurs at lowest field (δ 93.4—95.5) and C-6 at highest field (δ 62.9—63.8). The position of these signals is sensitive to the presence or absence of invariably they occur as singlets at δ 109.8—110.6 and δ 145.9—146.3 respectively) but those due to the various atoms C-1 (δ 120—122,) and C-4 (δ 138—139) are generally resolved. Like the signals due to the carbon atoms of the ester carbonyl groups they similarly provide a convenient index of the number of galloyl ester groups in the molecule (Figure 2). Structural analysis of galloyl-D-glucose derivatives in which the anomeric

TABLE 2

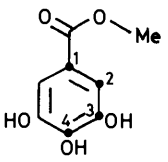
^{13}C N.m.r. characteristics of galloyl-β-D-glucose derivatives ^{13}C chemical shift (δ values from $SiMe_4$).

β-D-Glucopyranose derivatives	β-D-Glucopyranose carbon atoms						Galloyl carbonyl carbon atoms
	C-1	C-2	C-3	C-4	C-5	C-6	
1. 1,2,3,4,6-Pentagalloyl [a]	93.7	72.1	74.2	69.7	74.2	63.0	167.8, 167.1, 166.9 (2), 166.1
[b]	93.4	71.9	73.5	69.5	74.1	62.9	166.4, 165.7 (3), 165.0
2. 1,2,3,6-Tetragalloyl [a]	93.8	*76.5(2),	72.3,	69.6		63.9	168.1, 167.6, 167.1, 166.2
3. 1,2,4,6-Tetragalloyl [b]	93.4	*74.0,	73.8,	73.3,	71.7	63.1	166.3, 165.8 (2), 165.0
4. 1,2,6-Trigalloyl [b]	93.5	*76.0,	75.4,	73.8,	71.1	63.8	166.6, 165.9, 165.0
5. 1,3,6-Trigalloyl [23, b]	95.5	*78.6,	75.8,	72.3,	69.2	63.8	166.5 (2), 165.3
6. Neochebulinic Acid [33, b]	95.1	*77.4,	76.0,	73.4,	72.4,	62.9	166.6 (2), 165.4

[a] [$^2H_4$]Methanol. [b] [$^2H_6$]Acetone. * Signals not assigned.

an ester group at C-2 or C-4 respectively. Thus the signal due to C-1 shifts to lower field in β-1,3,6-trigalloyl-D-glucose and neochebulinic acid [33,34] (position 2 of D-glucopyranose free) and similarly that due to C-6 shows an analogous but generally smaller downfield shift when position 4 is unacylated (Table 2, β-1,2,6-trigalloyl-, β-1,3,6-trigalloyl-, and β-1,2,3,6-tetragalloyl-D-glucopyranose). Assignment of the ^{13}C resonances due to the carbon atoms of the aryl rings of the galloyl ester groups were made relative to those of methyl gallate (Figure 1). In polygalloyl esters of D-glucose the individual ^{13}C resonances due to C-2 and C-3 of the different galloyl ester groups are frequently not resolved at 25.15 MHz (*almost* hydroxy-group is not acylated is by comparison more difficult to achieve using the spectroscopic methods outlined above. In solution these derivatives may not only set up equilibrium mixtures of the α- and β-forms but they may also assume alternative conformations of the D-glucopyranose ring to that ($^4C_1$ or $^1C_4$) normally preferred.[29]

Table 3 lists the various galloyl-D-glucose derivatives which have now been isolated and identified in the leaves of plants. It is interesting to note amongst the partially esterified D-glucose derivatives now isolated the apparent predisposition of the ester groups to be located at positions 1, 2, and 6 on the D-glucopyranose ring. In the

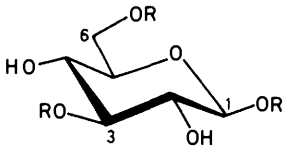


^{13}C δ/p.p.m. values from $SiMe_4$ in [$^2H_6$]acetone

Aryl C atom	[$^2H_6$]acetone	Calculated [31]
C-1	121.4	125.7
C-2	110.0	109.5
C-3	146.4	144.3
C-4	139.7	133.9

FIGURE 1 Chemical shift of the aryl carbon atoms of methyl gallate. Calculated values based on Stothers [31]

case of the 2 and 6 positions of the D-glucopyranose molecule these observations accord with the relative preferences shown for these positions in the *in vitro* esterification observed with acyl halides and α-methyl-D-glucopyranoside [35] and β-methyl-D-glucopyranoside.[36] These general observations prompted the suggestion that the structure of Aceritannin—isolated from *Acer ginnale* by Perkin and Uyeda [37] and assigned the structure of 3,6-di-*O*-galloyl-1,5-anhydro-D-glucitol by Kutani [38]—might require revision. The compound was isolated from leaves of *Acer saccharinium* and *Acer tartaricum* and ^1H n.m.r. spectroscopy with proton decoupling showed unequivocally that it indeed possessed the 2,6-bisgalloyl-1,5-anhydro-D-glucitol (9) structure. This same structure was confirmed during this work by Nielsen and his collaborators.[39] Accompanying the bisgalloyl ester in



R = CO·$C_6H_2(OH)_3$ -3, 4, 5

Aryl C atom	^{13}C δ/p.p.m. values from $SiMe_4$ in [$^2H_6$]acetone
C-1	120.7, 121.5, 121.9
C-2	110.0 (2), 110.2 (1)
C-3	145.9
C-4	138.2, 138.6, 138.9

FIGURE 2 Chemical-shift values of the aryl carbon atoms of the galloyl ester groups of β-1,3,6-tri-*O*-galloyl-D-glucose

