The Metabolism of Gallic Acid and Hexahydroxydiphenic Acid in Plants. Part 2.[1] Esters of (*S*)-Hexahydroxydiphenic Acid with D-Glucopyranose ($^4C_1$)

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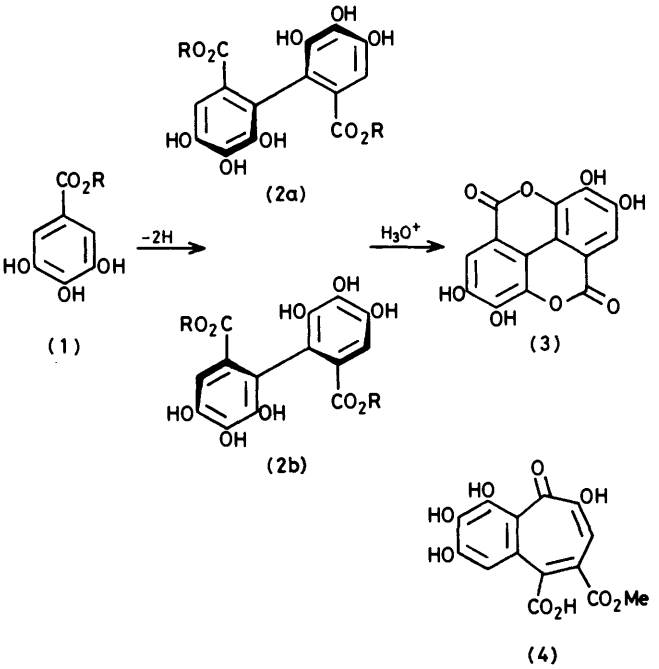
One form of further metabolism of β-penta-*O*-galloyl-D-glucose in higher plants is that in which intramolecular oxidative coupling takes place between adjacent galloyl ester groups (2,3, and 4,6). This route of biosynthesis is confined to certain plants and details of the structure elucidation of some of the principal metabolites are recorded.

A DISTINCTIVE feature which emerged from his chemotaxonomic survey of dicotyledonous plants and one to which Bate-Smith [2] drew particular attention was the widespread occurrence of ellagic acid (3) (Scheme 1) in the acid hydrolysates of plant extracts. Bate-Smith [2] inferred from this observation that the presumed precursor of ellagic acid—hexahydroxydiphenic † acid (2a,b; R = H), bound in ester form (2a,b)—was itself widely distributed amongst the phenolic metabolites of the plant kingdom. Although notable work in this particular area of natural product chemistry has been carried out by Schmidt and Mayer [3-5] in their classical work on the commercially important ellagitannins, no attempts have yet been made to explore the clearly more extensive part which hexahydroxydiphenic acid (2a,b; R = H) plays in plant metabolism overall. Observations on some facets of this role are recorded below; a preliminary account of some aspects of this work has been published.[6]

Whilst there is, as yet, no formal experimental proof it is generally assumed, following the hypothesis of Schmidt and Mayer,[7] that naturally occurring hexahydroxydiphenoyl esters (2a,b) are formed by oxidative coupling of two appropriately positioned galloyl ester groups in a suitable polygalloyl ester precursor (Scheme). Oxidation of esters of gallic acid has long been recognised [8,9] as an efficient method to prepare ellagic acid (3). Although many of these reactions probably proceed *via* the intermediate hexahydroxydiphenoyl esters (2a,b) there have been no reports of the isolation of such esters from this type of oxidation. This is doubtless due to the facile acid- or base-catalysed conversion of the intermediate esters (2a,b) into the very stable dilactone of ellagic acid (3). Support for the putative biogenetic pathway has now been obtained by oxidation of methyl gallate (1; R = Me) in aqueous media with potassium iodate [10] to give as one product of reaction dimethyl hexahydroxydiphenoate (2a,b; R = Me)‡. A further product isolated from this reaction was the purpurogallin carboxylic acid provisionally formulated as (4) on the basis of analytical and spectroscopic data and the mode of formation of purpurogallin derivatives.[10] Dimethyl hexahydroxydiphenoate (2a,b; R = Me) was characterised spectroscopically, by its ready transformation—heating to 150 °C or refluxing in aqueous media—to ellagic acid (3) and by its conversion with diazomethane into the known hexamethyl ether.[11] A significant



SCHEME Oxidative coupling of galloyl esters

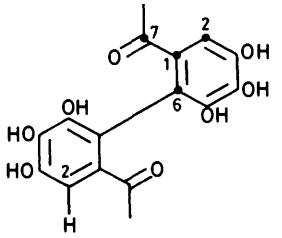
feature of the ^{1}H n.m.r. spectrum of (2a,b; R = Me) which has been used in subsequent structural work (*vide infra*) is the upfield shift of the C–H absorption of the ester methyl groups (Δδ 0.36) when compared to methyl gallate (1; R = Me).

Other significant spectroscopic features which aid the identification of a hexahydroxydiphenoyl ester group (2a,b) in a phenolic metabolite are derived from an examination of both ^{1}H and ^{13}C n.m.r. (Figure 1). In the ^{1}H n.m.r. spectra the two aryl protons appear as singlets almost invariably upfield ([^{2}H]acetone, δ 6.3—6.8, $SiMe_4$) from the two-proton singlet of an isolated galloyl ester group (1). Correspondingly in the ^{13}C n.m.r. signals due to the individual carbonyl carbon atoms appear downfield from $SiMe_4$ relative to those of simple galloyl esters (Figure 1, Table 2) and signals due to C-1,

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

‡ The conversion of (1; R = Me) to (2a,b; R = Me) has also now been achieved using the enzyme peroxidase (Professor W. Mayer, personal communication).

-2, and -6 are also diagnostic in their position. The number of hexahydroxydiphenoyl ester groups in a metabolite is generally most readily deduced from the number of aryl proton singlets (δ 6.3—6.8) or from the multiplicity of signals in the ^{13}C n.m.r. which arise from the carbon atoms which form the termini of the biphenyl linkage (C-6 δ 116.5). The bridging of two positions of a sugar or polyol ring by the biosynthesis of a hexahydroxydiphenoyl ester group results in the formation of a macrocyclic ring (ten or eleven membered dependent on 1,2- or 1,3-bridging of the polyol) and this, in turn, brings about a conformational restraint on the sugar or polyol. These effects are reflected in changes in both the ^{13}C and ^{1}H n.m.r. characteristics of the sugar or polyol component (Tables 1 and 2). No evidence has been obtained to suggest that there is significant conformational mobility in these macrocycles formed by oxidative coupling.



