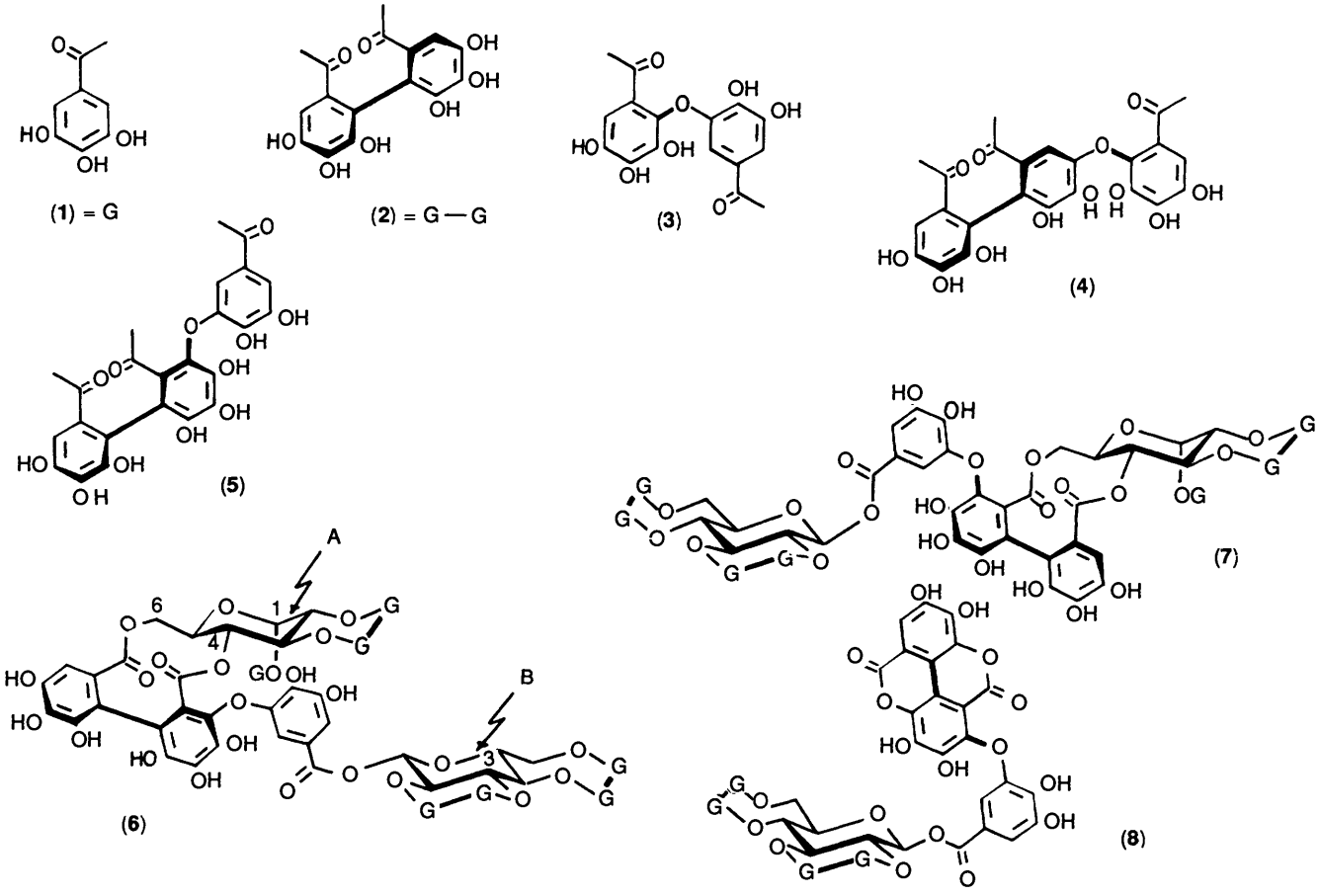
The Metabolism of Gallic Acid and Hexahydroxydiphenic Acid in Higher Plants Part 4;[1] Polyphenol Interactions Part 3.[2] Spectroscopic and Physical Properties of Esters of Gallic Acid and (*S*)-Hexahydroxydiphenic Acid with D-Glucopyranose ($^4C_1$)

Catriona M. Spencer, Ya Cai, Russell Martin, Terence H. Lilley, and Edwin Haslam*
Department of Chemistry, University of Sheffield, Sheffield S3 7HF

NMR spectroscopic methods, based upon ^1H and ^{13}C and two-dimensional long-range heteronuclear shift correlation, have been used accurately to define the positions of esterification to D-glucopyranose of structurally related phenolic acids in natural polyphenols. Quantitative measurements of physical properties *i.e.* gelling, distribution between octan-1-ol and water, and self-association of natural phenolic esters are described and related in some cases to features of putative biogenetic schemes for these metabolites.

Earlier papers in this series[1,3,4] demonstrated, within a suggested biogenetic framework, the extensive role which esters of both gallic and hexahydroxydiphenic acid (a convenient abbreviation for 6,6′-dicarbonyl-2,2′,3,3′,4,4′-hexahydroxydiphenic acid) play in higher plant metabolism. An increasing interest in the part which these particular metabolites play in the therapeutic action of traditional medicines of China and Japan[5,6] has engendered rapid growth of knowledge in this area. Many complex polyphenols including numerous 'dimeric' ellagitannins[7] such as rugosins D, E, and F,[8] sanguin H-6,[9] coriarin A,[10] agrimoniin,[11] and gemin A[12] have now been described and Okuda *et al.* have recently made reference[6] to 'tri- and tetra'-meric species, *i.e.* nobotannins J and K, respectively. Structures have generally been established by hydrolytic methods and by ^1H and ^{13}C NMR spectroscopy.[7,13,14,15] A crucial aspect of this work is the assignment of the precise



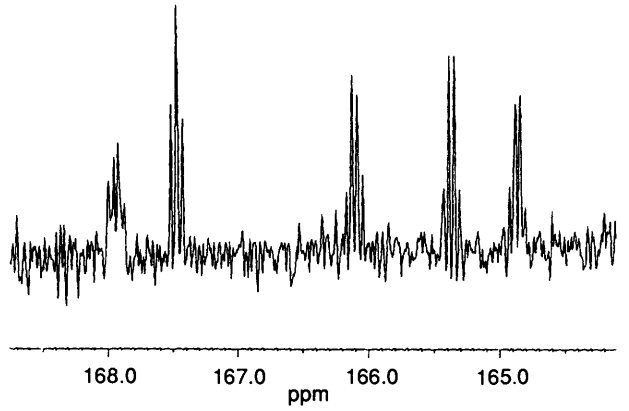


Figure 1. ^{13}C NMR spectrum of 1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose [eugeniin (**10**)] in ($^2H_6$)acetone at 20 °C, 0.2 mol dm^{-3}; the ester carbonyl region (165.0 to 168.0 ppm).