TABLE 3

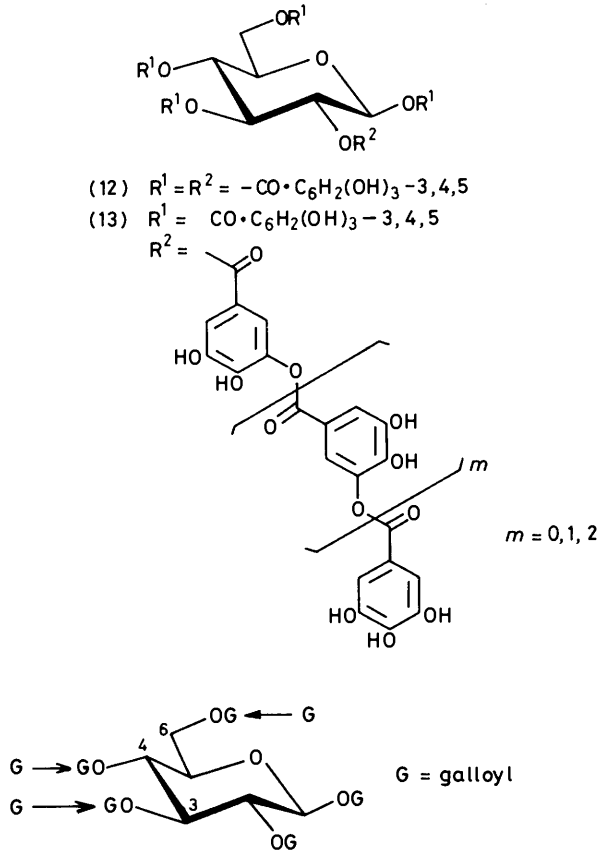
Naturally occurring galloyl esters of β-D-glucose and related polyols

	Galloyl ester	Plant	Plant family	Ref.
1.	β-1-*O*-Galloyl-D-glucose (β-D-glucogallin)	*Rheum officinale* (root)	Polygonaceae	22
2.	β-1,6-Di-*O*-galloyl-D-glucose	*Quercus infectoria* (galls)	Fagaceae	*a*
3.	β-1,2,6-Tri-*O*-galloyl-D-glucose	*Rubus fructicosus* (leaves)	Rosaceae	*a*
		Rubus idaeus (leaves)	Rosaceae	*a*
		Rosa canina (leaves)	Rosaceae	*a*
		Fuchsia sp. (leaves)	Onagraceae	*a*
		Epilobium angustifolium (leaves)	Onagraceae	*a*
4.	β-1,3,6-Tri-*O*-galloyl-D-glucose	*Terminalia chebula* (fruit)	Combretaceae	23
5.	β-1,2,3,6-Tetra-*O*-galloyl-D-glucose	*Fuchsia* sp. (leaves)	Onagraceae	*a*
		Epilobium angustifolium (leaves)	Onagraceae	*a*
		Quercus infectoria (galls)	Fagaceae	*a*
		Ceratonia siliqua (pods)	Leguminosae	*b*
6.	β-1,2,4,6-Tetra-*O*-galloyl-D-glucose	*Bergenia crassifolia* (roots and rhizomes)	Saxifragaceae	*a*
		Bergenia cordifolia (roots and rhizomes)	Saxifragaceae	*a*
7.	β-1,3,4,6-Tetra-*O*-galloyl-D-glucose	*Caesalpinia brevifolia* (pods)	Leguminosae	24
8.	β-Penta-*O*-galloyl-D-glucose	*Terminalia chebula* (fruit)	Combretaceae	23
		Acer platanoides (leaves)	Aceraceae	*a*
		Acer campestre (leaves)	Aceraceae	*a*
		Quercus infectoria (galls)	Fagaceae	*a*
		Quercus borealis (leaves)	Fagaceae	*a*
		Rhus semialata (galls)	Anacardiaceae	*a*
		Rhus coriaria (leaves)	Anacardiaceae	*a*
		Rhus typhina (leaves)	Anacardiaceae	*a*
		Cotinus coggyria (leaves)	Anacardiaceae	*a*
		Fuchsia sp. (leaves)	Onagraceae	*a*
		Epilobium angustifolium (leaves)	Onagraceae	*a*
		Rubus idaeus (leaves)	Rosaceae	*a*
		Rubus fructicosus (leaves)	Rosaceae	*a*
		Rosa canina (leaves)	Rosaceae	*a*
		Geranium robertanium (leaves)	Geraniaceae	*a*
9.	2,6-Di-*O*-galloyl-1,5-anhydro-D-glucitol (Aceritannin)	*Acer ginnale* (leaves)	Aceraceae	37,38,39
		Acer saccharinum (leaves)	Aceraceae	*a*
		Acer tartaricum (leaves)	Aceraceae	*a*
10.	2′,5-Di-*O*-galloyl-D-hamamelose (Hamameli tannin)	*Hamamelis virginiana* (bark)	Hamamelidaceae	*c*
		Quercus rubra (bark)	Fagaceae	*c*
		Castanea sativa (bark)	Fagaceae	*c*

[a] Isolated in this work. [b] Personal communication D. Magnolato. [c] W. Mayer, N. Kunz, and F. Loebich, *Liebigs Ann. Chem.*, 1965, **688**, 232.

the leaves of *Acer saccharinum* and *Acer tartaricum* are the 2- and 6-*O*-galloyl esters of the anhydro-sugar.