^{1}H n.m.r.	[$^{2}H_6$]Acetone	[$^{2}H_4$]Methanol
H-2	6.3—6.8	
^{13}C n.m.r.		
C-1	125.5—127.0	
C-2	107.0—109.0	
C-6	114.0—116.5	
C-7	167.8—169.5	168.9—171.2

(δ values in p.p.m. from $SiMe_4$)

FIGURE 1 The hexahydroxydiphenoyl ester group: n.m.r. characteristics

Leaf extracts from over 150 plants have been examined by two-dimensional paper chromatography and h.p.l.c.[1] In the paper chromatographic analysis a spray reagent of nitrous acid was specifically used to detect esters of hexahydroxydiphenic acid (2a,b) and their derivatives. This reagent is based on the distinctive colour test devised originally by Procter and Paessler [12] for ellagic acid and used later by Bate-Smith.[13] Esters (2a,b) react with the nitrous acid to give initially a carmine-red changing through green, brown, and purple to give finally (5—10 min) a moderately stable indigo-blue colour. Amongst plants surveyed on the basis of Bate-Smith's earlier observations [2] several distinctive paper chromatographic 'fingerprints' of hexahydroxydiphenoyl esters, and their variants, were discerned. One of these (here named group B) predominated. The principal components and the composite paper chromatographic 'fingerprint' of group B metabolites are shown in Figure 2; the metabolic pattern has been distinguished in the plant families Hamamelidaceae, Fagaceae, Theaceae, Rosaceae, Saxifragaceae, Myrtaceae, Onagraceae, Combretaceae, Cornaceae, Elaegnaceae, and Juglandaceae. Compounds (5) and (6) were first

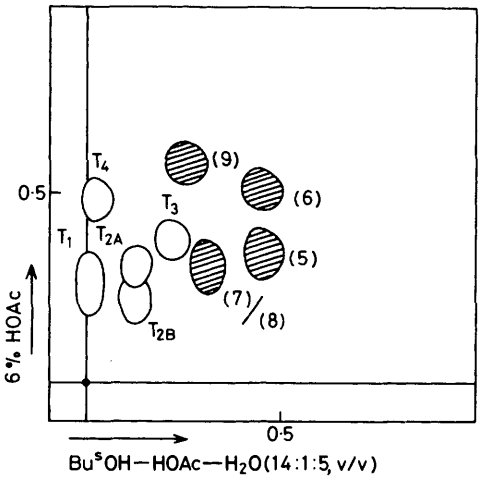


FIGURE 2 Natural (*S*)-hexahydroxydiphenoyl-D-glucose esters—Group B. Paper chromatographic fingerprint

(5) β-1,2,3-Tri-*O*-galloyl-4,6-(*S*)-hexhydroxydiphenoyl-D-glucose
(6) 2,3-Di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose
(7) β-1-*O*-Galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose
(8) α-1-*O*-Galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose
(9) 2,3:4,6-Bis-(*S*)-hexahydroxydiphenoyl-D-glucose

briefly reported by Wilkins and Bohm [14] from *Tellima grandiflora* and were assigned respectively the structures of β-1,2,3-tri-*O*-galloyl-4,6-hexahydroxydiphenoyl-D-glucose and 2,3-di-*O*-galloyl-4,6-hexahydroxydiphenoyl-D-glucose. Recently, Nonaka, Harada, and Nishioka [15] have reported the isolation of (5) from cloves (*Eugenia caryophyllata*) and both (5) and (6) have been obtained [16] from the fruit of *Cornus officinalis*. The metabolites (7) and (8) have not previously been reported and compound (9) is identical with pendunculagin (syn. Juglanin) isolated variously by Schmidt [17] from oak galls, by Hillis and Siekel [18] from eucalyptus wood and by Jurd [19] from walnut pellicles. The variations in the patterns of occurrence of these substances and the associated metabolites (T_1—T_4), Figure 2, may be used as a molecular taxonomic guide.[20]

Hydrolysis of each of the esters (5)—(9), isolated from the leaves of a range of plants, with the enzyme tannase [21] gave gallic acid, ellagic acid, and D-glucose and thus confirmed their identity as hexahydroxydiphenoyl-D-glucose derivatives. The structure of compound (5) was confirmed as that of β-1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose on the basis of spectroscopic measurements although the arguments employed differ from those recorded by Wilkins and Bohm.[14] The β-D-glucopyranose conformation (*C*-1 or $^{4}C_1$) was shown by the various proton coupling constants of the D-glucopyranose ring.[22] The signals due to 4-H and one of the diastereotopic protons at C-6 showed, relative to the analogous signals in β-penta-*O*-galloyl-D-glucose (10), a displacement upfield (Δδ 0.4—0.5, Table 1) and this suggested (*vide supra*) that the hexahydroxydiphenoyl ester bridged the 4,6-positions of the D-glucopyranose ring. The remaining C-6 proton showed a correspond-

TABLE 1

^{1}H N.m.r. characteristics of (*S*)-hexahydroxydiphenoyl esters of D-glucopyranose: δ values form $SiMe_4$ in [$^2H_6$] acetone; *J* values in Hz.

	D-Glucopyranose derivative	D-Glucopyranose protons							Aromatic protons	
		1-H	2-H	3-H	4-H	5-H	6-H	6-H	Galloyl (2 H)	Hexahydroxydiphenoyl (1 H)
1	β-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)	4.60 (m)	4.45 (dd, *J* 12.0, *J* 3.0)	4.6 (m)	7.02, 7.08, 7.10 7.17, 7.23	—
2	β-1,2,3-Trigalloyl-4,6-hexahydroxydiphenoyl	6.20 (d, *J* 9.5)	5.60 (t, *J* 9.5)	5.85 (t, *J* 9.5)	5.23 (t, *J* 9.5)	4.54 (dd, *J* 5.0, *J* 9.5)	5.38 (dd, *J* 12.5, *J* 5.0)	3.87 (bd, *J* 12.5, *J* 1.0)	6.98, 7.00, 7.12	6.48 6.57
3	β-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl	6.20 (d, *J* 9.0)	5.14 (t, *J* 9.0)	5.45 (t, *J* 9.0)	5.18 (t, *J* 9.0)	4.48 (dd, *J* 6.0 *J* 9.0)	5.36 (dd, *J* 13.0, *J* 6.0)	3.86 (bd, *J* 13.0, *J* 1.0)	7.17	6.36, 6.46, 6.53, 6.67
4	α-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl	6.62 (d, *J* 3.5)	5,40 (dd, *J* 9.5, *J* 3.5)	5.59 (t, *J* 9.5)	5.22 (t, *J* 9.5)	4.64 (dd, *J* 6.0, *J* 9.5)	5.31 (dd, *J* 12.5, *J* 6.0)	3.82 (bd, *J* 12.5, *J* 1.0)	7.22	6.37, 4.47, 6.58 6.67
5	α-1-Galloyl-2,3-hexahydroxydiphenoyl	6.53 (d, *J* 3.5)	5.18 (dd, *J* 9.5, *J* 3.5)	5.47 (t, *J* 9.5)	4.04 (t, *J* 9.5)	3.90 (dd, *J* 6.0, *J* 9.5)	3.84 (m)	3.84 (m)	7.21	6.46, 6.72
6	β-1-Galloyl-2,3-hexahydroxydiphenoyl	6.13 (d, *J* 9.0)	5.00 (t, *J* 9.0)	5.22 (t, *J* 9.0)	←—	3.80 —→	←—	4.00 —→	7.14	6.43, 6.71
7	2,3-Hexahydroxydiphenoyl-α	5.39 (d, *J* 3.5)	4.90 (dd, *J* 9.0, 3.5)	5.02 (t, *J* 9.0)	←—	3.50 —→	←—	4.00 —→	—	6.63, 6.71
	β	5.33 (d, *J* 9.0)	4.70 (t, *J* 9.0)	4.98 (t, *J* 9.0)	←—	3.50 —→	←—	4.00 —→		6.63, 6.71
8	*Rubus* polyphenol (16)									
	α-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl-residue	6.57 (d, *J* 4.0)	5.32 (dd, *J* 9.0, *J* 4.0)	5.12 (t, *J* 9.0)	5.06 (t, *J* 9.0)	4.30 (m) —	5.61 (dd, *J* 13.0, *J* 6.5)	3.93 (bd, *J* 13.0, *J* 1.0)	7.14 (1 H, d) 7.28 (1 H, d) (*J* 3)	6.28, 6.30, 6.38 6.47, 6.50, 6.78
	β-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl-residue	6.20 (d, *J* 9.0)	5.22 (t, *J* 9.0)	5.39 (t, *J* 9.0)	5.12 (t, *J* 9.0)	4.38 (dd, *J* 9.0, *J* 6.5)	5.25 (dd, *J* 13.0, *J* 6.5)	3.82 (bd, *J* 13.0, *J* 1.0)	7.12	6.76