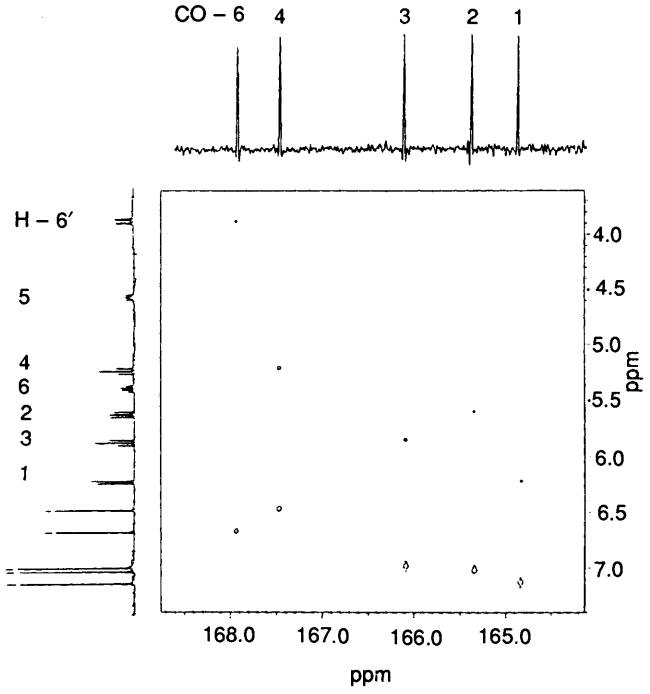


Figure 2. Two-dimensional COLOC C–H correlations for 1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose [(eugeniin (**10**)] in ($^2H_6$)acetone at 20 °C, 0.2 mol dm^{-3}, covering the carbonyl region in the carbon domain and the whole of the proton domain.

location on the D-glucopyranose core of the various closely related phenolic groups [galloyl (**1**); (*S*)-hexahydroxydiphenoyl (**2**); dehydrodigalloyl (**3**); valoneoyl (**4**); sanguisorbyl (**5**)]. Present methods rely heavily on analogy and empirical reasoning using the spectroscopic characteristics of known metabolites.[4,5,7,8] Investigations of polyphenol complexation, aimed at the determination of structure–activity relationships[16,17,18] and the selectivity of intermolecular recognition,[18] have also highlighted the same need to identify unequivocally and distinguish apparently identical groups [*e.g.* (**1**)–(**5**), all based on the 3,4,5-trihydroxybenzoyl radicle] in a given metabolite.

The chemistry of the polyphenolic substance T_1, isolated from various *Rubus* species[4,7] and shown subsequently to be identical with sanguin H-6 obtained from *Sanguisorba officinalis* by Nishioka,[9] typifies these two key problems. Hydrolytic and spectroscopic studies delineated[4] its structure, except that of the orientation of the sanguisorbyl ester group on the $^4C_1$-D-glucopyranose ring [*i.e.* (**6**) or (**7**)]. The subsequent isolation of (**8**), by aqueous hydrolysis (90 °C, 48 h, 5%) of T_1

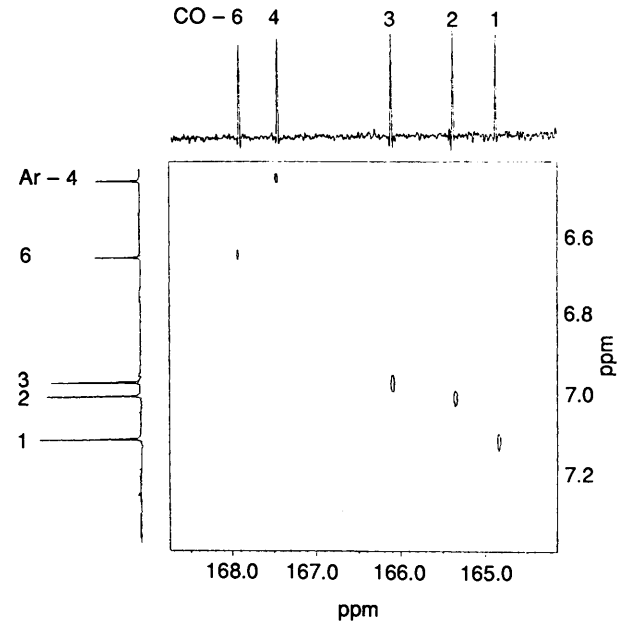


Figure 3. Two-dimensional COLOC C–H correlations for 1,2,3-tri-*O*-galloyl-4,6-(*S*)-β-hexahydroxydiphenoyl-D-glucopyranose [(eugeniin (**10**)] in ($^2H_6$)acetone at 20 °C, 0.2 mol dm^{-3}, covering the carbonyl region in the carbon domain and the aromatic ester protons in the proton domain.

further supports these conclusions. Likewise, detailed analysis of the complexation of T_1 (sanguin H-6) with caffeine (**9**)[19] clearly shows that the overwhelmingly preferred sites of association on the polyphenolic substrate are at the single free galloyl ester group (ring A C-1) and with just two of the nine remaining aromatic rings defined by the sanguisorbyl (**5**) and three (*S*)-hexahydroxydiphenoyl (**2**) ester groups. The present paper describes general procedures, based upon the technique of six-bond proton–proton correlations *via* two three-bond carbon–proton chemical shift correlations, using one- and two-dimensional NMR spectroscopy, which go a substantial way towards the solution of both of these problems. Brief reference has already been made to these techniques.[16,17,20]

Results and Discussion

Eugeniin[21] [*syn*-tellimagrandin-2,[22] (**10**)] is typical of the many simpler phenolic esters under consideration. Two-dimensional proton–carbon correlation spectroscopy enables the connectivity between the proton(s) of the aromatic ring of a 3,4,5-trihydroxybenzoyl ester [or a derivative such as (**2**)–(**5**)] and the proton at the position of esterification to the D-glucopyranose core to be established by virtue of the ^3J couplings to the aromatic proton(s) from the carbonyl carbon atom and likewise to the D-glucopyranose ring proton. The value of $^3J_{C-H}$ was determined from a fully coupled ^{13}C NMR spectrum as $^3J_{C-H}$ = 5.5 Hz (D-glucopyranose). The ^{13}C NMR spectrum of (**10**) thus gives rise to four quartets for the carbonyl carbon atoms associated with the four phenolic esters (at C-1, -2, -3, and -6) and one triplet from the carbonyl atom of the ester at C-4, (Figure 1). The two-dimensional long-range heteronuclear chemical-shift correlation uses the COLOC pulse sequence,[23]

$$^1H = D\text{–}90\text{–}t_{\frac{1}{2}}\text{–}180\text{–}t_{\frac{1}{2}}\text{–}(\Delta_1 - t_1)\text{–}90 \qquad BB$$

$$^{13}C = \text{————}180\text{————}90\text{–}\Delta_2\text{–FID}$$

with delay Δ_1 chosen to optimize for $^3J_{C-H}$ = 5.5 Hz *i.e.* 0.0909 s. The results are illustrated for eugeniin (**10**) in Figures 2 and 3. The proton assignments around the D-glucopyranose core were

Table 1. (*a*) Polyphenols: proton chemical shifts [($^2H_6$)acetone; $SiMe_4$].