For a great many plants the metabolism of gallic acid begins and ends with their ability to synthesise esters with D-glucose and occasionally other polyols and phenols. However those plants able to biosynthesise β-penta-*O*-galloyl-D-glucose (12) are noteworthy: this intermediate appears to mark a biogenetic watershed from which other biosynthetic pathways subsequently diverge.[12] The ability to metabolise polyphenols in which additional gallic acid molecules are esterified to a pre-existing galloyl ester, such as (12), in the form of *meta*-depsides (Figure 3)



(14) ⟶ attachment of depside galloyl groups

represents one such biosynthetic capacity. It is here referred to as group A and the plant surveys suggest that it is limited to a comparatively few plant families. In the earlier literature the few examples then encountered were invariably referred to as gallotannins.[10,11] The structure of the complex polyphenol derived from the twig galls of *Rhus semialata* (Chinese gallotannin, *syn*-tannic acid) as a core of β-penta-*O*-galloyl-D-glucose (12) to which up to five other galloyl ester groups are linked in depside fashion was first proposed by Fischer and Freudenberg [40] and later refined by Haworth [26] and Haslam [41,42] to suggest that the depsidically bound galloyl ester groups are in the form of a chain of variable length, probably located at C-2 on the D-glucopyranose ring (13). Molecules with similar polyester structures based on β-penta-*O*-galloyl-D-glucose (12) have now been obtained from several additional plants (Table 4). The

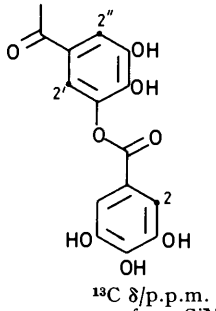
TABLE 4

Polygalloyl esters based upon β-penta-*O*-galloyl-D-glucose (12)

Molar ratio of methyl gallate : β-penta-*O*-galloyl-D-glucose [a]	Plant	Plant family
1.8	*Rhus semialata* (galls)	Anacardiaceae
2.0	*Rhus coriaria* (leaf)	Anacardiaceae
1.7	*Rhus typhina* (leaf)	Anacardiaceae
1.6	*Cotinus coggyria* (leaf)	Anacardiaceae
1.2	*Acer platanoides* (leaf)	Aceraceae
1.1	*Acer campestre* (leaf)	Aceraceae
1.2	*Acer rubrum* (leaf)	Aceraceae
1.1	*Arctostaphylos uva-ursi* (leaf)	Ericaceae
1.3	*Pelargonium* sp. (leaf)	Geraniaceae
1.3	*Paeonia officinalis* (leaf)	Paeoniaceae
1.5	*Hamamelis mollis* (leaf)	Hamameliadaceae
1.5	*Parrottia persica* (leaf)	Hamameliadaceae

[a] Determined after methanolysis [26] by h.p.l.c.

presence of depsidically linked galloyl ester groups is indicated by methanolysis,[26] ^{1}H n.m.r.[41,42] and by ^{13}C n.m.r. measurements. In the ^{13}C n.m.r. spectrum the change in chemical shift of C-2′ and C-2″ in the aromatic ring of depsidically linked galloyl esters, compared to C-2 in methyl gallate, is characteristic (Figure 3). Analo-



Aryl C atom	^{13}C δ/p.p.m. values from $SiMe_4$ in [$^2H_6$]acetone
C-2	110.8
C-2′	117.7
C-2″	114.8

FIGURE 3 Chemical-shift values of the aryl carbon atoms of depside galloyl esters

gous changes are observed in the associated protons in the ^{1}H n.m.r. spectrum [41,42] and under high resolution (220 and 400 MHz) the positions of absorption of the seven protons of the β-D-glucopyranose ring are virtually identical to those of the parent β-penta-*O*-galloyl-D-glucose (12) (Table 1). Methanolysis [26] splits the depsidically linked galloyl ester groups from the β-penta-*O*-galloyl-D-glucose core (12) and the number average value of depsidically linked galloyl ester groups may be determined by quantitative h.p.l.c. to give the molar ratios of (12) and methyl gallate (Table 4).

Although the principal structure in which gallic acid is found combined in depside form is that in association with β-penta-*O*-galloyl-D-glucose (12), other esters of this class have been described.[43,44] A further important example has been isolated from *Acer saccharinum* and