TABLE 2

^{13}C N.m.r. characteristics of (*S*)-hexahydroxydiphenoyl esters of D-glucopyranose: δ values in p.p.m. from $SiMe_4$ in (a) [$^2H_6$]acetone and (b) [$^2H_6$]methanol

	D-Glucopyranose derivative		D-Glucopyranose C-atoms 1	2	3	4	5	6	Carbonyl C-atoms: Galloyl	Carbonyl C-atoms: Hexahydroxydiphenoyl
1.	β-1,2,3,4,6-Pentagalloyl	(a)	93.4	71.9	73.5	69.5	74.1	62.9	166.4, 165.7 (3×), 165.0	—
		(b)	93.7	72.1	74.2	69.7	74.2	63.0	167.8, 167.1, 166.9 (2×), 166.1	
2.	β-1,2,3-Trigalloyl-4,6-hexahydroxydiphenoyl	(b)	94.0	73.9,	73.5,	72.3,	71.2	63.6	167.3, 166.7, 166.0	169.3, 169.0
3.	β-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl	(a)	92.2	77.2,	75.9,	73.4,	69.2	63.0	165.1	169.4, 169.7, 168.2 (2×)
4.	α-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl	(a)	90.9	76.2,	74.3,	71.2,	69.4	63.5	165.1	169.5, 169.6, 168.2, 167.9
5.	α-1-Galloyl-2,3-hexahydroxydiphenoyl	(b)	91.4	79.1,	76.6,	74.5,	68.0	61.8	166.4	171.5, 170.1
6.	2,3-Hexahydroxydiphenoyl-	(b)								
		α	91.9	81.2,	78.9,	78.5 (2×)		62.3		171.2, 170.7
		β	95.3	76.1,	73.3,	68.8 (2×)		62.3		
7.	*Rubus* Polyphenol (16)	(a)								
	α-1-Galloyl-2,3:4,6-bis-hexahydroxydiphenoyl-residue.		90.8	75.9,	73.9,	71.3,	69.4	63.3	165.5, 165.0	169.2, 168.3, 168.1, 167.9
	β-1-Galloyl-2,3:4,6-bis-hexahydroxy diphenoyl-residue		92.7	77.4,	75.4,	73.6,	69.0	62.9		167.9, 167.8, 165.6

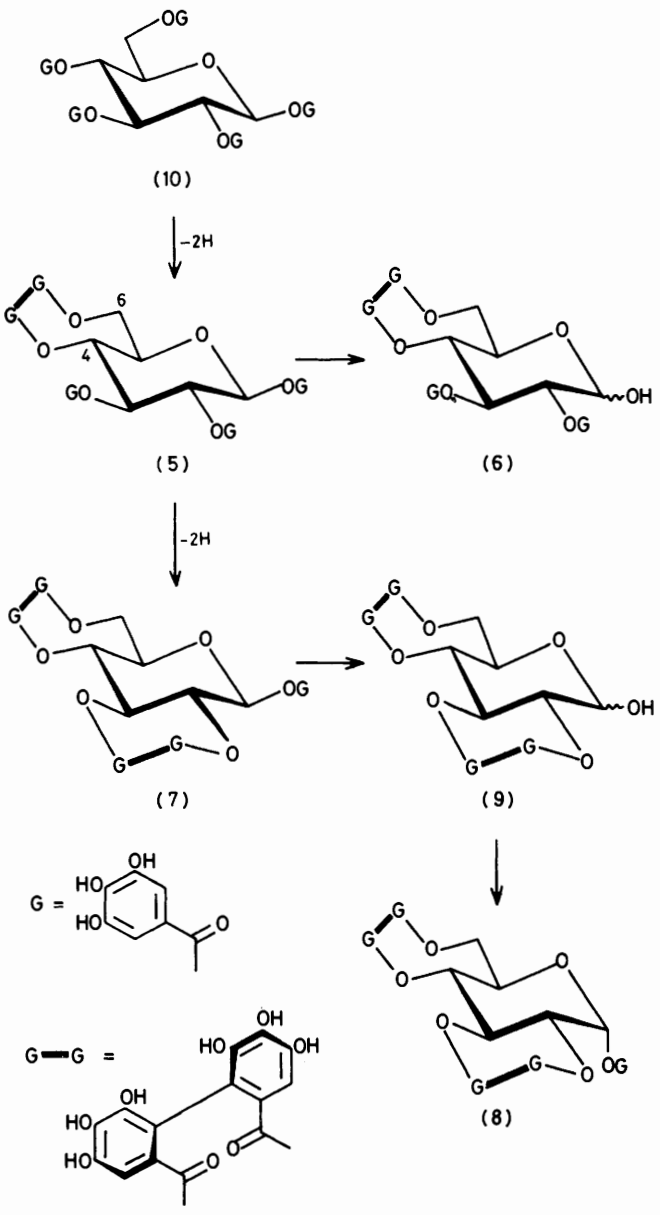


FIGURE 3 Naturally occurring derivatives of (*S*)-hexahydroxydiphenic acid: postulated metabolic relationships

ingly strong downfield shift of 0.58 p.p.m. (Table 1). This observation is readily explicable by an examination of molecular models which show that when the hexahydroxydiphenoyl ester group bridges the 4,6-positions of D-glucopyranose the carbonyl group which esterifies the C-6 hydroxy-group is aligned parallel to the pro-*R*-H-6 and thus places this proton in a strongly deshielding environment. The ester (5) was further characterised as its pentadeca-acetate and pentadecamethyl ether (prepared with diazomethane) derivatives. Hydrolysis of (5) in water at 100 °C for 48 h gave gallic and ellagic acids and two other major products, one of which was isolated and shown to be identical with the metabolite (6). Wilkins and Bohm [14] suggested the structure of 2,3-di-*O*-galloyl-4,6-hexahydroxydiphenoyl-D-glucose (6) for this compound, although its spectroscopic properties (analogous to those reported by Wilkins and Bohm [14]), did not permit an unequivocal assignment. The absence of a lowfield signal for the anomeric proton in the D-glucopyranose structure implied that the anomeric centre was not acylated and hence in solution this metabolite exists as an equilibrium mixture of α- and β-forms and thus gives a complex ^{1}H n.m.r. pattern.[1] This evidence is consistent with the formation of compound (6) by the relatively facile hydrolysis of the 1-*O*-galloyl ester group from the tri-*O*-galloyl ester (5); similar to the reaction noted earlier by Haworth and his collaborators [23] with β-penta-*O*-galloyl-D-glucose (10). In agreement with this hypothesis the trideca-acetate of (6) was shown by ^{1}H n.m.r. analysis (integration of the signals due to the anomeric protons of the α- and β-forms) to be a mixture of the α- and β-D-glucopyranose forms in approximately equal amounts.