Polyphenol (β-D-glucopyranose)	D-Glucopyranose ring protons							
	H-1	H-2	H-3	H-4	H-5	H-6	H-6	Conc./mol dm^{-3}; T/°C
1,3,6-Tri-*O*-galloyl-	5.82	3.89	5.31	3.87	4.01	4.56	4.38	0.2; 20[a]
1,2,6-Tri-*O*-galloyl-	5.98	5.25	3.98	3.77	3.93	4.58	4.48	0.3; 20
1,2,4,6-Tetra-*O*-galloyl- (**11**)	6.16	5.44	4.50	5.48	4.39	4.55	4.28	0.3; −23
1,2,3,4,6-Penta-*O*-galloyl- (**18**)	6.35	5.62	6.02	5.67	4.59	4.54	4.41	0.2; 20
1,2,3-Tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl- [eugeniin (**10**)]	6.22	5.61	5.86	5.23	4.57	5.38	3.90	0.2; 20
1,6-(*S*)-Hexahydroxydiphenoyl-2,3,4-tri-*O*-galloyl- (davidiin)	6.15	5.50	5.78	5.21	4.59	4.85	4.41	0.2; 45
Sanguin H-6 (**6**) ring A	6.54	5.29	5.04	5.02	4.22	5.60	3.91	0.2; 20
ring B	6.17	5.21	5.37	5.11	4.36	5.24	3.79	0.2; 20

(*b*) Galloyl ester protons: hexahydroxydiphenoyl ester protons.

Polyphenol (β-D-glucopyranose)	Position of ester group				
	1	2	3	4	6
1,3,6-Tri-*O*-galloyl-	7.11	—	7.14	—	7.07
1,2,6-Tri-*O*-galloyl-	7.05	7.08	—	—	7.13
1,2,4,6-Tetra-*O*-galloyl- (**11**)	7.10	7.13	—	7.17	7.19
1,2,3,4,6-Penta-*O*-galloyl- (**18**)	7.12	7.03	6.98	7.06	7.19
1,2,3-Tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl- [eugeniin (**10**)]	7.13	7.02	6.99	6.47	6.67
1,6-(*S*)-Hexahydroxydiphenoyl-2,3,4-tri-*O*-galloyl- (davidiin)	6.88	7.18	7.14	7.17	6.89
Sanguin H-6 (**6**) ring A	7.09	6.39	6.31	—	6.78
ring B	7.29, 7.14	6.47	6.30	6.50	6.76

[a] ($^2H_6$)acetone–D_2O (3:2 v/v).

established by conventional proton decoupling commencing with the anomeric proton (1-H, δ 6.22 ppm). The carbonyl group at C-6 shows a correlation (Figure 2) into just one of the diastereoisomeric protons at C-6 (δ 3.90 ppm) and this is due to the shorter T_1 relaxation time for the other C-6 proton (δ 5.38 ppm). Tables 1 and 2 show the accumulated ^1H and ^{13}C chemical shift data for a range of natural phenolic esters examined in this way. An alternative approach to this same problem is also illustrated for β-1,2,4,6-tetra-*O*-galloyl-D-glucopyranose (**11**). Figure 4 shows the heteronuclear shift correlation experiment for the aromatic region of (**11**); Figure 5 depicts the results of a one-dimensional decoupling difference experiment when the D-glucopyranose protons are decoupled. Figure 5(*a*) shows the fully coupled ^{13}C NMR spectrum (including a 'folded' peak marked with an asterisk); Figures 5(*b*)–5(*d*) are selectively decoupled difference spectra using very low decoupling power at D-glucopyranose protons 2, 4, and 1, respectively. Application of these procedures to sanguin H-6 (compound T_1) confirms (**6**) as the structure of the 'dimeric' ellagitannin. High-resolution (400 MHz) proton NMR spectroscopy leads to a complete assignment of the 14 aliphatic protons of the two D-glucopyranose rings; seven are associated with each of the two D-glucopyranose rings—A(α-configuration) and B(β-configuration)—both of which have a $^4C_1$ conformation. The sole ester carbonyl carbon atom (^{13}C, δ 165.4 ppm) with a doublet structure when fully proton-coupled, collapsed when 4-H on ring A (δ 5.02 ppm) was irradiated.

Application of these same procedures to the 'dimeric' ellagitannin rugosin D,[6,24] isolated earlier in this work[4] from *Rosa* sp. and *Filipendula ulmaria*, does not lead similarly to an unequivocal structural solution. Within the spectroscopic and chemical evidence two structures are compatible [(**12** or **13**)] dependent similarly on the precise orientation of the linking valeonyl group. Okuda and his colleagues[24] favour (**13**) on the basis of the analysis of the relative changes in chemical shift in ($^2H_6$)acetone–D_2O upon the progressive addition of ($^2H_5$)-pyridine. The relative stability towards hydrolysis of the 'dimers' (**6**) and (**12** or **13**) are nevertheless worthy of brief comment. Rugosin D (**12** or **13**) is hydrolysed rapidly in water at 90 °C to give tellimagrandin-1 (**14**)[22] and a compound, identical with

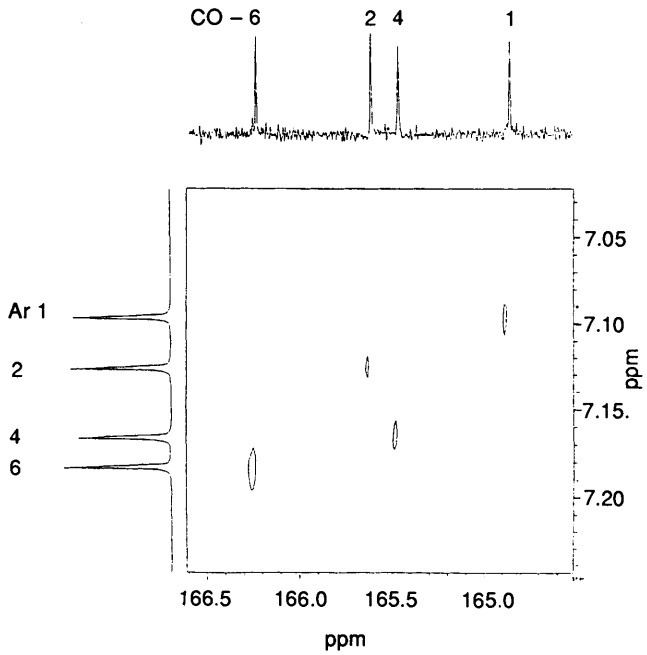


Figure 4. Two-dimensional long-range heteronuclear chemical shift correlation spectrum of 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose covering the carbonyl region in the carbon domain and the aromatic ester protons in the proton domain.

Table 2. Polyphenols: ^{13}C chemical shifts [($^2H_6$)acetone; $SiMe_4$].