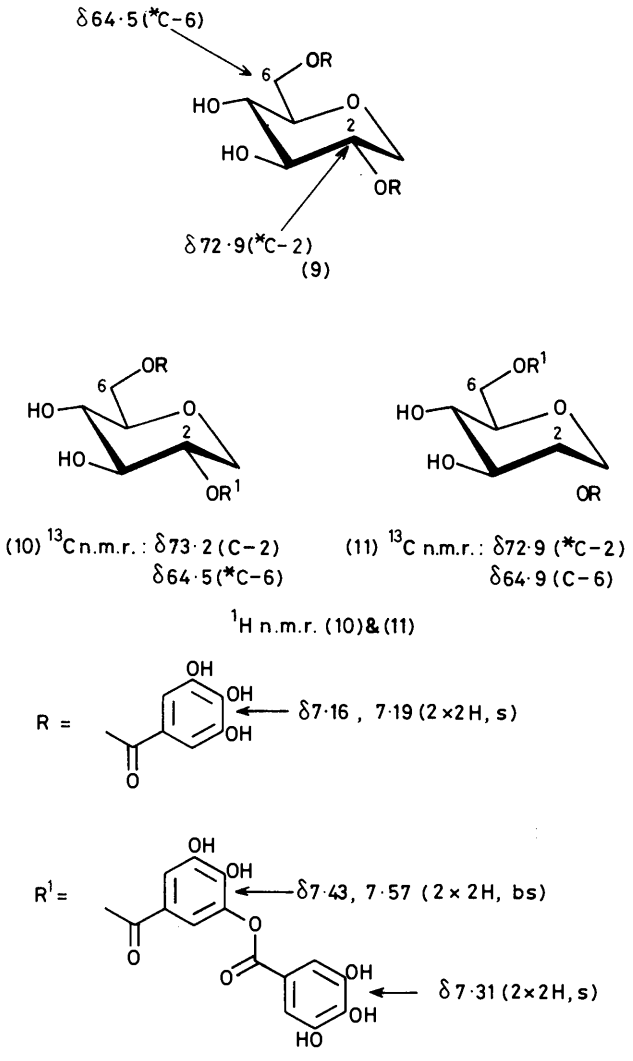


FIGURE 4 Depsides from *Acer saccharinum*. ^{1}H and ^{13}C N.m.r. data

Acer tartaricum along with the bisgalloyl ester (9). The structural assignment of this product as an approximately equimolar mixture of the two trigalloyl esters (10) and (11) is based upon the following considerations. Methanolysis [26] gave 2,6-bisgalloyl-1,5-anhydro-D-glucitol (9) and methyl gallate in equivalent molar proportions (h.p.l.c.). In the ^{1}H n.m.r. spectrum (Figure 4) the product showed a series of signals from the aliphatic protons analogous to those for compound (9), but the aromatic region displayed signals due to two non-equivalent *m*-digalloyl ester groups and two non-equivalent simple galloyl ester groups. In the ^{13}C n.m.r. spectrum signals at δ 114.5, 117.5, and 117.3 were attributed (*vide supra*) to the presence of depside galloyl ester groups and the signals associated with the aliphatic carbon atams of the anhydro-sugar were identical to those recorded for (9). Significantly, however, the signals due to C-2 and C-6 occurred as sharp doublets in the proton-decoupled spectrum (Figure 4). Each doublet has a separation of 0.3—0.4 p.p.m. and one half of each doublet was a signal coincident with that due to C-2 or C-6 in (9) (Figure 4). The remainder of each doublet, with the small perturbation in ^{13}C chemical shift, has been attributed to the presence of species in which C-2 or C-6 is esterified to *m*-digallic acid (*i.e.* (10) and (11) respectively] as opposed to gallic acid. This analysis was confirmed by the observation in the ^{13}C n.m.r. of Δδ(Me) 0.4 p.p.m. between the methyl esters of gallic and *m*-digallic acids.

This observation has been used as the basis for a critical re-examination of the structure (13) proposed in the 1960's [41,42] for the polyesters based on β-penta-*O*-galloyl-D-glucose (12) (Table 4). Samples of each polyester were isolated by methods previously described [15,26] and examined by ^{13}C n.m.r. spectroscopy. They showed striking similarities such as to reaffirm the belief that they are all based on a common structural pattern related to compound (12). The ^{13}C signals due to several of the aliphatic carbon atoms of the D-glucopyranose ring, however, displayed small but significant differences from analogous signals in the spectrum of (12). The complex polyester from the leaves of Norway maple (*Acer platanoides*) was obtained by chromatography on polyamide [15,26] and analysed as a hexa- to

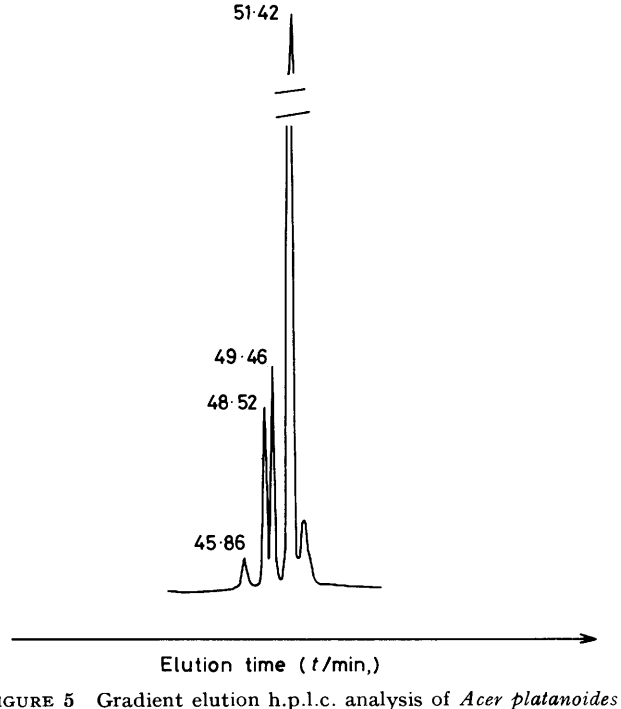


FIGURE 5 Gradient elution h.p.l.c. analysis of *Acer platanoides* gallotannin. Fraction 4. t_R 45.86 min, β-penta-*O*-galloyl-D-glucose; t_R 48.52, 49.46 min, hexagalloyl-D-glucoses; t_R 51.42 min, heptagalloyl-D-glucoses

TABLE 5

Fractionation of gallotannin from *Acer platanoides*

Fraction	H.p.l.c. analysis Mole fraction galloyl–glucose penta	hexa	hepta	^{13}C N.m.r.-β-D-glucopyranose (δ values, $SiMe_4$; in [$^2H_6$]acetone) C-1	C-2	C-3	C-4	C-5	C-6	Mole ratio methyl gallate [a]
1	0.80			93.4 (1.0)	71.9 (1.16)	73.5 (1.02)	69.5 (1.01)	74.1 (1.11)	62.9 (0.80)	
2	0.82			93.5	71.9	73.5	69.5	74.1	62.9	
3	0.23	0.64	0.03	93.4	71.8	73.4 *	69.7 69.4	74.1 ‡	62.9	0.7
4	0.07	0.38	0.43	93.4	71.9	73.4 *	69.7 69.4	74.0 ‡	62.9 63.3	1.2
5	0.01	0.16	0.77	93.4	71.9		69.7 69.4	74.0 ‡	62.9 63.4	1.6
6	0.02	0.18	0.74	93.4	71.9		69.7 69.4	74.0 ‡	62.8 63.4	1.5
7	0.01	0.14	0.81	93.4 (1.0)	71.8 (1.16)		69.7 (0.35) 69.4 (0.44)	74.0 (2.2)	62.8 (0.22) 63.4 (0.54)	1.8

[a] Mole ratio of methyl gallate : β-penta-O-galloyl-D-glucose produced on methanolysis.[41] * Signal intensity decreased. ‡ Signal intensity increased. Figures in parenthesis indicate relative signal strength to that of C-1.