The metabolite (9) presented similar problems of structural analysis to (6) and the complication is once again a direct result of the absence of an aroyl ester group at the anomeric centre. The compound was however identical in all respects with pedunculagin (*syn.* juglanin) isolated previously by Schmidt,[17] Seikel and Hillis,[18] and Jurd.[19] Partial hydrolysis of pedunculagin (9) in water at 100 °C (72 h) gave, after chromatography, 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (11) in good yield (30%). The structure of this degradation product follows directly from high resolution ^{1}H n.m.r. spectroscopy (220 and 400 MHz) which delineates the α- and β-forms in solution ([$^2H_6$]acetone) in a ratio of approximately 1 : 1. Spin decoupling locates the 2,3 positions as the points of esterification. Formation of the nona-acetate gave an α,β-mixture containing roughly 1 : 2 parts of the two anomeric forms. Only traces of the isomeric 4,6-hexahydroxydiphenoyl-D-glucose were detectable in the hydrolysis of (9) and this demonstrates the relative ease of hydrolysis of the 4,6- as opposed to the 2,3-hexahydroxydiphenoyl ester group.

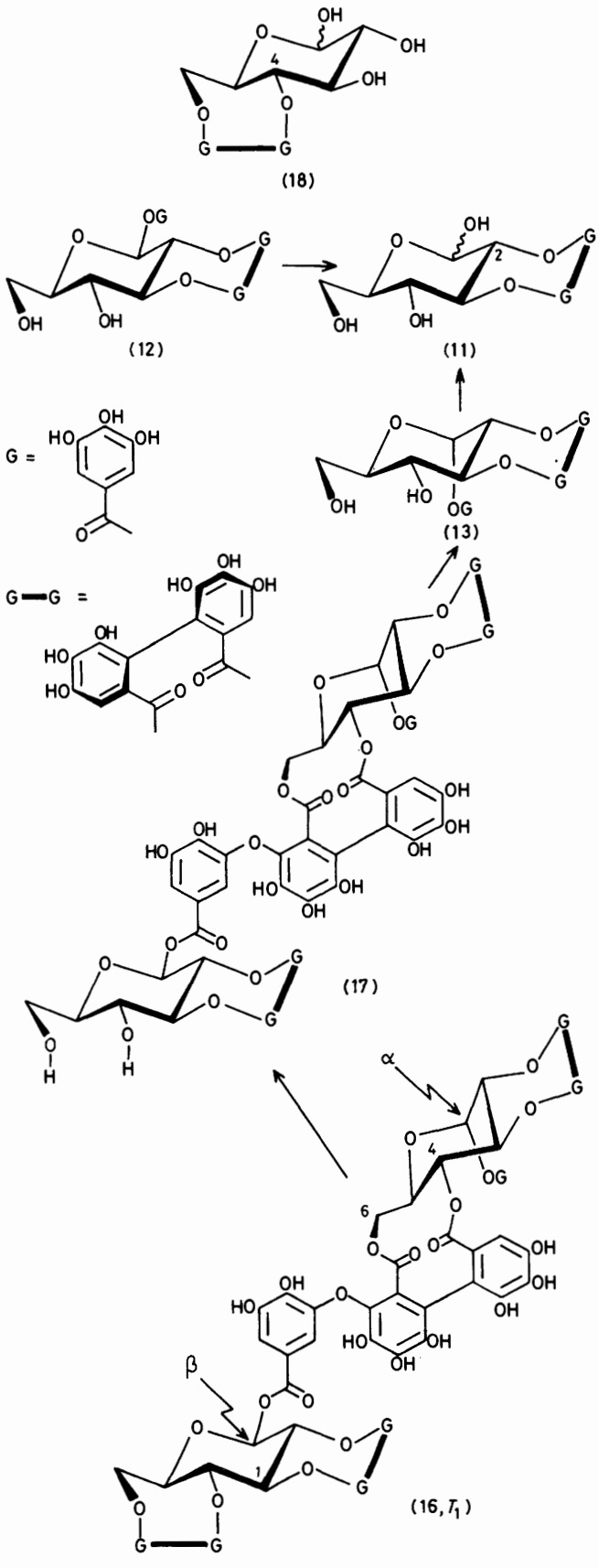
The metabolite (7) was first isolated from the young shoots of *Rosa canina* and other *Rosa* sp. and was formulated on the basis of both ^{1}H and ^{13}C n.m.r. measurements as β-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose with the D-glucopyranose ring adopting the *C*-1 or $^4C_1$ conformation. Using the ^{1}H n.m.r. chemical-shift criteria established above the two hexahydroxydiphenoyl ester groups in (7) were located, by comparison with the ^{1}H n.m.r. spectra of β-penta-*O*-galloyl-D-glucose (10) and β-1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose (5), at the 4,6- and 2,3-positions (Table 1). Support for this structural assignment was derived from studies of the partial hydrolysis of (7) in water at 100 °C. Similarly to compound (9) the 4,6-hexahydroxydiphenoyl ester group was most rapidly cleaved and 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (11) was isolated (20%, 72 h). After 24 h an intermediate was obtained, and although not fully characterised, its ^{1}H n.m.r. spectrum was entirely consistent with its formulation as β-1-*O*-galloyl-2,3-(*S*)-hexahydroxydi-

phenoyl-D-glucose (12). Further hydrolysis of this intermediate gave gallic acid and (11). In later work with other members of the plant family Rosaceae the metabolite (7) was found in association with a closely related metabolite (8) from which it was not readily separated and from which it was not distinguished by paper chromatography (Figure 2). Compound (8) has been isolated along with (7) from the leaves and fruit of *Rubus idaeus* and *Rubus fructicosus*. Hydrolysis in water (100 °C, 72 h) gave 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (11) and an intermediate in the hydrolysis isolated (24 h) and characterised (Tables 1 and 2) as α-1-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (13) from its spectroscopic properties and its further hydrolysis to (11). The metabolite (8) is therefore formulated as α-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose. It is the sole example in the work to date of the isolation of an α-D-glucopyranoside derivative.

The group of phenolic metabolites (5)—(9) constitute key substances in a pattern of metabolism of gallic acid which has been widely encountered in plants. In several cases β-penta-*O*-galloyl-D-glucose (10) has also been isolated from the same plants and its co-occurrence with the same metabolites (5)—(9) has prompted the suggestion [1,6] that this is the biosynthetic precursor of the various hexahydroxydiphenoyl esters. In the context of this particular proposal 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* metabolises in very small amount β-penta-*O*-galloyl-D-glucose (10)—identified by h.p.l.c. and paper chromatography—but none of the metabolites (5)—(9) which leaves and other tissues of the fully differentiated plant *Quercus robur* metabolise. This situation appears analogous to that reported for example in cell suspension cultures of *Baptisia australis* where only those tetracyclic quinolizidine alkaloids which occur early in the biosynthetic sequence which operates in the plant were found to accumulate.[24]

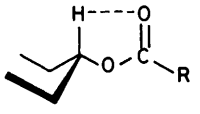
Bearing in mind the hypothesis of Schmidt and Mayer [7] a logical biogenetic relationship of the phenolic metabolites (5), (6), (7), and (9) to their putative precursor β-penta-*O*-galloyl-D-glucose (10) is shown in Figure 3. It is assumed that the D-glucopyranose ring in (10) adopts the most stable *C*-1 or $^4C_1$ conformation and that pairs of adjacent galloyl ester groups (2,3 and 4,6) are oxidatively coupled to give hexahydroxydiphenoyl ester groups. The origin of the α-glucoside (8) remains an enigma and Figure 3 incorporates a tentative suggestion as to its derivation.

Schmidt has shown [17] that the chirality of the hexahydroxydiphenoyl ester groups linked 2,3 and 4,6 to D-glucopyranoside in pedunculagin (9) corresponds to the laevorotary form and following the work of Okuda [25] they must therefore possess the *S*-configuration. These observations are also borne out by c.d. studies.[26] It is interesting to note that the same stereochemical conclusions follow from theoretical considerations if the



following assumptions are made in the oxidative coupling of galloyl ester groups.