Polyphenol (β-D-glucopyranose)	Position of esterification on D-glucopyranose[a] 1	2	3	4	6	Conc./mol dm^{-3}; T/°C
1,3,6-Tri-*O*-galloyl-	165.98	—	167.28	—	167.35	0.2; 20[b]
1,2,6-Tri-*O*-galloyl-	165.06	165.91	—	—	166.58	0.3; 20
1,2,4,6-Tetra-*O*-galloyl- (**11**)	164.86	165.61	—	165.47	166.24	0.2; −23
1,2,3,4,6-Penta-*O*-galloyl- (**18**)	164.85	165.55	165.78	165.48	166.32	0.2; 20
1,2,3-Tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl- [eugeniin (**10**)]	164.86	165.37	166.11	167.47	167.94	0,2; 20
1,6-(*S*)-Hexahydroxydiphenoyl-2,3,4-tri-*O*-galloyl- (davidiin)	166.19	165.67	165.57	166.22	168.28	0.2; 45
Sanguin H-6 (**6**) ring A	164.74	168.18	167.85	165.44	167.56	0.2; 20
ring B	165.32	167.76	169.05	167.56	167.70	
ring A	164.76	168.20	167.95	165.35	167.59	0.2; 42
ring B	165.35	167.79	169.09	167.53	167.71	

[a] Ester carbonyl carbon atoms. [b] ($^2H_6$)acetone: D_2O (3:2, v/v).

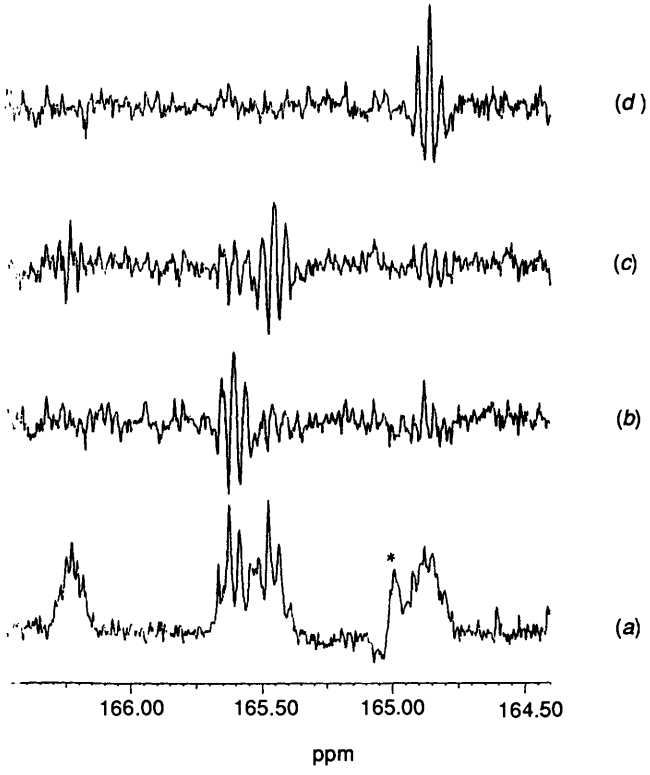


Figure 5. ^{13}C NMR spectra of the carbonyl region of β-1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose. Spectrum (*a*) shows the fully coupled ^{13}C spectrum including a folded peak marked with an asterisk. Spectra (*b*)–(*d*) are selectively decoupled difference spectra using very low decoupling power at D-glucopyranose ring protons 2,4, and 1, respectively.

rugosin A,[24,25] formulated as (**15a**), or its regioisomer (**15b**). Chromatography on Sephadex LH-20 in methanol at ambient temperature similarly leads to methanolysis of rugosin D and formation of (**14**) and the ester (**16a** or **b**). Molecular models clearly indicate that the linking valoneoyl ester group (**4**) is readily able to adopt a conformation (**17**) in which neighbouring hydroxy-group participation facilitates solvolytic action at the terminal ester group. By contrast, sanguin H-6 (**6**) is very slowly hydrolysed[4] by water at 90 °C; molecular models show that geometrical constraints totally inhibit such a solvolytic reaction in the isomeric sanguisorbyl ester group (**5**).

With the proliferation of novel 'dimeric' ellagitannin structures such as (**6**) and (**12** or **13**) reported over the past seven years[6,7] it is timely to seek to expand and define in greater detail the biogenetic framework which unifies them. The following general observations may now be made.

Table 3. Polyphenols: properties in aqueous media.
Dimerisation constants (K_D) in deuterium oxide; distribution coefficients between octan-1-ol and water (K_{oct}/H_2O) at 20 °C.

Polyphenol	K_D/dm^3 mol^{-1}	K_{oct}/H_2O
Flavan-3-ol derivatives		
(−)-Epiafzelechin	—	6.1
(−)-Epicatechin	4.39[a]	1.6
(−)-Epigallocatechin	4.23[a]	0.5
(−)-Epigallocatechin 3-*O*-gallate	8.56[a]	5.2
(+)-Catechin	3.35[a]	4.6
(+)-Catechin 3-*O*-gallate	9.75[a]	17.0
(+)-Gallocatechin	3.89[a]	1.0
Procyanidin B-2 (epicat-4β-8-epicat)	—	0.2
Procyanidin B-3 (cat-4α-8-cat)	—	0.3
D-Glucopyranose derivatives		
1,3,6-Trigalloyl-	6.89[b]	1.5
1,2,6-Trigalloyl-	6.54[b]	—
1,2,4,6-Tetragalloyl- (**11**)	18.17[b]	10.1
1,2,3,4,6-Pentagalloyl- (**18**)	26.70[b]	31.7
Corilagin	7.74[b]	0.1
Davidiin	54.92[b]	0.6
Eugeniin (**10**)	25.55[b]	4.4
Compound (**15a**) or (**15b**)	24.38[b]	—
Vescalagin	—	0.1
Casuarictin (**20**)	—	2.1
Sanguin H-6 (**6**)	7.51[b]	0.5
Rugosin D (**12** or **13**)	6.47[b]	1.2

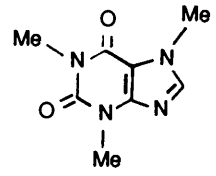
[a] 45 °C. [b] 60 °C.

(*i*) The great majority of these metabolites appear to be derived formally, in the biogenetic sense, by progressive dehydrogenation of β-1,2,3,4,6-penta-*O*-galloyl-D-glucose (**18**), for example, sanguin H-6 (**6**) from two molecules of (**18**) less ten hydrogen atoms.

(*ii*) Many metabolites which contain a 2,3:4,6-bis[(*S*)-hexahydroxydiphenoyl]-substituted D-glucopyranose ring also have the unique α-configuration at the anomeric centre, for example, sanguin H-6 (**6**), potentillin (**19**),[9] agrimoniin,[9] and gemin A.[10]

(*iii*) Invariably, co-occurring with (*ii*) are polyphenols in which the galloyl ester group at the anomeric centre is absent,[6] for example, pedunculagin (**21**)[26] and tellimagrandin-1 (**14**).[22]

(*iv*) *Intra*molecular oxidative coupling between phenolic ester



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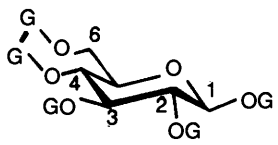

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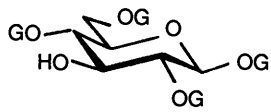

