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GALLOYLHAMAMELOSES AND PROANTHOCYANIDINS FROM *HAMAMELIS VIRGINIANA**

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Key Word Index—*Hamamelis virginiana*; Hamamelidaceae; bark; proanthocyanidins; galloylhamameloses; hamamelitannin

Abstract—The unique series of C-1 acylated galloylhamameloses is extended by the discovery of 1-*O*-(4-hydroxybenzoyl)-2′,3,5-tri-*O*-galloyl-α-D-hamamelofuranose and its 1-*O*-β-anomer. They are accompanied by the parent 2′, 3, 5-tri-*O*-galloyl-D-hamamelofuranose and the first pyranose analogue, shown to be 2′, 4-di-*O*-galloyl-D-hamamelopyranose. Assignment of ^{1}H NMR data for the peracetates of hamamelitannin is revised on the basis of a NOESY experiment. Structural examination of the proanthocyanidins present in the bark of *Hamamelis virginiana* reveals the occurrence of the novel epicatechin-(4β → 8)-catechin-3-*O*-(4-hydroxy)benzoate, the uncommon prodelphinidins, epigallocatechin-(4β → 8)-catechin, 3-*O*-galloyl epigallocatechin-(4β → 8)-catechin and 3-*O*-galloyl epigallocatechin-(4β → 8)-gallocatechin, together with the procyanidins, 3-*O*-galloyl epicatechin-(4β → 8)-catechin and catechin-(4α → 8)-catechin, and a tentatively characterized oligomer. The structures of these compounds were established by chemical and spectroscopic methods.

INTRODUCTION

The popular medicinal plant, *Hamamelis virginiana* L., has a long tradition of use in the treatment of a range of ailments, such as gastrointestinal disorders and inflammatory skin conditions [1]. Extracts of the bark and leaves of this species are highly valued in a number of countries for their curative properties. They are employed as a local haemostyptic agent and as an astringent, aiding, for example, the healing of wounds, burns and inflammation. From clinical studies and traditional medical practices, the volatile components and the polar fraction of the plant are of particular interest, containing the alleged biologically active substances. Earlier work on polyphenolic extractives provided proof of the occurrence of flavan-3-ols and a complex mixture of proanthocyanidins and hydrolysable tannins [2–7]. In a recent study, we have reported on the isolation and characterization of a series of highly unstable novel C-1 acylated galloylhamameloses and related metabolites, obtained from the ethyl acetate fraction [8]; the present work is a continuation of this investigation.

RESULTS AND DISCUSSION

Previous fractionation procedures of the ethyl acetate-soluble portion of the bark of *H. virginiana*, alternating between chromatography on Sephadex LH-20 and medium-pressure liquid chromatography (MPLC) on RP-18 using ethanol and gradient systems of H_2O–methanol, had afforded 1-*O*-(4-hydroxybenzoyl)-2′,5-di-*O*-galloyl-α-D-hamamelofuranose, its 1-*O*-β-anomer, 1,2′,5-tri-*O*-galloyl-α-D-hamamelofuranose and a tentatively characterized di-*O*-galloyl-D-hamamelopyranose, together with other unresolved chromatographic fractions [8]. In a modified work-up procedure, the ethyl actate-soluble extractives were processed following a similar combination of chromatographic techniques as described above to yield three additional tri-*O*-galloylhamameloses and six proanthocyanidin dimers, besides a chromatographically homogeneous oligomer. Owing to the complexity of the phenolic mixture from this source and difficulties in purification as free phenols, the metabolites were identified as the corresponding peracetates, the additional chromatographic stage offered by such an approach being a prerequisite for compound purity.

Known compounds amongst the monomers included (+)-catechin and (+)-gallocatechin, which were readily identified by physical properties (^{1}H NMR and optical

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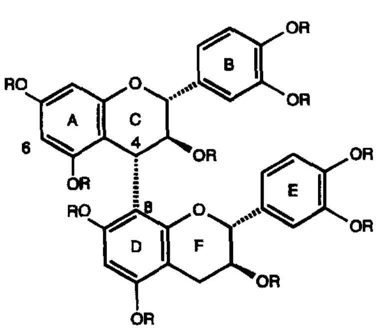
*Part of the projected Ph.D. thesis of C. Hartisch. Dedicated

O-gallate in our plant material [2, 3]. The absence of the putative 2,3-*cis* nucleophiles as potential precursors of associated proanthocyanidins may be rationalized by rapid turnover rates, e.g. complete conversion into oligomers. Similar cases are to be found in *Salix* [10] and *Rhus* [11] species.