(i) The carbonyl groups of the hexahydroxydiphenoyl esters have, like other ester groups,[27,28] a *preference* for the eclipsed conformation (14) with a hydrogen atom at the point of esterification on the D-glucopyranose ring; and (ii) there is a *preference* for conformations in the hexahydroxydiphenoyl ester group in which the two carbonyl groups are *anti* and with the $\overrightarrow{C-O}$ dipoles aligned antiparallel (15).



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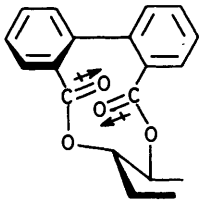
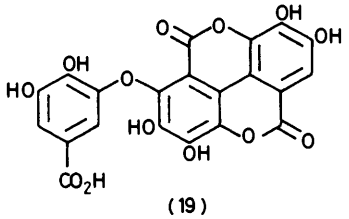


(15)



(19)

If these stereochemical features control the oxidative coupling reactions of galloyl esters of D-glucopyranose then molecular models show that the geometrical constraints of the sugar ring lead directly to the formation of the (*S*)-hexahydroxydiphenoyl ester group by oxidative coupling between the 2,3 and the 4,6 positions respectively.

Substantial variations in the ratio of the phenolic metabolites [(5)—(9), Figure 2] are observed from plant to plant and with these variations there are parallel patterns of occurrence of the substances denoted as T_1—T_4 (Figure 2).[20] In several members of the Rosaceae—*Rubus idaeus*, *Rubus fructicosus*, *Geum rivale*, and various *Potentilla* sp.—the bis-(*S*)-hexahydroxydiphenoyl esters (7)—(9) predominate and alongside these esters is found exclusively the metabolite T_1 and this is, overwhelmingly, the principal phenolic metabolite occurring in such plants. As such it is doubtless largely responsible for the astringency of the raspberry and blackberry and for the characteristic properties and features of the herbal remedies prepared from the fruit and leaves of these plants.[29]

The phenolic substance T_1 has been isolated from various *Rubus* sp. Chemical and spectroscopic evidence leads to its formulation as (16)—formed it is supposed by C-O oxidative coupling of the metabolites (7) and (8). High-resolution ^{1}H n.m.r. with proton decoupling leads to the definition of both structural entities (7) and (8) in the overall structure (Table 1) and to the conclusion that the structure contains one isolated galloyl ester group and three hexahydroxydiphenoyl ester groups. A critical feature of the ^{1}H n.m.r. analysis is the presence of two *meta*-coupled doublets (J 3 Hz) in the galloyl ester region each equivalent to one proton and an additional one-proton singlet in the hexahydroxydiphenoyl ester region. This evidence has been interpreted in terms of the presence of a C-O bond between the galloyl ester group of the β-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose fragment (7) and the hexahydroxydiphenoyl ester group linking the 4,6-positions in the fragment (8). Hydrolysis of (8) (water, 100 °C) leads eventually to its complete breakdown but after 48 h products which have been isolated are the ester (17), 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (11), α 1-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (13) and in addition 4,6-(*S*)-hexahydroxydiphenoyl-D-glucose (18) has been identified by paper-chromatographic comparison.[18] The isolation of (13) from the partial hydrolysis of (16) clearly shows that the galloyl ester group of the fragment (7) is involved in the C-O bond between (7) and (8) and also pinpoints the only two possible points of attachment to the fragment (8). No evidence has yet been obtained to distinguish between these two possibilities but the structure as written (16) shows just one. Analogously the acid (19) which is a postulated product of hydrolytic breakdown of T_1 and is isomeric with valoneic acid dilactone [30] has not yet been isolated. Further work on these aspects of the structural problem and on the identification of the additional phenolic metabolites T_2—T_4 (Figure 2) is in progress.

EXPERIMENTAL

General Methods.—Chromatographic and isolation procedures were as previously described.[1] Hexahydroxydiphenoyl esters and their derivatives were characterised using a spray of nitrous acid—an ice-cold 10% sodium nitrite solution (100 ml) to which glacial acetic acid (5—10 drops) was added. The spray is used immediately after preparation and hexahydroxydiphenoyl esters are revealed by the immediate formation of a rose-red spot which changes rapidly through green, brown and purple to indigo-blue (5—10 min). Glucose was determined by an adaptation of the anthrone procedure of Park and Johnson [31] after hydrolysis of the natural ester with the enzyme tannase.[21,23] Hydrolysis of hexahydroxydiphenoyl esters was also carried out by refluxing in glass-distilled water under a stream of nitrogen.

Dimethyl Hexahydroxydiphenoate.—Potassium iodate solution (2.0 g, in water, 75 ml) was added dropwise during 45 min to a rapidly stirred solution of methyl gallate (4.0 g) in water (175 ml) at room temperature. The solution was kept at 20 °C for 16 h and then extracted with ethyl acetate (5 × 200 ml). After drying (Na_2SO_4) the organic solvent was removed at 30 °C to give a brown gum which was dissolved in ethanol (50 ml) and left at 0—4 °C for 48 h. Ellagic acid (0.35 g) was removed by filtration and the ethanol filtrate added to a column of Sephadex LH-20 (50 × 3.0 cm) in ethanol. The column was eluted with ethanol and fractions (10 ml) collected and analysed by paper chromatography.[1] Fractions were combined as appropriate. Fractions 30—39

contained as a major component a substance [R_F(A) 0.30; R_F(B) 0.69] [1] which gave a light blue fluoresence on chromatograms when viewed under u.v. light, changing to green when fumed in ammonia. The residue after removal of ethanol was filtered in methanol through a short column of polyamide Woelm (15 × 2 cm). Removal of the methanol and crystallisation from aqueous acetone gave compound (4) as rosettes of yellow needles (0.25 g), m.p. 285—290 °C (decomp.) (Found: C, 51.1; H, 2.8. $C_{15}H_8O_9 \cdot H_2O$ requires C, 51.4; H, 2.9%), R_F(A) 0.30, R_F(B) 0.69; $\nu_{max.}$ (Nujol) 1 650 and 1 700—1 750 cm^{-1}; ^1H n.m.r. ([$^2H_6$]-acetone $SiMe_4$): δ 6.95, 7.09 (2 H, s, aromatic), and 3.91 (3 H, s, OMe); ^{13}C n.m.r. ([$^2H_4$]methanol/$SiMe_4$) δ 170.4, 160.4, 158.2, 154.5, 150.2, 143.0, 121.6, 113.8, 113.0, 104.0, and 53.6; ([$^2H_6$]acetone) δ 168.8, 158.9, 157.5, 154.2, 149.4, 142.3, 134.6, 122.1, 113.4, 113.0, 103.8, and 53.1. Compound (4) gave (acetic anhydride–pyridine) a *triacetate* which crystallised from acetone–methanol as pale-yellow needles, m.p. 208 °C (Found: C, 54.5; H, 3.1. $C_{21}H_{14}O_{12}$ requires C, 55.0; H, 3.1%), ^1H n.m.r. ([$^2H_6$]acetone) δ 7.51, 7.90 (2 H, s, aromatic), 4.01 (3 H, s, OMe), and 2.35, 2.38, and 2.45 (3 × 3 H, s, COMe); ($CDCl_3$) δ 7.32, 7.71 (2 H, s, aromatic), 4.02 (3 H, s, OMe), 2.38 (3 H), and 2.31 (6 H, s, COMe); ^{13}C n.m.r. ($CDCl_3$/$SiMe_4$) δ 167.4, 166.8, 166.5, 157.3, 156.5, 153.3, 146.6, 136.5, 131.9, 128.2, 119.6, 103.6 53.7, 20.6, and 20.3 p.p.m. The mass spectrum did not show a molecular ion but gave an (M − 1) peak at 447 and fragment ions at *m/e* 405, 363, 321 (successive loss of 3 acetate groups as keten, 42) and a peak at *m/e* 416 (M − 1 loss of OMe).