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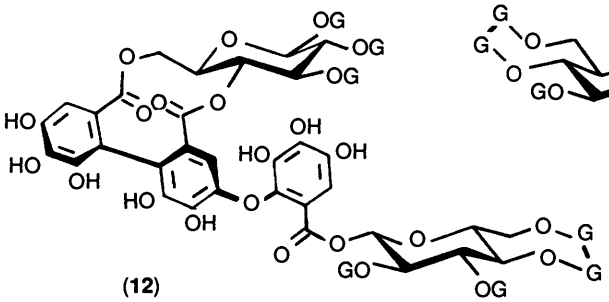

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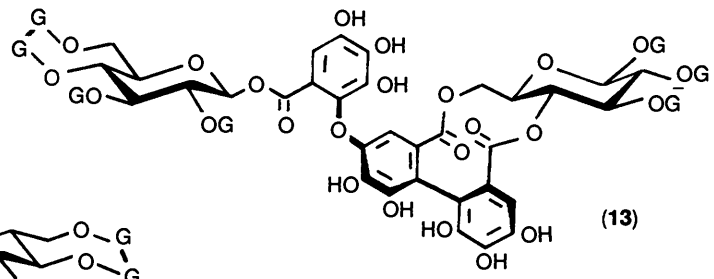

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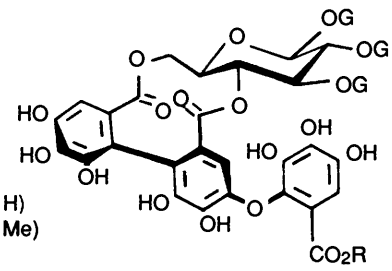
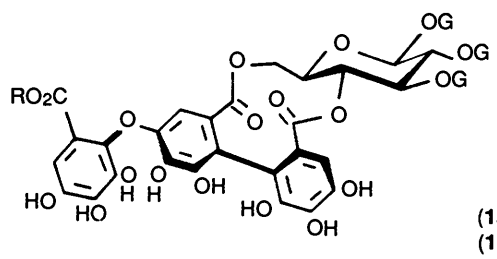


(15a; R = H)
(16a; R = Me)



(15b; R = H)
(16b; R = Me)

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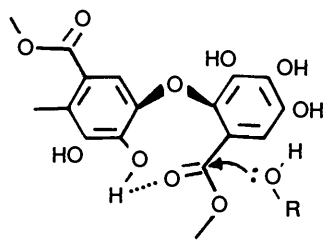

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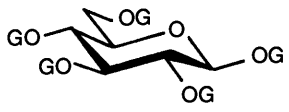

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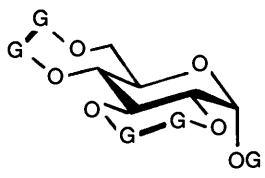

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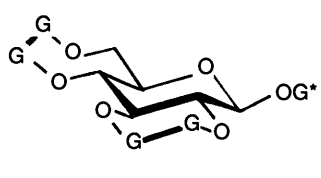

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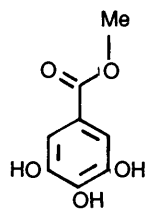

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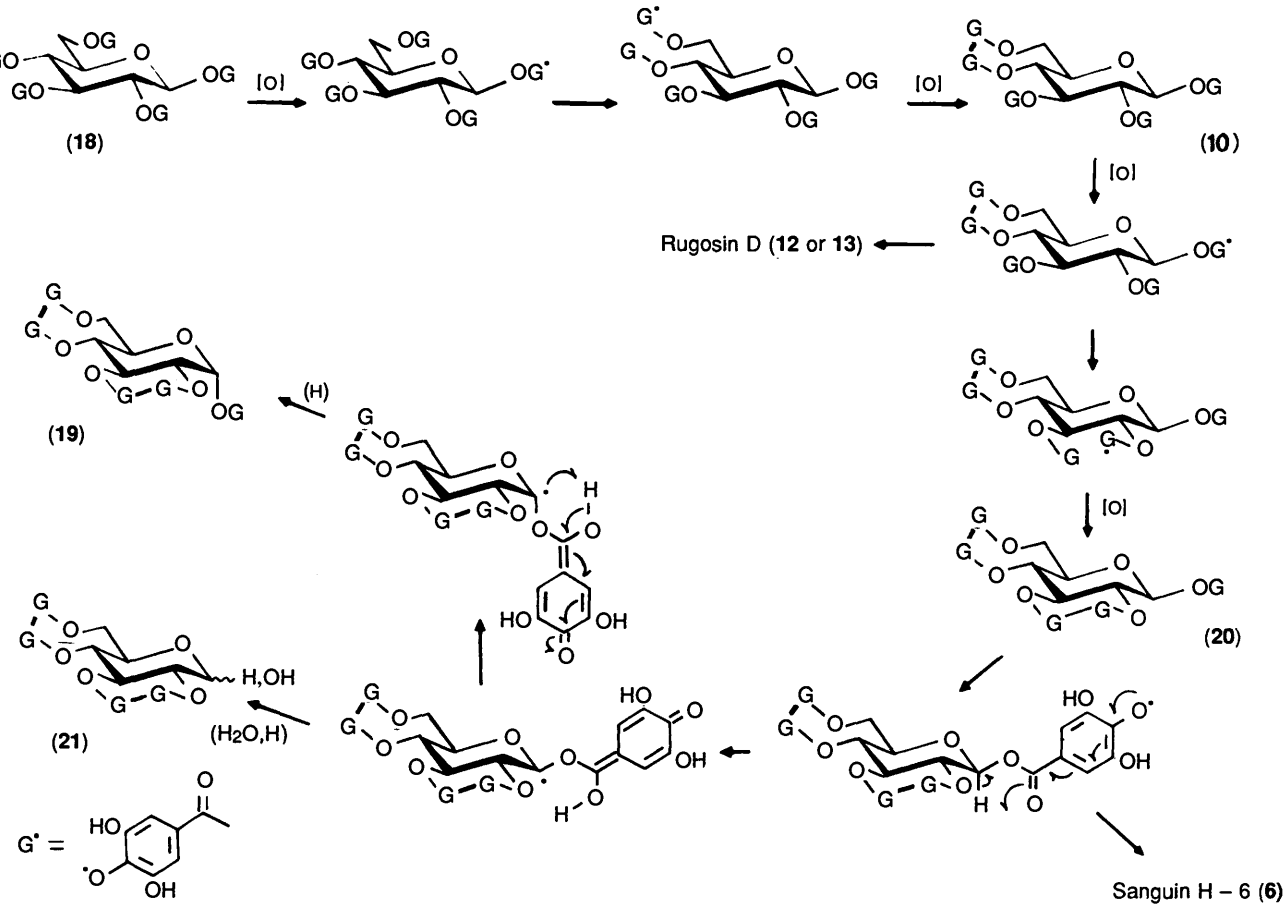


Scheme. Oxidative metabolism of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (**18**): some biogenetic proposals.

Table 4. Gel point of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (**18**).