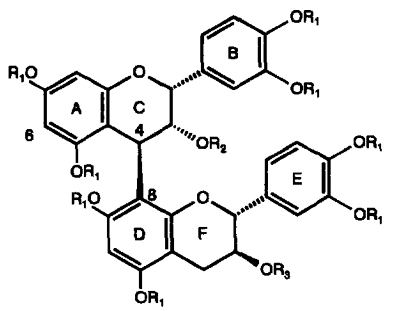
Structural assessment of the procyanidins **1**–**3** has been achieved by examination of the spectroscopic data [^{1}H NMR, mass spectrometry and circular dichroism (CD)] of their peracetates, supported by degradative studies. Identification of catechin-(4$\alpha \rightarrow$8)-catechin (**1**), isolated at very low concentrations, was made by comparison of the physical data of its acetate **1a** with those of an authentic specimen [12].

The dimers (**2** and **3**) were found to be acyl derivatives of the known epicatechin-(4$\beta \rightarrow$8)-catechin (**4**) as indicated by the close analogy of signal and coupling patterns of the ^{1}H NMR spectra of their peracetates **2a**–**4a**. Coupling constants of the heterocyclic protons of the C-ring ($J_{2,3} = J_{3,4} \leq 2$ Hz) confirmed the relative 2,3-*cis*-3,4-*trans*-configuration of the upper constituent units, while the 2,3-*trans*-stereochemistry of the lower units was evident from the large coupling constants ($J_{2,3} = 9.8$–9.9 Hz). Both compounds gave cyanidin on reaction with 5% HCl in ethanol and yielded catechin on treatment with 0.1 M ethanolic HCl under the conditions employed [13]. Accordingly, the degradation studies in conjunction with ^{1}H NMR data suggested a biflavanoid constitution with an 'upper' epicatechin and 'lower' catechin constituent unit in each instance. A diagnostic feature in the ^{1}H NMR spectrum of **2a** was the presence of a sharp low-field two-proton singlet (δ 7.54) attributable to the equivalent 2- and 6-protons of a galloyl group. Supporting evidence for the presence of a galloyl group and the B- and E-ring oxygenation pattern was available from acid hydrolysis and DCI-mass spectrometry ($[M + 18]^+$ *m/z* 1252) of the acetate derivative. Location of the galloyl group at C-3(C) followed from a significant downfield shift of the H-3(C) proton ($\Delta\delta$ 0.26) as compared with the equivalent proton of the acetate of the parent procyanidin **4a**. The upfield position of the aromatic A-ring proton resonances (H-6 and H-8: δ 6.06 and 6.33, respectively) was consistent with the (4$\rightarrow$8)-interflavanyl linkage, as was the chemical shift of H-2(F) (δ 4.36) [14, 15]. Confirmation of the 4β-DEF moiety and, thus, the 4*R* absolute configuration was obtained via the positive Cotton effect in the diagnostic wavelength region 200–240 nm of the CD spectrum of compound **2a**. Collectively, these features characterized **2** as 3-*O*-galloyl epicatechin-(4$\beta \rightarrow$8)-catechin, its natural occurrence being hitherto demonstrated by its isolation from rhubarb [16], *Polygonum multiflorum* [17], *Quercus miyagii* [18] and *Vitis vinifera* [19].



1 R = H

1a R = Ac

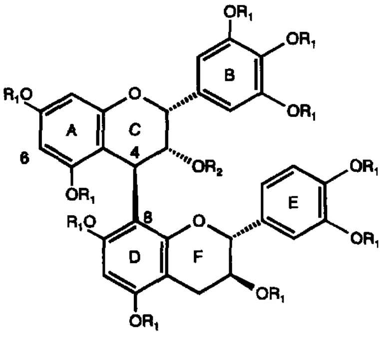


2	$R_1 = R_3 = H$;	R_2 = galloyl
2a	$R_1 = R_3 = Ac$;	R_2 = galloylperacetate
3	$R_1 = R_2 = H$;	R_3 = (4-hydroxy)-benzoyl
3a	$R_1 = R_2 = Ac$;	R_3 = (4-hydroxy)-benzoylperacetate
4	$R_1 = R_2 = R_3 = H$	
4a	$R_1 = R_2 = R_3 = Ac$	