hepta-galloyl-D-glucose derivative (Table 4). The ester was further fractionated on Sephadex LH-20 to give fractions of approximately equal mass which were examined by ^{1}H n.m.r. and ^{13}C n.m.r. (Table 5) spectroscopy and by gradient elution h.p.l.c. analysis (Figure 5 and Experimental section). The h.p.l.c. traces obtained for each fraction were interpreted to show the ratios of penta-, hexa-, and hepta-galloyl-D-glucose species in each fraction (Table 5). Particularly significant are the changes observed in the ^{13}C n.m.r. signals from the individual carbon atoms of D-glucose in each fraction and the overall changes noted in passing from fractions 1 and 2 (largely β-penta-*O*-galloyl-D-glucose) to fractions 6 and 7 (largely heptagalloyl-D-glucose species). Signals due to C-1 and C-2 remained as sharp singlets unchanged in their width and intensity (1 : 1.16). Changes were however observed in the signals due to C-3, C-4, and C-6. The spectra showed for each of these atoms the appearance of a satellite signal, shifted downfield from $SiMe_4$, in passing from fraction 1 to 7 (Table 5)—it is presumed that the satellite signal from C-3 merges with that due to C-5 at 74.0 p.p.m. from the change in relative intensity of this particular signal. On the basis of the earlier arguments noted above the legitimate conclusion of these observations is that the polyester from *Acer platanoides* is a mixture of very closely related species ranging from β-penta-*O*-galloyl-D-glucose (12) to hepta- and possibly octa-galloyl-D-glucose species in which the additional galloyl ester groups are linked as *meta*-depsides preferentially to the galloyl ester groups at C-3, C-4, and C-6 (14). Similar analyses, with analogous conclusions, have been performed on the polygalloyl esters from *Acer campestre*, *Pelargonium* sp. and *Paeonia officinalis* (Table 4).

The weight of evidence thus favours the view that the heterogeneity of the gallotannins derives from a ' partly random ' addition of further gallic acid molecules, by esterification as *meta*-depsides, to the β-penta-*O*-galloyl-D-glucose core (12). It therefore supports the view, originally expressed by Emil Fischer,[45] that these substances are not only mixtures of isomers but also of substances of differing empirical formulae.

EXPERIMENTAL

General Methods.—Chromatographic methods were as previously described.[26,46] Paper chromatography was carried out using Whatman No. 2 paper (27.5 cm^2) in the solvent systems A, 6% acetic acid and B, butan-2-ol–acetic acid–water (14 : 1 : 5, v/v) at 20 ± 3 °C. Galloyl esters were detected by their absorbtion or violet fluorescence (enhanced by fuming with ammonia) under u.v. light, by various phenolic spray reagents,[26,46] and by a spray of a saturated aqueous potassium iodate which revealed galloyl esters as red to pink spots [47] and gallic acid as an orange-red (purpurogallin carboxylic acid). The enzyme tannase was prepared as previously described [27,48] and the hydrolysis of galloyl esters was conducted in 0.1M-acetate buffer (pH 5.0) at 30 °C.[26,46] Glucose was determined by an adaptation of the anthrone procedure of Park and Johnson.[49] Methanolysis of galloyl esters was conducted by refluxing in methanol (7 days) or by stirring in deoxygenated 0.5M, pH 6.0, acetate buffer methanol (1 : 10, v/v) at 37 °C for 7 days.[26]

High-pressure Liquid Chromatography.—All solvents were redistilled before use. Acetonitrile was u.v. grade and phosphoric acid analytical grade. Isocratic (reverse phase) separations were carried out with a Du Pont 860 machine using a Zorbax-NH_2 column (250 mm × 4.6 mm) fitted with a guard column. Samples (*ca.* 1 mg/ml) were filtered before injection (~5 μl). With a flow rate of 1.5 ml/min and a solvent composition of acetonitrile–distilled water–phosphoric acid (90 : 10 : 0.1, v/v) the following retention times were recorded: methyl gallate (2.3 min); 2,6-digalloyl-1,5-anhydro-D-glucitol (2.6 min); β-1,2,4,6-tetragalloyl-D-glucose (2.7 min); β-pentagalloyl-D-glucose (2.9 min). Using a solvent composition of acetonitrile–distilled water–phosphoric acid (95 : 5 : 0.1, v/v) the following retention times were recorded: methyl gallate (2.6 min); 2,6-digalloyl-1,5-anhydro-D-glucitol (3.6 min); β-1,2,4,6-tetragalloyl-D-glucose (4.6 min), and β-pentagalloyl-D-glucose (5.2 min).

Gradient elution h.p.l.c. was performed in a system built from an Altex 110 pump with u.v. detector, integrator, and microprocessor, and utilised a Brownlee RP 18 Lichrosorb column 10 μm (250 × 4.6 mm) and guard column. Samples were eluted with a gradient of increasing acetonitrile concentration (0—100%) in distilled water containing 0.05% phosphoric acid and with a flow rate of 1.4 ml/min. Components were identified by the addition of known standards. The gradient profile was programmed as follows: (i) 1—5 min (0% acetonitrile), (ii) 5—50 min (0—30% acetonitrile), (iii) 50—60 min (30—100% acetonitrile). Under these conditions the following retention times were recorded: gallic acid (15.6 min), methyl gallate (26.8 min), β-1,3,6-trigalloyl-D-glucose [23] (37.1 min), (α,β)-2,3,4,6-tetragalloyl-D-glucose (41.0 min), β-1,2,4,6-tetragalloyl-D-glucose (43.4 min), and β-pentagalloyl-D-glucose (45.8 min). In the analysis of the 'gallotannin' fractions of *Acer platanoides* peaks at 45.8—46.0 min were assigned to β-pentagalloyl-D-glucose, twin peaks at 48.6—48.8 and 49.6—49.8 min were assigned to hexagalloyl-D-glucose species and a peak at 51.2—51.4 min to heptagalloyl-D-glucose species (Figure 5). These assignments are based on the incremental retention times of galloyl-D-glucose derivatives relative to β-pentagalloyl-D-glucose, the ratio of methyl gallate to β-pentagalloyl-D-glucose from methanolysis, and the ^{13}C n.m.r. analysis (Table 5).