Solution	Gel point/°C
(**18**) 5 mg cm^{-3}	22.5
(**18**) 5 mg cm^{-3} + NaCl (1 mol dm^{-3})	31.8
(**18**) 5 mg cm^{-3} + aspartame (3 mg cm^{-3})	27.8
(**18**) 5 mg cm^{-3} + octyl-β-D-glucopyranose (3 mg cm^{-3})	17.3

groups occurs to give new C–C bonds, whereas *inter*molecular oxidative coupling yielding 'dimeric' ellagitannins takes place *via* one of the anomeric galloyl ester groups to give new C–O bonds. Thus (**6**) is formally derived from (**19**) and (**20**) by oxidation at the galloyl ester (marked with an asterisk), at C-1 of (**20**).[7]

These facets of structure may be rationalised if *all* the oxidative metabolism of (**18**) is initiated at the anomeric galloyl ester group (*C*-1). Intramolecular radical transfer thus leads to 4,6 and then 2,3 C–C coupling (preferred on stereochemical grounds) to form (*S*)-hexahydroxydiphenoyl esters, to isomerisation at the anomeric centre or oxidatively mediated hydrolysis[27] of the C-1 galloyl ester group (Scheme).

The range of polyphenolic substrates now available makes possible for the first time the systematic study of the association of polyphenols with macromolecules such as proteins and polysaccharides.[5,16,17] These processes of molecular recognition depend essentially on the differences between the interactions of the isolated molecules and their environment (usually water) and the binding interactions in the complexes. Fersht[28] has suggested that the principal driving force for the association of macromolecules is the hydrophobic effect and that hydrogen bonds generally provide selectivity and specificity. In order to assess whether the association of polyphenols (with a multiplicity of hydrogen-bond donating and accepting groups) is a simple global hydrophobic process or has important and specific contributions from complementary hydrogen-bonding interactions, studies of the behaviour of polyphenolic substrates in aqueous media have been conducted. Hansch *et al.*[29] earlier studied the binding of a range of simple phenols to BSA. They observed that the adsorption of phenols onto the protein closely paralleled the transfer of the phenols from a water phase to octan-1-ol. They suggested that the hydroxy group(s) may not therefore play a specific role. Molyneux and Frank[30] similarly suggested that the binding of aromatic compounds to polyvinylpyrrolidone (PVP) in aqueous media involved two main effects, one of which was the entropy gain due to the formation of hydrophobic interactions. The transfer of a range of polyphenols between octan-1-ol and water has been measured (Table 3). Although there are significant exceptions polyphenols generally show a perhaps surprising affinity for partition into the octan-1-ol phase. These observations strongly support the view that water solubility[5,16,17] and associated hydrophobic effects are important factors in polyphenol complexation. Polyphenols partition exclusively nevertheless to the aqueous phase when the isomeric di-n-butyl ether constitutes the organic phase.

Esters such as β-1,2,3,4,6-penta-*O*-galloyl-D-glucopyranose (**18**) and β-1,2,3,6-tetra-*O*-galloyl-D-glucopyranose at concentrations of 2–5 mg cm^{-3} (*ca.* 2–5 × 10^{-3} mol dm^{-3}) readily form gels when aqueous solutions are cooled from 50–60 °C to ambient temperature. The gel point (determined spectro-

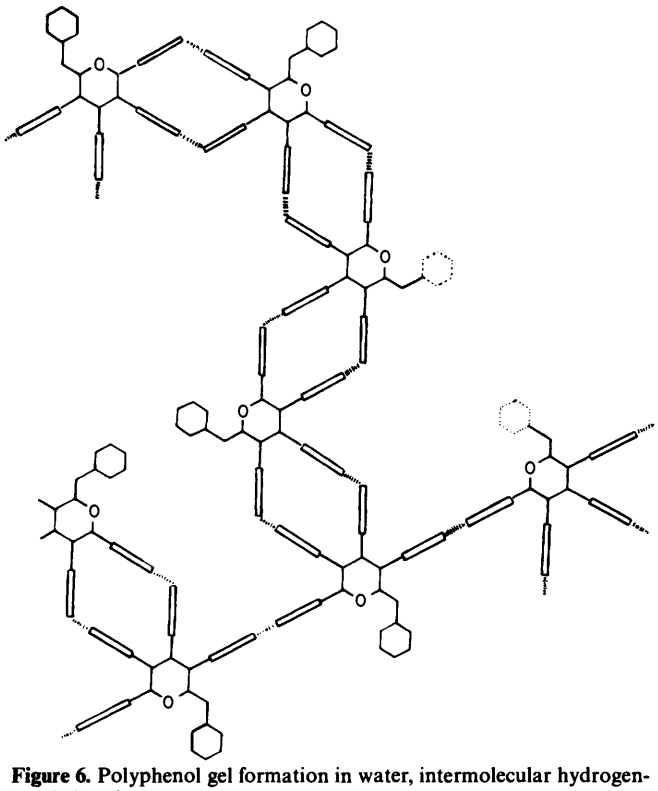


Figure 6. Polyphenol gel formation in water, intermolecular hydrogen-bonded lattice.

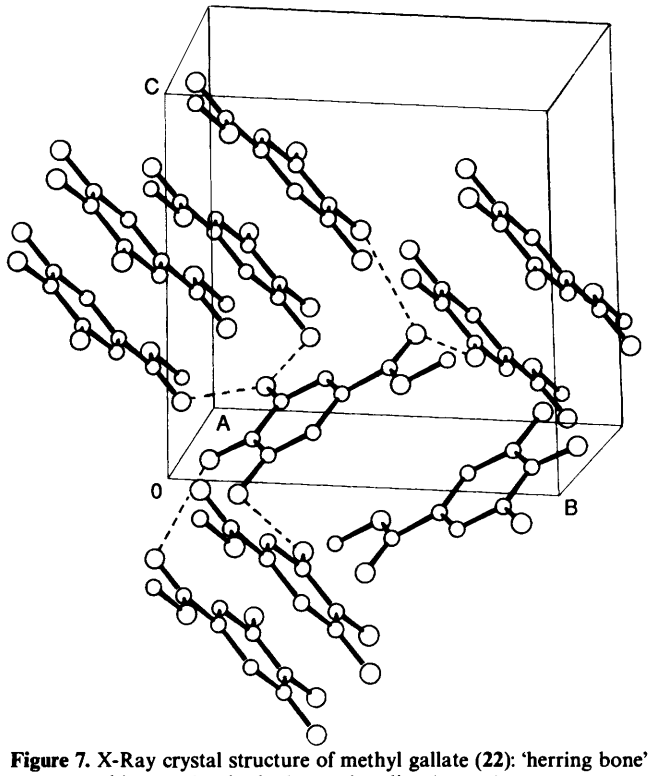


Figure 7. X-Ray crystal structure of methyl gallate (**22**): 'herring bone' pattern and intermolecular hydrogen bonding (- - - -).