A protocol of using appropriate ^{1}H parameters and CD in combination with chemical methods similarly (4$\beta \rightarrow$8)-catechin structure, with resonances at δ 5.99(A), 6.29(A) and 4.32(F), in conjunction with the dominance of one conformer characteristic of a (4$\beta \rightarrow$8)-interflavanoid linkage [13–15], and accompanied by resonances and coupling constants readily assigned to heterocyclic C- and F-rings of the constituent units as indicated above. Notable differences included replacement of the two-proton galloyl singlet by A_2B_2-type aromatic signals at δ 7.31 and 8.13, signifying the presence of a 1,4-disubstituted benzene moiety. Allocation of these signals followed from a ^{1}H–^{1}H COSY experiment which permitted unambiguous assignment of the coupling pattern of overlapping signals in the aromatic region. Detection of 4-hydroxybenzoic acid as the cleavage product upon acetylation of the chromatographically homogeneous sample provided proof of the *p*-hydroxybenzoyl residue in **3**, while the facile elimination of the acyl substituent reflected constitution. Positive ion FAB-mass spectrometry of **3a** provided successful M_r information, showing the peak of highest mass at m/z 1059, which can be accommodated in terms of loss of an acetyl fragment from the expected $[M+H]^+$. Comparison of the ^{1}H NMR data for the peracetate **3a** with those for **4a** indicated a little downfield shift of H-3(F) ($\Delta\delta$ 0.05) in **3a**, thereby locating the *p*-hydroxybenzoyl group at this position. In this context, it should be noted that deshielding of H-3(F) induced by 3-*O*-acyl residues on ring F in proanthocyanidins with 2,3-*cis* extender units is less pronounced (*ca* $\Delta\delta$ 0.10–0.18) than that of H-3(C) of 'upper' 2,3-*cis* and 2,3-*trans* constituent units (*ca* $\Delta\delta$ 0.23–0.50) by equivalent substituents on ring C (unpublished results). This differential shift behaviour should be of direct value in defining the point of attachment of acyl groups to either constituent flavanyl unit in dimers. While the chemical shift criteria of aromatic A-ring proton resonances and H-2(F) strongly supported the presence of a 4β-substituted epicatechin entity [14], the negative sign of the low-wavelength Cotton effect in the CD spectrum contrasts with such an orientation of the interflavanyl linkage. Reversal of the Cotton effect in the diagnostic wavelength region may well be the result of unconventional conformational behaviour, for example, significant contributions by a non-preferred A-conformation of the 'upper' epicatechin moiety. Owing to both line-broadening of signals and small coupling constants associated with 2,3-*cis*-3,4-*trans*-proanthocyanidin units, evidence for E–A conformational inversion of the C ring is lacking. At present, we cannot satisfactorily explain the irregularities regarding the CD data for $4\beta \rightarrow 8$ linked proanthocyanidin peracetates possessing 'upper' 2,3-*cis*-3,4-*trans* moieties, being apparently caused by the presence of a bulky acyl group, such as galloyl or 4-hydroxybenzoyl residues at C-3 of the 'lower' flavanyl unit. Similar peculiar chiroptical properties have very recently been reported for epigallocatechin-$(4\beta \rightarrow 8)$-epigallocatechin-3-*O*-gallate [20]. Taken in conjunction, these features collectively characterized **3** as the noval epicatechin-$(4\beta \rightarrow 8)$-catechin-3-*O*-(4-hydroxy)benzoate. Although *p*-hydroxybenzoic acid has already been reported as an uncommon acyl residue of monomeric flavan-3-ols [20, 21], this is the first example of a proanthocyanidin dimer possessing a *p*-hydroxybenzoyl moiety.

The remaining proanthocyaninids **5**–**7** gave delphinidin on reaction with alcoholic hydrochloric acid. Comparison of the ^{1}H NMR data for their peracetates **5a**–**7a** with those for the well-documented peracetate of epicatechin-$(4\beta \rightarrow 8)$-catechin (**4a**) [12, 22] revealed their close structural resemblance to this procyanidin derivative. The ^{1}H NMR spectra of **4a** and **5a** were virtually superposable, except for the presence of only one AMX-system characteristic of the 3′,4′-oxygenation pattern on the B-ring of constituent flavanyl units an 'upper' flavanyl unit possessing a pyrogallol-type element. The chemical shift of the characteristic two-proton singlet of the equivalent 2′-H and 6′-H of the B-ring (δ 7.17) was fully consistent with such an arrangement [23]. This constitution was further supported by a $[M+18]^+$ peak at m/z 1074 in the DCI-mass spectrum of **5a**. The ^{1}H NMR spectral properties of **5a** proved to be identical to those recently described [24]. These data taken in conjunction with the strong positive Cotton effect at low wavelengths in the CD spectrum, established the structure of **5** to be epigallocatechin-$(4\beta \rightarrow 8)$-catechin. Compound **5** has previously been reported from *Pinus sylvestris* [25], *Ginkgo biloba* [26] and *Cistus incanus* [24].