Fractions 40—46 from the chromatography on Sephadex LH-20 contained as major product a substance [R_F(A) 0.85; R_F(B) 0.49] [1] which gave a strong rose-red colour with nitrous acid. Removal of the ethanol gave a gum which was dissolved in ethanol (10 ml) and stood at 0—4 °C for 16 h. Ellagic acid (0.1 g) which separated was removed and the residue after evaporation of the ethanol at 30 °C, was filtered in glass-distilled water through a Polyamide-Woelm column (5 × 2 cm). The water was lyophilised from the filtrate and the remaining solid was crystallised either from water, care being taken not to heat above 60—70 °C for more than 1 min, or from ethyl acetate–light petroleum (b.p. 60—80 °C). Dimethyl hexahydroxydiphenoate (0.12 g) crystallised as small prisms, softens 150—180 °C with decomposition, resolidifies 220—230 °C, no m.p. <280 °C [Found (after drying at 100 °C, 0.5 mmHg over P_2O_5): C, 52.1, 51.9; H, 3.9, 4.1. Calc. for $C_{16}H_{14}O_{10}$: C, 52.4; H, 3.9%], R_F(A) 0.85, R_F(B) 0.49; $\nu_{max.}$ (Nujol) 1670—1690 cm^{-1}; ^1H n.m.r. ([$^2H_6$]acetone): δ 7.09 (1 H, s, aromatic) and 3.44 (3 H, s, OMe); ([$^2H_4$]methanol) δ 7.06 (1 H, s, aromatic) and 3.58 (3 H, s, OMe); ^{13}C n.m.r. ([$^2H_4$]methanol) δ 51.9 (OMe), 110.7 (C-2), 114.1 (C-6), 122.4 (C-1), 138.5 (C-4), 141.3 and 144.7 (C-3, C-5), and 169.8 (CO). Dimethyl hexahydroxydiphenoate (0.05 g) when heated in water (100 °C, 15 min) gave ellagic acid (0.03 g) which was characterised as its tetra-acetate by t.l.c. Dimethyl hexamethoxydiphenoate, prepared with diazomethane in acetone and isolated by t.l.c. on silica using benzene–acetone (8 : 1, v/v) as eluant, crystallised from aqueous methanol as needles, m.p. 109—110 °C (Found: C, 58.8; H, 5.9. Calc. for $C_{22}H_{26}O_{10}$: C, 58.7; H, 5.8%).

2,3-(S)-*Hexahydroxydiphenoyl*-D-*glucose*.—This compound was obtained from the hydrolysis (water, 100 °C, 24—48 h) of β-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose (7), α-1-*O*-galloyl-2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose (8), 2,3:4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose (9), and the substance T_1 (16) (Figure 2). The hydrolysis products were chromatographed in ethanol on Sephadex LH-20 and 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose was derived as a light brown amorphous powder after repeated evaporation from acetone [Found (after drying at 60 °C, 0.5 mmHg, over P_2O_5): C, 47.8; H, 4.3. $C_{20}H_{18}O_{14} \cdot H_2O$ requires C, 48.0; H, 4.0%], $[\alpha]_D^{20}$ +50.5° (*c* 0.8, methanol); R_F(A) 0.69; R_F(B) 0.16; ^1H n.m.r. ([$^2H_6$]acetone) showed an α : β ratio of *ca.* 1 : 1; δ 5.38 (½ H, d, *J* 3.0 Hz, 1α-H), 4.80 (½ H, dd, *J* 3.0, 9.0 Hz, 2α-H); δ 5.02 (½ H, t, *J* 9.0 Hz, 3α-H), 5.34 (½ H, d, *J* 9.5 Hz, 1β-H), δ 4.70 (½ H, t, *J* 9.5 Hz, 2β-H), 4.99 (½ H, t, *J* 9.5 Hz, 3β-H), 3.50—3.95 [4 H, m, 4-,5-,6*R*,6*S*-H (α and β)] and 6.63 and 6.71 (2 × 1 H, s, aromatic); ^{13}C n.m.r. ([$^2H_4$]methanol) δ 95.3 (C-1α), 91.9 (C-1β), 81.2, 78.5, 78.5, 78.9, 73.3, 76.1, 68.8, 68.7, 62.3, and 62.3 (C-2—6, α and β), δ 171.2 and 170.7 (CO). The *nona-acetate* was prepared in acetic anhydride–pyridine and separated by t.l.c. [silica, ethyl acetate–light petroleum (b.p. 60—80 C°), 4 : 1, v/v] and then crystallised from aqueous methanol as a white solid with no definite m.p. (Found: C, 53.4; H, 4.2. Calc. for $C_{38}H_{36}O_{23}$: C, 53.0; H, 4.2%); ^1H n.m.r. ($CDCl_3$) showed an α : β ratio of *ca.* 1 : 2; δ 7.21 and 7.09 (2 × 1H, s, aromatic), 6.41 (⅓ H, d, *J* 3.0 Hz, 1α-H) and 5.87 (⅔ H, d, *J* 9.5 Hz, 1β-H). The compound and its acetate were chromatographically identical (paper and t.l.c. on silica respectively) to compound D-1 and its acetate described by Seikel and Hillis from *Eucalyptus delegatensis* wood.[18]

β-1,2,3-*Tri*-O-*galloyl*-4,6-(S)-*hexahydroxydiphenoyl*-D-*glucose*.—This compound was obtained from the phenolic extracts prepared from the leaves and shoots of various *Quercus* sp., *Rosa canina*, *Cornus alba*, *Fuchsia* sp., *Filipendula ulmaria*, *Epilobium angustifolium*, and *Tellima grandiflora* (kindly donated by Professor B. A. Bohm, University of British Columbia), by chromatography in ethanol on Sephadex LH-20.[1] The ester was obtained as a light-brown amorphous powder after evaporation from acetone [Found (after drying at 60 °C, 0.5 mmHg, over P_2O_5): C, 51.2; H, 3.6. Calc. for $C_{41}H_{30}O_{26} \cdot H_2O$: C, 51.5; H, 3.4%], $[\alpha]_D^{20}$ +10° (*c* 0.85 in methanol); R_F(A) 0.35, R_F(B) 0.37. The *pentadeca-acetate* crystallised from methanol as small prisms, softens 160—165 °C, m.p. 180—182 °C [lit.,[14] m.p. 180—184 °C (decomp.)] (Found: C, 54.6; H, 3.5. Calc. for $C_{71}H_{60}O_{41}$: C, 54.3; H, 3.8%), $[\alpha]_D^{20}$ −5° (*c*, 0.6 in chloroform); ^1H n.m.r. ($CDCl_3$): δ 7.80, 7.68, and 7.68 (3 × 2 H, s, aromatic) 7.37 and 7.47 (2 × 1 H, s, aromatic), 6.11 (1 H, *J* 9.5 Hz, 1-H), 5.78 (1 H, t, *J* 9.5 Hz, 3-H), 5.65 (1 H, t, *J* 9.5 Hz, 2-H), 5.40 (2 H, m 4-, 6-H), 4.30 (1 H, bt, 5-H), 3.98 (1 H, bd, *J* 12.0 Hz, 6-H), and 2.02—2.20 (45 H, m, COMe). The *pentadecamethyl ether* (prepared with diazomethane and after t.l.c. on silica) crystallised from methanol as small prisms m.p. 131—132 °C (Found: C, 58.1; H, 5.4. $C_{56}H_{60}O_{26}$ requires C, 58.5; H, 5.2%), $[\alpha]_D^{20}$ +13.0° (*c* 1.2 in acetone); ^1H n.m.r. ($CDCl_4$): δ 7.13, 7.13, and 7.19 (3 × 2 H, s, aromatic) 6.67 and 6.77 (2 × 1 H, s, aromatic), 6.09 (1 H, d, *J* 9.5 Hz, 1-H), 5.75 (2 H, bt 2-, 3-H), 5.50 (2 H, m, 4-, 6-H), 4.32 (1 H, m 5-H), and 3.66—3.92 (46 H, m, 6-H, OMe).