photometrically*) is dependent upon the rate of cooling and solute concentration. Such gels presumably arise from the ability of polyphenols to form extensive three-dimensional cross-linked lattices by stacking and by intermolecular hydrogen-bonding between galloyl ester groups, with the localisation and exclusion of solvent (Figure 6). Support for this proposal derives from X-ray crystal structure analyses of simple phenols such as methyl gallate [(**22**); analysis by Dr. N. A. Bailey and Mr. H. Adams, University of Sheffield] in which the molecules are arranged in a classical 'herring-bone' fashion linked to nearest neighbours by hydrogen bonds (dashed line, Figure 7) involving each phenolic group as donor or acceptor[31] in turn. Polyphenol gels may be disrupted by being shaken or by the addition of solutes such as urea and β-octyl-D-glucopyranose (Table 4), (*cf.* proteins unfold in the presence of urea which it is believed decreases hydrophobic effects[32,33] in aqueous media). Gel formation is promoted by salts and by solutes such as caffeine (**9**) and the peptide sweetener, aspartame, presumably by facilitating formation of lattices of mixed composition.[34]

The ability to form gels is a particularly striking characteristic of many polyphenols and this ability for self-association has been determined by the quantitative evaluation of polyphenol homotactic dimerisation constants in water (Table 3). The technique uses the chemical-shift changes induced by magnetic anisotropic effects as the molecules associate and employs these in a modified form of the Benesi–Hildebrand equation[35] to determine K_D. There are no immediate parallels between these values and the distribution coefficients for polyphenols between octan-1-ol and water (Table 3). In this context, however, self-association may well be a prelude to oxidative dimerisation in the biogenesis of the 'dimeric' ellagitannins. If this proceeds *via* 'face-to-face' complexation then molecular models clearly demonstrate that steric effects are minimised if the complexes take the form shown [(**23**)] for eugeniin (**10**). This would lead specifically, as in the analogous case of sanguin H-6 (**6**), to the biosynthetic formation of an ether linkage between ester groups at C-1 (ring B) and at C-4 (ring A) and hence to the alternative structure (**12**) for rugosin D.[6,24] The preferential formation of 'face-to-face' complexes of this type [(**23**)], if they are generally maintained in solution for the 'dimeric' ellagitannins themselves [*e.g.* (**6**) and (**12**)], would explain the relatively high dimerisation constants for species such as (**10**) and (**18**) when compared with (**6**) and (**12**).

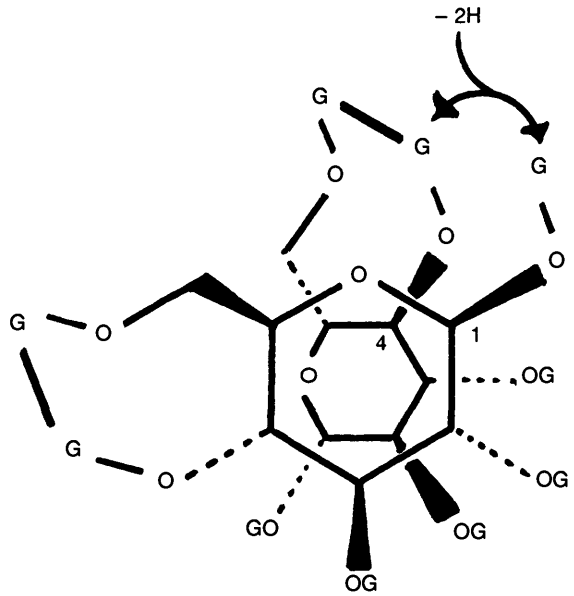
The important role that solvation plays in the determination of the properties of polyphenols is also demonstrated by the changes in proton NMR characteristics as the solvent is progressively changed from 100% ($^2H_6$)acetone or ($^2H_4$)methanol to deuterium oxide. Different phenolic nuclei are clearly solvated to quite different extents (Table 5). Proton NMR displacements were followed by systematic change of solvent composition from 100% ($^2H_6$)acetone [or ($^2H_4$)methanol] to 70% deuterium oxide content (at ambient temperature), to 70% deuterium oxide content (at 60 °C) and finally to 100% deuterium oxide at 60 °C (Table 5). These measurements now make possible the detailed examination of the selectivity of polyphenol complexation with substrates such as caffeine (**9**), α- and β-cyclodextrins and various peptides and these will be discussed in subsequent papers. However, it is significant to note, in the context of earlier proposals (*vide supra*) that in deuterium oxide caffeine binds highly selectively to sanguin H-6 (**6**) in the region of the galloyl ester group (C-1, ring A) and the two aromatic rings (C-6, ring A; C-3, ring B). Such an observation is readily accommodated if these two rings form a sandwich-type structure with the caffeine molecule.[34] The conformation of (**6**) required to permit such a complex is derived with very little perturbation from the conformation which results from the 'face-to-face' complex [*e.g.* (**23**)], postulated for the biogenesis of (**6**) and related 'dimeric' ellagitannins. This observation therefore adds further weight to the suggestions that these 'dimeric' ellagitannins adopt 'face-to-face' sandwich-

* With Miss S. Hope.

Table 5. Polyphenols; proton chemical shifts (ppm TSP); 0.003 mol dm^{-3} in deuterium oxide at 60 °C.

Polyphenol (β-D-glucopyranose)	Position of ester group[a]				
	1	2	3	4	6
1,3,6-Tri-*O*-galloyl-	7.15	—	7.12	—	7.28
1,2,6-Tri-*O*-galloyl-	7.00	7.13	—	—	7.16
1,2,4,6-Tetra-*O*-galloyl- (**11**)	7.11	7.16	—	7.13	7.09
1,2,3,4,6-Penta-*O*-galloyl- (**18**)	7.08	7.02	6.94	6.92	6.97
1,2,3-Tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl- [eugeniin (**10**)]	7.12	7.00	7.01	6.65	6.81
1,6-(*S*)-Hexahydroxydiphenoyl-2,3,4-tri-*O*-galloyl- (davidiin)	6.51	6.93	7.05	6.92	6.84
Sanguin H-6 (**6**) ring A	6.94	6.57	6.52	—	6.85
ring B	7.21, 6.99	6.468	6.474	6.77	6.87

[a] Galloyl and hexahydroxydiphenoyl ester protons.



(**23**)

like conformations in solution, and that the principal mode of polyphenol self-association in the galloyl-D-glucopyranose series is *via* similar 'face-to-face' complexation.

Experimental

General Methods.—Chromatographic methods (paper, HPLC, and Sephadex LH-20 separations) were as previously described.[3,36] Polyphenols were isolated and purified by repeated chromatography on Sephadex LH-20 until judged homogeneous by paper chromatography, HPLC, ^{13}C NMR spectroscopy, and microanalysis. Compounds were produced as follows: β-1,2,3,4,6-penta-*O*-galloyl-D-glucopyranose (**18**) by methanolysis of tannic acid (galls, *Rhus semialata*);[3,27] 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose by methanolysis of the gallotannin from Aleppo (galls, *Quercus infectoria*);[3,37,38] 1,2,-4,6-tetra-*O*-galloyl-β-D-glucopyranose (**11**) from roots and rhizomes of *Bergenia* sp;[3,39] corilagin by partial hydrolysis (H_2O, 90 °C, 0.5 h) of 1-*O*-galloyl-2,4:3,6-bis(hexahydroxydiphenoyl)-β-D-glucopyranose derived from geraniin by hydrogenation;[1] 1,3,6-tri-*O*-galloyl-β-D-glucopyranose by partial hydrolysis of chebulinic acid;[40] β-1,2,6-tri-*O*-galloyl-β-D-glucopyranose from leaves and fruit of *Rubus fructicosus*;[3,39] davidiin from the leaves and fruit of *Davidia involucrata*;[1] (+)-3-*O*-galloylcatechin from roots and rhizomes of *Bergenia* sp.;[41] (−)-epigallocatechin and (−)-3-*O*-galloylepigallocatechin from green tea[42] (*Camellia sinensis*); rugosin D (**12** or **13**), eugeniin (**10**) and compound (**15a**) or (**15b**) from leaves and inflorescences of *Filipendula ulmaria*;[3] sanguin H-6 (**6**) and casuarictin (**20**) from leaves and fruit of *Rubus* sp.;[3] (−)-epiafzelechin was a gift from Professor I. Nishioka (Kyushu University, Japan).