General Procedure for the Isolation of Galloyl Esters.—Phenolic extracts from freshly picked leaves (1—2 kg) were prepared as described previously.[46] The phenolic extract (*ca.* 20 g) was dissolved in a minimum of ethanol (25—50 ml) and applied to a column of Sephadex LH-20 in the same solvent (column: 45 × 6.0 cm). The column was eluted with ethanol and fractions (10—15 ml) collected. The progress of the chromatography was monitored by sampling of every 5th tube and paper chromatographic analysis in systems A or B as appropriate. The contents of tubes were combined, evaporated at 30 °C to yield individual components. Further purification was achieved by rechromatography or, where appropriate, by crystallisation or separation from water, or by precipitation from anhydrous ethyl acetate with light petroleum (b.p. 60—80 °C). Samples recovered from chromatography were evaporated repeatedly from acetone (AnalaR). Samples were finally dried at 100 °C/0.01 mmHg for 48 h over phosphorus pentoxide or potassium hydroxide. The order of elution of galloyl esters from Sephadex LH-20 in ethanol generally parallels the degree of galloylation of the sugar mono > bis > tris > tetrakis > pentakis. Sources of various galloyl-D-glucose derivatives are shown in Tables 3 and 4. Polygalloyl esters (gallotannins) were obtained from phenolic extracts by chromatography on polyamide (Woelm) as earlier described.[15, 26] Fractionation was subsequently carried out by chromatogagphy on Sephadex LH-20 in ethanol.

β-*Penta*-O-*galloyl*-D-*glucose* (12).—This compound was obtained as an off-white granular solid from aqueous solutions kept at 5 °C for 3—4 days. It had no m.p. <250 °C (Found: C, 52.1, 52.3; H, 3.7, 3.5; glucose 18.8. $C_{41}H_{32}O_{26}$ requires C, 52.3; H, 3.4; glucose 19.2%), $[\alpha]_D^{20}$ +18.2°, $[\alpha]_{578}^{20}$ +20.5° (*c*, 1.0 in acetone), R_F (A) 0.06; R_F (B) 0.50.

The pentadeca-acetate was obtained from methanol as a white granular solid, softens 120—140 °C and m.p. (decomp.) >250 °C (Found: C, 53.8; H, 3.70. $C_{71}H_{62}O_{41}$ requires C, 54.3; H, 4.0%), $[\alpha]_D^{20}$ +1.2° (*c*, 0.8 in chloroform) (lit.,[50] values +5.6, +4.1°; ^1H n.m.r. ($CDCl_3$) δ 7.66, 7.68, 7.71, 7.78, and 7.81 (all 2H, singlets, ArH), 6.12 (1 H, d, *J* 9.5 Hz, 1-H), 5.89 (1 H, t, *J* 9.5 Hz, 3-H), 5.70 (1 H, t, *J* 9.5 Hz, 2-H), 5.58 (1 H, t, *J* 9.0, 9.5 Hz, 4-H), 4.58 (1 H, br d, *J* 12.0 Hz, 6-H), 4.26 (1 H, m, 5-H), 4.38 (1 H, dd, *J* 12.0, 5.0 Hz, 6-H), 2.2—2.28 (65 H, m, acetate CH_3). The pentadecamethyl ether crystallised from benzene as slender needles, m.p. and mixed m.p. 132—134 °C (Found: C, 58.0; H, 5.5. $C_{56}H_{62}O_{26}$ requires C, 58.4; H, 5.4%), $[\alpha]_D^{20}$ +16.5° (*c* 0.75 in acetone) (lit.,[48] +17.1°), ^1H n.m.r. ($CDCl_3$) δ 7.09, 7.15 (2×), 7.28, and 7.30 (all 2 H s, ArH), 6.20 (1 H, d, *J* 9.5 Hz, 1-H), 6.01 (1 H, t, *J* 9.5 Hz, 3-H), δ 5.79 (1 H, t, *J* 9.5 Hz, 2-H), 5.65 (1 H, t, *J* 9.5, 9.0 Hz, 4-H), δ 4.79 (1 H, br d, *J* 12.5 Hz, 6-H), 4.40 (2 H, m, 5-, 6-H) and 3.7—3.9 (65 H, m, OMe).

β-1,2,3,6-*Tetragalloyl*-D-*glucose*.—This compound separated from water as very fine needles which had no m.p. <250 °C (Found: C, 51.4; H, 4.1; glucose 22.3. Calc. for $C_{34}H_{28}O_{22}$, C, 51.8; H, 3.6; glucose 22.8%), $[\alpha]_D^{20}$ +41° (*c* 0.8 in methanol), R_F (A) 0.08, R_F (B) 0.48.

The *trideca-acetate* was obtained from methanol as a granular white solid, softens 125—135 °C, m.p. (decomp.) 220—225 °C (Found: C, 53.6; H, 4.2. Calc. for $C_{60}H_{54}O_{35}$: C, 54.0; H, 4.1%), $[\alpha]_D^{20}$ +38° (*c* 0.6 in chloroform), ^1H n.m.r. ($CDCl_3$) δ 7.69, 7.71, 7.80, and 7.83 (all 2 H, s, ArH), 6.11 (1 H, d, *J* 9.5 Hz, 1-H), 5.75 (1 H, t, *J* 9.5 Hz, 4-H), 5.61 (1 H, t, *J* 9.5 Hz, 3-H), 5.39 (1 H, t, *J* 9.5 Hz, 2-H), and 4.50 and 4.15 (3 H, m, 5-H, 6-H_2).