2,3-*Di*-O-*galloyl*-4,6-(S)-*hexahydroxydiphenoyl*-D-*glucose*.—This compound was obtained from phenolic extracts prepared from leaf and shoots of various *Quercus* sp., *Rosa canina*, *Cornus alba*, *Fuchsia* sp., *Epilobium angustifolium*, *Filipendula ulmaria* and *Tellima grandiflora*. The compound was obtained as a light-brown solid after chromatography on Sephadex LH-20 in ethanol and, finally, evaporation from acetone (Found: C, 50.5; H, 3.8. Calc. for

$C_{34}H_{26}O_{22}\cdot H_2O$: C, 50.7; H, 3.5%), $[\alpha]_D^{20}$ +121.4° (*c* 1.3 in methanol); δ 7.06—6.98 (1 H, m, aromatic) δ 6.94, 6.66, 6.46, and 6.44 (2 H, m, aromatic), 5.89 (1 H, bt, *J* 9.5 Hz, 3-H), 5.57 (⅔ H, d, *J* 3.0 Hz, 1β-H), and 5.60 (⅓ H, d, *J* 9.5 Hz, 1β-H); R_F(A) 0.48; R_F(B) 0.40. The *trideca-acetate* [separated by t.l.c., R_F 0.5 on silica, with ethyl acetate–light petroleum (b.p. 60—80 °C) (9 : 1, v/v) as eluant] separated from ethanol as a white granular solid, m.p. 165—175 °C (Found: C, 54.3; H, 3.8. $C_{60}H_{52}O_{35}$ requires C, 54.1; H, 3.9%), ^{1}H. n.m.r. ($CDCl_3$) indicated a ratio of α and β anomeric forms of 2 : 1, $[\alpha]_D^{20}$ +54.1° (*c* 0.5 in $CHCl_3$).

β-1-O-*Galloyl*-2,3:4,6-*bis*-(S)-*hexahydroxydiphenoyl*-D-*glucose*.—This compound was isolated from phenolic extracts of the leaf and shoots of *Rosa canina*, *Quercus infectoria* (galls), *Rubus fructicosus*, and *Rubus idaeus* (fruit and leaves) by chromatography on Sephadex LH-20 in ethanol–methanol. The *ester* was obtained after evaporation from acetone as a light brown amorphous powder [Found (after drying at 60 °C, 0.5 mmHg, over P_2O_5): C, 51.2, H, 3.5; glucose, 18.2. $C_{41}H_{28}O_{26}\cdot H_2O$ requires C, 51.6; H, 3.1; glucose, 18.9%], $[\alpha]_D^{20}$ +34.9° (*c* 0.6 in methanol); R_F(A) 0.29, R_F(B) 0.26). Hydrolysis in water at 100 °C gave after 12 h ellagic acid, gallic acid, 2,3-(S)-hexahydroxydiphenoyl-D-glucose and a compound provisionally identified from the ^{1}H n.m.r. (Table 1) as β-1-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-D-glucose; R_F(A) 0.52; R_F(B) 0.26.

The *pentadeca-acetate* separated from methanol as a white granular solid, m.p. 182—185 °C (Found: C, 54.0; H, 4.0. $C_{71}H_{58}O_{41}$ requires C, 54.4; H, 3.6%), $[\alpha]_D^{20}$ −12.9° (*c* 0.4 in $CHCl_3$); R_F 0.39 (silica, acetone–chloroform, 1 : 6 v/v); ^{1}H n.m.r. ($CDCl_3$): δ 7.78 (2 H, s, aromatic), 7.45, 7.38, 7.20, and 7.15 (4 × 1 H, s, aromatic), 6.20 (1 H, d, *J* 9.0 Hz, 1-H), 5.56 (1 H, t, *J* 9.0 Hz, 3-H), 5.32 (3 H, m, 2-, 4-, 6-H), 4.20 (1 H, dd, *J* 6.0, 9.0 Hz; 5-H), 3.88 (1 H, bd, *J* 13.0 Hz, 6-H), and 2.05—2.30 (45 H, m, COMe).

α-1-O-*Galloyl*-2,3:4,6-*bis*-(S)-*hexahydroxydiphenoyl*-D-*glucose*.—This compound was isolated by chromatography on Sephadex LH-20 from the phenolic extracts of leaves and fruit of *Rubus fructicosus* and *Rubus idaeus*. The ester was obtained as a pale brown amorphous solid (Found: C, 51.3; H, 3.4; glucose 18.0. $C_{41}H_{28}O_{26}\cdot H_2O$ requires C, 51.6; H, 3.1; glucose 18.9%), $[\alpha]_D^{20}$ +125.8° (*c* 1.4 in methanol), R_F(A) 0.28; R_F(B) 0.25. The pentadeca-acetate separated from methanol as a white granular solid, m.p. 185—192 °C (Found: C, 54.5; H, 3.6. $C_{71}H_{58}O_{41}$ requires C, 54.4; H, 3.7%), ^{1}H n.m.r. ($CDCl_3$): δ 7.80 (2 H, s, aromatic), 7.48, 7.38, 7.13, and 7.21) (4 × 1 H, s, aromatic), 6.55 (1 H, d, *J* 3.5 Hz, 1-H), 5.65 (1 H, t, *J* 9.5 Hz, 3-H), 5.47 (1 H, dd, *J* 3.5, 9.5 Hz, 2-H), 5.32—5.38 (2 H, m, 4-, 6-H), 4.47 (1 H, dd, *J* 6.0, 9.5 Hz, 5-H), and 3.82 (1 H, bd, *J* 12.5, 1.0 Hz, 6-H).