Bislactone (**8**). This compound was isolated as an off-white amorphous powder by chromatography of the partial hydrolysate[3] (H_2O, 100 °C, 24 h) of sanguin H-6 (**6**). *m/z* (FAB, negative ion) 1 235 ($M-H^-$), $[\alpha]_D^{20}$ −63° (0.8, MeOH *c*). ^1H NMR [$(CD_3)_2CO$] 7.58 (1 H, s), 7.30 (1 H, d, *J* 2.0 Hz), 6.87 (1 H, d, *J* 2.0 Hz), 6.64 (1 H, s), 6.50 (1 H, s), 6.35 (1 H, s), 6.29 (1 H, s); D-glucopyranose protons, 6.03 (1 H, d, *J* 8.5 Hz), 5.06 (1 H, t, *J* 8.5 Hz), 5.36 (1 H, dd, *J* 8.5 and 9.5 Hz), 5.10 (1 H, t, *J* 9.5 Hz), 4.39 (1 H, m), 5.28 (1 H, dd, *J* 7.5 and 13.0 Hz) and 3.80 (1 H, dd, *J* 2.0 and 13.0 Hz). ^{13}C NMR [$(CD_3)_2CO$] (carbonyl carbon atoms) 169.2, 168.4, 168.0, 167.8, 164.9, 160.1, 156.9.

Gel-point Determination.—(With Miss S. Hope.) Solutions of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (**18**) (5 mg cm^{-3}, 5.3×10^{-3} mol dm^{-3}) were kept at 60 °C in a thermostat for 1 h. Samples in standard silica cells (1 cm) were analysed using a Perkin-Elmer 559 UV–VIS spectrophotometer equipped with an automatic temperature control system. The sample was maintained at 60 °C for 15 min until the absorbance was constant and then cooled at a constant rate (0.5 °C min^{-1}). The absorbance at $\lambda = 500$ nm was recorded continuously. A plot of temperature *vs.* absorbance and extrapolation gave the gel initiation point (Table 4). Other substrates (sodium chloride, urea, aspartame, octyl-β-D-glucopyranoside, methyl β-D-glucoside, potassium thiocyanate) were added as necessary to the original polyphenol solution.

NMR Experiments.—All experiments were carried out on a Bruker WH-400 spectrometer equipped with an Aspect 2000 computer. Temperatures were accurately measured using a Comark series 5000 electronic thermometer attached to a copper–constantan thermocouple in an NMR tube containing the chosen solvent.

In the two-dimensional long-range heteronuclear chemical-shift correlation experiments, COLOC,[23] delays were chosen to optimise for coupling constants of 4–6 Hz and the digitisation employed was determined by the proximity of the signals under investigation in each compound. Typical values were 1 Hz/point in the carbon domain and 3 Hz/point in the proton domain.

Experimental times were normally *ca.* 16 h. Specific conditions employed are shown in Tables 1, 2, and 5. Typical experiments were carried out as described below for eugeniin (**10**), Figures 1, 2, and 3, and for 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose, Figures 4 and 5. Spectra of 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose were obtained in 0.25 mol dm^{-3} (100 mg/0.5 cm^3) solution in ($^2H_6$)acetone at −23 °C. The delays Δ_1 and Δ_2 were 0.125 and 0.0625 s, respectively. Two hundred scans were measured for each of 256 t_1 increments using 212 Hz in f_1 and 635 Hz in f_2. The total experimental time was 16 h. Selectively decoupled spectra (Figure 5) were measured using 3 360 scans for each spectrum in multiples of 96 to ensure good subtraction. The experiment took 16 h. The COLOC pulse sequence was employed for 1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranoside (**10**), 0.2 mol dm^{-3} in ($^2H_6$)acetone at 20 °C, with delays of Δ_1 and Δ_2 of 0.909 and 0.0455 s, respectively. The spectral width in f_2 was 468 Hz and in f_1 it was 764 Hz with a digital resolution of 1.83 Hz per point for carbon and 2.98 Hz per point for proton, 320 scans were taken for each of the 256 increments and the total experimental time was 33 h.

The temperatures and solvent mixtures used in the two-dimensional COLOC carbon–proton correlation experiments were chosen to give adequate separation of signals and to allow unambiguous assignment of the aromatic proton singlets of galloyl and related phenolic ester groups. In the case of sanguin H-6 (**6**) in addition to experiments conducted at 20 °C, further experiments were performed at 42 °C which gave better separation of the ester carbonyl groups of C-6 (ring A) and C-4 (ring B) and of the aromatic proton resonances of the ester groups at position 3 (rings A and B). An additional check on the assignments of the ester carbonyl signals of C-6 (ring A) and C-4 (ring B) was carried out at −20 °C where these two signals in the ^{13}C spectrum were sufficiently well resolved to permit selective decoupling when the associated D-glucopyranose ring protons were irradiated. The movement of all signals between −20 and +42 °C was monitored.

All samples in deuterioacetone were referenced to $(CHD_2)_2CO$ at 2.04 ppm and to the methyl group of $(CD_3)_2CO$ at 29.8 ppm. In deuterium oxide spectra were referenced to TSP (1,1,2,2-tetradeuteriotrimethylsilyl propionate, sodium salt) at 0.000 ppm in an external capillary. In the solvent titration experiments, the lock signal and reference were provided by hexadeuterioacetone (100% to 40%). Deuterium oxide lock and external TSP capillary were used for reference from 40% D_2O to 100% D_2O. This technique provided a degree of internal consistency whilst changing lock and reference system.

The self-association of polyphenols was measured using ^1H NMR chemical shift changes in solutions of increasing polyphenol concentration. Ten polyphenol solutions (*ca.* 1.5 to 12.0 × 10^{-3} mol dm^{-3}) were prepared in deuterium oxide. Samples were allowed to spin in the NMR probe (set at 45 °C for flavan-3-ols and at 60 °C for polyphenolic esters) for 20 min to reach thermal equilibrium, Spectra were then recorded (Bruker 250 MHz) and referenced with respect to an external standard (TSP). ^1H NMR chemical shift changes of selected signals in the spectrum of the polyphenol were analysed as a function of the increasing polyphenol concentration using a standard non-linear least square curve-fitting procedure. Assuming that in addition to monomer (P) the principal species additionally present in solution was dimer $(P)_2$ the association constant for dimerisation was obtained by a modified procedure based on the Benesi–Hildebrand method.[35]

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