The *dodecamethyl ether* (prepared with diazomethane) separated from methanol as a white solid, m.p. 85—87 °C (Found: C, 57.5; H, 5.5. Calc. for $C_{46}H_{52}O_{22}$: C, 57.7; H, 5.4%), $[\alpha]_D^{20}$ +37° (*c* 0.5 in $CHCl_3$), ^1H n.m.r. ([$^2H_6$]acetone) δ 7.21, 7.29, 7.31, and 7.38 (all 2 H, s, ArH), 6.23 (1 H, d, *J* 9.5 Hz, 1-H), 5.86 (1 H, t, *J* 9.5 Hz, 3-H), 5.67 (1 H, t, *J* 9.5 Hz, 2-H), 5.35 (1 H, m, 4-H), 4.87 (1 H, br d, *J* 12.5 Hz, 6-H), 4.47 (1 H, dd, *J* 12.5, 5.0 Hz, 6-H), and 4.28 (1 H, m), 5-H).

β-1,2,4,6-*Tetragalloyl*-D-*glucose*.—This compound was obtained as fine white needles from water, m.p. >250 °C (decomp.) (Found: C, 51.3; H, 4.0; glucose 22.2. Calc. for $C_{34}H_{28}O_{22}$: C, 51.8; H, 3.6; glucose 22.8%), $[\alpha]_D^{20}$ −2.0° (*c* 0.5 in methanol), R_F (A) 0.11, R_F (B) 0.45.

β-1,2,6-*Trigalloyl*-D-*glucose*.—This compound was obtained as a buff amorphous solid after repeated evaporation from anhydrous acetone (Found: C, 49.2; H, 4.4; glucose 27.6. Calc. for $C_{27}H_{24}O_{18}\cdot H_2O$: C, 49.5; H, 4.0; glucose 27.5%), $[\alpha]_D^{20}$ +10.3° (*c* 0.5 in acetone), R_F (A) 0.30; R_F (B) 0.35.

The *undeca-acetate* separated from methanol as a white granular solid which softened to a glass at 150—160 °C, m.p. 235—240 °C (decomp.) (Found: C, 53.2; H, 4.4. Calc. for $C_{49}H_{46}O_{29}$: C, 53.6; H, 4.2%), $[\alpha]_D^{20}$ +1.8° (*c* 0.4 in chloroform), ^1H n.m.r. ($CDCl_3$) δ 7.71, 7.78, and 7.80 (all 2 H, s, ArH), 6.02 (1 H, d, *J* 9.5 Hz, 1-H), 5.52 (2 H, m, 2-H, 3-H), 4.22 (1 H, t, *J* 9.5 Hz, 4-H), 4.50 (1 H, br d, *J* 12.5 Hz, 6-H), 4.39 (1 H, dd, *J* 12.5, 5.0 Hz, 6-H), and 4.1 (1 H, m, 5-H).

β-1,6-*Di*-O-*galloyl*-D-*glucose*.—This compound was obtained as a buff amorphous solid after repeated evaporation from anhydrous acetone (Found: C, 47.4; H, 4.7; glucose 34.9. Calc. for $C_{20}H_{20}O_{14}\cdot H_2O$: C, 47.9; H, 4.4; 'glucose 35.9%), $[\alpha]_D^{20}$ −3.5° (*c* 0.8 in methanol), R_F (A) 0.43; R_F (B) 0.43.

The nona-acetate separated from methanol as minute prisms, m.p. 105—107 °C (Found: C, 52.8; H, 4.6. Calc. for $C_{38}H_{38}O_{23}$: C, 52.9; H, 4.4%), $[\alpha]_D^{20}$ +13° (*c* in 0.5 in

chloroform), ^{1}H n.m.r. ($CDCl_3$) δ7.73 (4 H, s, ArH), 5.88 (1 H, d, *J* 9.5 Hz), 5.1—5.3 (3 H, m, 2-, 3-, 4-H), 4.46 (1 H, br d, *J* 12.5 Hz, 6-H), δ 4.34 (1 H, dd, *J* 12.5, 5.0 Hz, 6-H), 4.0 (1 H, m, 5-H), 1.98—2.20 (27 H, m, acetate methyl).

2,6-*Di*-O-*galloyl*-1,5-*anhydro*-D-*glucitol*.—This compound was obtained from leaves of *Acer saccharinium* and *Acer tartaricum* and crystallized from methanol–water to give needles, m.p. 164—166 °C (decomp.) (lit.,[37] m.p. 164—166 °C) $[\alpha]_D^{20}$ +20° (*c* 0.5 in acetone), R_F (A) 0.26, R_F (B) 0.55, ^{1}H n.m.r. ([$^2H_6$]acetone) δ 7.18, 7.20 (each 2 H s, ArH), 4.86 (1 H, dt, *J* 10.0 and 5.5 Hz, 2-H), 4.64 (1 H, d, *J* 12.0 Hz, 6-H), 4.45 (1 H, dd, *J* 12.0 and 5.0 Hz, 6-H), 4.13 (1 H, dd, *J* 11.0 and 5.5 Hz, 1-$H_{eq.}$), 3.90 (1 H, t, *J* 9.5 Hz, 3-H), 3.75 (2 H, m, 4-, 5-H), 3.39 (1 H, t, *J* ≈10.0 Hz, 1-$H_{ax.}$); ^{13}C n.m.r. ([$^2H_6$]acetone, $SiMe_4$) δ 166.7 and 166.3 (Aryl CO), 79.4, 76.5, 72.9, 71.5, 67.3, and 64.5 (aliphatic C atoms, 1,5-anhydro-D-glucitol).