Hydrolysis of the phenolic ester in water (100 °C) for 12 h gave ellagic acid and α-1-O-*galloyl*-2,3-(S)-*hexahydroxydiphenoyl*-D-*glucose* which was isolated as a light-buff powder after chromatography of the hydrolysis products in ethanol on Sephadex LH-20 (Found: C, 49.4; H, 4.0. $C_{27}H_{22}O_{18}\cdot H_2O$ requires C, 49.7; H, 3.7%), $[\alpha]_D^{20}$ +86° (*c* 0.6 in methanol); R_F(A) 0.51, R_F(B) 0.25. The *undeca-acetate* was separated by t.l.c. on silica [ethyl acetate–light petroleum (b.p. 60—80 °C), 9 : 1, v/v: R_F 0.81] and crystallised from ethyl acetate–light petroleum (b.p. 60—80 °C) as a white granular solid, m.p. 152—154 °C (Found: C, 53.5; H, 4.2. $C_{49}H_{44}O_{29}$ requires C, 53.7; H, 4.0%) $[\alpha]_D^{20}$ +42° (*c* 0.3 in chloroform); ^{1}H n.m.r. ($CDCl_3$): δ 7.84 (2 H, s, aromatic) 7.25 and 7.27 (2 × 1 H, s, aromatic), 6.58 (1 H, d, *J* 3.5 Hz, 1-H), 5.62 (1 H, t, *J* 9.0 Hz, 3-H), 5.45 (1 H, dd, *J* 3.5, 9.0 Hz, 2-H), 5.38 (1 H, t, *J* 9.0 Hz, 4-H), 4.35 (1 H, dd, *J* 3.0, 9.0 Hz, 5-H), 4.05—4.18 (2 H, m, 6-H_2), and 2.04—2.30 (33 H, m, COMe). Hydrolysis of the original phenol (48 h, 100 °C in water) gave 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose; R_F (A) 0.71, R_F(B) 0.17 and gallic acid.

2,3:4,6-*Bis*-(S)-*hexahydroxydiphenoyl*-D-*glucose*.—This compound was isolated from phenolic extracts of the leaf and shoots of *Rubus fructicosus*, *Rubus idaeus* and various *Quercus* sp. as a pale brown solid after chromatography on Sephadex LH-20 in ethanol–methanol (Found: C, 50.5; H, 3.6; glucose 21.7. Calc. for $C_{34}H_{24}O_{22}\cdot H_2O$: C, 50.9; H, 3.2; glucose 22.4%), $[\alpha]_D^{20}$ +97.4° (*c* 1.2 in methanol); R_F(A) 0.56; R_F(B) 0.15. The compound was identical (paper chromatographically) with pedunculagin [17] and D-2 (kindly donated by Dr. W. E. Hillis) [18] and with juglanin (kindly donated by Dr. L. Jurd).[19] The trideca-acetate was isolated by t.l.c. [silica, ethyl acetate–light petroleum (b.p. 60—80 °C), 8 : 1, v/v] and separated from methanol as a white granular solid (Found: C, 53.8; H, 3.8. Calc. for $C_{60}H_{50}O_{35}$: C, 54.1; H, 3.8%), $[\alpha]_D^{20}$ −15° (*c* 0.7 in $CHCl_3$); ^{1}H n.m.r. ($CDCl_3$) indicated an α : β ratio of anomeric forms of *ca.* 3 : 2.

Hydrolysis of the phenolic ester in water (100 °C, 12 h) gave 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose [R_F(A) 0.70; R_F(B) 0.17], and ellagic acid.

Phenolic Ester T_1 (16).—This compound was obtained from the young shoots, leaves and fruit of *Rubus fructicosus* and *Rubus idaeus* as a buff coloured powder after chromatography on Sephadex LH-20 in methanol (Found: C, 52.0, 52.6; H, 2.8, 2.9; glucose 18.2. $C_{82}H_{54}O_{52}$ requires C, 52.6; H, 2.9; glucose 19.3. $C_{82}H_{54}O_{52}\cdot H_2O$ requires C, 52.1; H, 3.0; glucose 19.1%), $[\alpha]_D^{20}$ +65.2° (*c* 1.4 in methanol), R_F(A) 0.29, R_F(B) 0.02. Hydrolysis of the phenolic ester T_1 (water, 100 °C, 40 h) gave, after chromatography on Sephadex LH-20 in ethanol, gallic acid, ellagic acid, 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (9%), α-1-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (5%) and the phenolic ester (17) (10%).

The *acetate* derivative of T_1 separated from methanol as a white granular solid m.p. 258—260 °C (decomp.) (Found: C, 53.9, 53.7; H, 3.6, 3.7. $C_{140}H_{112}O_{81}$ requires C, 54.4; H, 3.6%), $[\alpha]_D^{20}$ −7.5° (*c* 0.8 in $CHCl_3$); ^{1}H n.m.r. ($CDCl_3$): δ 7.88 (2 H, s, aromatic), 7.58 and 7.26 (2 × 1 H, d, *J* 3.0 Hz aromatic), 7.61, 7.48, 7.29, 7.25, 7.18, 7.13, and 7.12 (7 × 1 H, s, aromatic); glucose (A): δ 6.05 (1 H, d, *J* 9.0 Hz, 1β-H), 5.44 (2 H, bd, 2-, 3-H), δ 5.35 (1 H, m, 6-H), 5.02 (1 H, t, *J* 9.0 Hz, 4-H), 4.15 (1 H, dd, *J* 6.5, 9.0 Hz, 5-H), 3.89 (1 H, bd, *J* 12.0, 1.0 Hz, 6-H); glucose (B): δ 6.43 (1 H, d, *J* 3.5 Hz, 1α-H), 5.35 (2 H, m, 2-, 6-H), 5.15 (1 H, t, *J* 9.0 Hz, 3-H), 5.02 (1 H, t, *J* 9.0 Hz, 4-H), 3.82 (1 H, bd, *J* 12.0 Hz, 6-H), and 3.76 (1 H, dd, *J* 9.0, 6.5 Hz, 5-H).

Phenolic Ester (17).—This compound was obtained as a pale-brown powder after chromatography of the products of partial hydrolysis of the ester T_1 on Sephadex LH-20 in methanol (Found: C, 51.4; H, 3.5. $C_{69}H_{48}O_{44}\cdot H_2O$ requires C, 51.8; H, 3.1%), R_F(A) 0.42, R_F(B) 0.04; ^{1}H n.m.r. ([$^2H_6$]pyridine) δ 7.89 and 7.70 (2 × 1 H, d, *J* 3.0 Hz, aromatic), 7.65 (2 H, δ, aromatic), 7.28, 7.13, 7.07, 7.00, and 7.00 (5 × 1 H, s, aromatic), 6.79 (1 H, d, *J* 3.0 Hz, 1α-H), 6.52 (1 H, d, *J* 9.5 Hz, 1β-H), 6.12 (1 H, t), 5.97 (1 H, t), 5.65—5.78 (5 H, m), 4.54 (1 H, t), 4.36 (1 H, bd), 4.27 (1 H, bd), and 3.80 (1 H, bd). The acetate derivative separated from methanol as a white granular solid (Found: C, 51.7; H, 3.8. $C_{119}H_{98}O_{79}$ requires C, 51.2; H, 3.5%), ^{1}H n.m.r.

($CDCl_3$) δ 7.85 (2 H, s, aromatic), 7.53, 7.39, 7.23, 7.15, and 7.12 (5 × 1 H, s, aromatic), 7.64 and 7.19 (2 × 1 H, d, *J* 2.0 Hz, aromatic), 6.45 (1 H, d, *J* 3.0 Hz, 1α-H), and 5.92 (1 H, d, *J* 9.5 Hz, 1β-H).

The authors thank I.D.R.C. (Ottawa) for a fellowship (R. K. G.) and the Iraq government for financial support (S. M. K. Al-S); Mr. D. Williams (Curator, Sheffield Botanical Gardens) and the Directors of the Royal Botanical Gardens at Kew and Edinburgh for the provision and identification of plant species; Dr. B. A. Bohm for a gift of *Tellima grandiflora;* Dr. W. E. Hillis and Dr. L. Jurd for the provision of samples for identification purposes, and Dr. B. E. Mann and Dr. B. F. Taylor for ^1H and ^{13}C n.m.r. measurements and interpretations.

[2/024 *Received, 7th January,* 1982]

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