The *octa-acetate* crystallised from methanol, m.p. 154 °C (lit.,[38] m.p. 154—155 °C), $[\alpha]_D^{20}$ +32.4° (*c* 1.8 in acetone); ^{1}H n.m.r. ($CDCl_3$) δ 7.72, 7.80 (2 H, s, ArH), 5.42 (1 H, t, *J* 9.5 Hz, 3-H), 5.19 (1 H, dt, *J* 9.5 and 5.0 Hz, 2-H), 5.11 (1 H, t, *J* 9.5 Hz, 4-H), 4.50 (1 H, d, *J* 12.0 Hz, 6-H), 4.36 (1 H, dd, *J* 12.0 and 5.0 Hz, 6-H), 4.30 (1 H, dd, *J* 10.0 and 5.5 Hz, 1-$H_{eq.}$), 3.75 (1 H, m, 5-H), 3.39 (1H, t, *J* 10.0 Hz, 1-$H_{ax.}$), 2.29 (18 H), 2.04 (3 H, s), and 2.00 (3 H, s, acetate Me).

Trigalloyl-1,5-*anhydro*-D-*glucitol* (10), (11).—This compound was obtained as an amorphous off-white powder by repeated evaporation from anhydrous acetone (Found: C, 50.5; H, 4.4. Calc. for $C_{27}H_{24}O_{17}\cdot 1H_2O$: C, 50.8; H, 4.1%), $[\alpha]_D^{20}$ −4.5° (*c* 1.2 in methanol), R_F (A) 0.13, R_F (B) 0.59; ^{1}H n.m.r. ([$^2H_6$]acetone δ 7.53 (1 H, bs), 7.42 (1 H, bs), 7.32 (2 H, s) (depside ArH), 7.20 (1 H, s), 7.17 (1 H, s, Ar ester H), 4.92 (1 H, dt, 2-H), 4.6 (2 × d, 1 H, 6-H), 4.45 (1 H, m, 6-H), 4.10 (1 H, dd, 1-$H_{eq.}$), 3.90 (1 H, bt, 3-H), 3.75 (2 H, m, 4-, 5-H), and 3.40 (1 H, bt, *J* 9.5 Hz, 1-$H_{ax.}$); ^{13}C n.m.r. ([$^2H_6$]acetone) δ 64.5, 64.9 (C-6), 72.9, 73.2 (C-2), 67.3, 71.5, 76.5, and 79.4 (C-1, -3, -4, -5), 114.6, 117.3, 117.5 (depside aryl C), 166.6, 166.3, 166.1, 165.8, 164.8 (aromatic carbonyl C). Methanolysis[26] gave after 7 days methyl gallate (m.p. and mixed m.p. 157 °C and 2,6-digalloyl-1,5-anhydro-D-glucitol (m.p. and mixed m.p. 164—165 °C) after separation on Sephadex LH-20. H.p.l.c. analysis showed a ratio of approximately 1 : 1 of methyl gallate and 2,6-digalloyl-1,5-anhydro-D-glucitol after methanolysis.

The *deca-acetate* separated from methanol as a white solid (Found: C, 54.0; H, 4.4. Calc. for $C_{47}H_{44}O_{27}$: C, 54.2; H, 4.2%), $[\alpha]_D^{20}$ +29.7 (*c* 1.7 in acetone); ^{1}H n.m.r. ($CDCl_3$) δ 7.78—7.98 (4 H, m, depside aromatic H), 7.80 and 7.70 (2 × 1H, s, aromatic ester H), 5.42 (1 H, t, 3-H), 5.1—5.25 (2 H, m, 2-, 4-H), 4.30—4.55 (3 H, m, $1_{eq.}$-H, 6-H_2), 3.85 (1 H, m, 5-H), 3.50 (1 H, t, *J* 9.5, $1_{ax.}$-H), 2.30 and 2.22 (24 H), and 2.02 and 2.00 (2 × 3 H, s, acetate methyl).

Isolation of Gallotannins.—Polygalloyl esters of the gallotannin class were isolated as described previously[15, 26] by chromatography on Polyamide (Woelm). The esters were isolated as buff amorphous powders by repeated evaporation from acetone, R_F (A) 0.00—0.35 and R_F (B) 0.35—0.50. Acetate derivatives were prepared by precipitation from chloroform–methanol. *Acer platanoides* ester (Found: C, 51.9; H, 3.6; glucose 15.8%), $[\alpha]_D^{20}$ +14.2° (*c*, 1.3 in acetone); acetate (Found: C, 54.5; H, 3.6%).

Acer campestre ester (Found: C, 51.6; H, 3.8; glucose 15.8%), $[\alpha]_D^{20}$ +15.0° (*c*, 0.8 in acetone); acetate (Found: C, 54.2; H, 3.7%).

Pelargonium sp. ester (Found: C, 51.9; H, 3.8; glucose 15.5%), $[\alpha]_D^{20}$ +13.5° (*c*, 0.7 in acetone); acetate (Found: C, 54.4; H, 3.8%).

Paeonia officinalis ester (Found: C, 52.0; H, 3.4; glucose 15.0%), $[\alpha]_D^{20}$ +13.8° (*c*, 0.4 in acetone); acetate (Found: C, 54.3; H, 3.8%).

Hexagalloyl-D-glucose ($C_{48}H_{36}O_{30}\cdot 1H_2O$ requires C, 51.9; H, 3.4; glucose 16.2%); acetate ($C_{82}H_{70}O_{47}$ requires C, 54.5; H, 3.9%).

Heptagalloyl-D-glucose ($C_{55}H_{40}O_{34}\cdot 1H_2O$ requires C, 52.3; H, 3.3; glucose 14.3%); acetate ($C_{93}H_{78}O_{53}$ requires C, 54.7; H, 3.8%).

Octagalloyl-D-glucose ($C_{62}H_{44}O_{38}\cdot 1H_2O$ requires C, 52.6; H, 3.3; glucose 12.7%); acetate ($C_{104}H_{86}O_{59}$ requires C, C, 54.8; H, 3.8%).

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