The ^{1}H NMR spectrum of the structurally related peracetate **6a** differed from that of **5a** with respect to the presence of an additional two-proton singlet at δ 7.54, attributable to the equivalent protons of a galloyl group. Supporting evidence was provided by acid hydrolysis of **6**, furnishing gallic acid which was identified by co-chromatography with an authentic specimen. DCI-mass spectrometry of **6a** gave an [M +

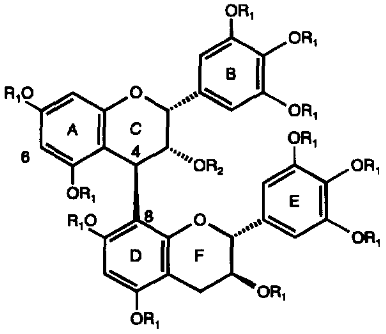


5 $R_1 = R_2 = H$

5a $R_1 = R_2 = Ac$

6 $R_1 = H$; $R_2 =$ galloyl

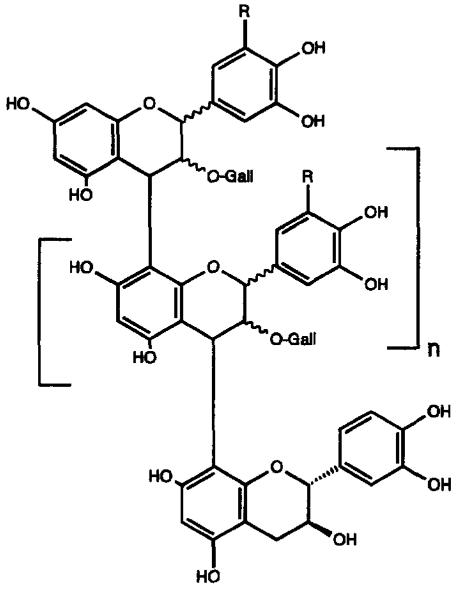
6a $R_1 = Ac$; $R_2 =$ galloylperacetate



$18]^+$ ion peak at m/z 1310, fully consistent with the anticipated chemical structure. The point of attachment of the galloyl group in **6a** at the C-3(C) position was indicated by a significant downfield shift ($\Delta\delta$ 0.25) of H-3(C) as compared with the chemical shift of the equivalent proton in the parent prodelphinidin **5a**. Also, the H-2(C) and H-4(C) in **6a** are clearly shifted downfield ($\Delta\delta$ 0.12 and 0.10, respectively) in comparison with **5a**, providing further evidence for the localization of the galloyl group at this position. The upfield position of the aromatic A-ring proton resonances H-6 and H-8 (δ 6.06 and 6.34, respectively) and of H-2(F) (δ 4.35) [14, 15], when taken in conjunction with the dominance of one conformer [13], indicated a ($4\beta\rightarrow8$) interflavanoid linkage. The chemical shift evidence in conjunction with ^{1}H NMR coupling constants (C-ring: $J_{2,3}$ and $J_{3,4} < 2$ Hz; F-ring: $J_{2,3}$ 9.6 Hz) and degradative studies suggested a biflavanoid constitution consisting of a 3-*O*-galloyl epigallocatechin 'upper' unit linked to the C-8 of a catechin 'lower' unit. The assignment of the structure 3-*O*-galloyl epigallocatechin-($4\beta\rightarrow8$)-catechin to **6** was finally made by the high-amplitude positive Cotton effect in the CD spectrum at 200–240 nm. Compound **6**, being reported from a plant source for the second time, has recently been isolated from *Q. dentata* [27].

Structural assessment of the prodelphinidin 3-*O*-galloyl epigallocatechin-($4\beta\rightarrow8$)-gallocatechin (**7**) was similarly achieved by combination of the ^{1}H NMR data for its peracetate (**7a**) and the strong positive Cotton effect in the diagnostic wavelength region, supported by chemical degradation studies that suggested an 'upper' gallocatechin-type unit linked to the terminal gallocatechin moiety, but also the presence of a galloyl group in **7**. DCI mass spectral analysis indicated a $[M+18]^+$ ion at m/z 1369, corresponding to a monogalloylated prodelphinidin structure. Replacement of the 3',4'-oxygenation pattern in **6a** by a pyrogallol element in **7a** was evident from two sharp two-proton singlets (δ 6.86 and 7.19) in the B-ring region of the ^{1}H NMR spectrum of **7a**, while the additional aromatic low-field singlet at δ 7.55 was again indicative of the presence of a galloyl group. The notable downfield shift of H-3(C) ($\Delta\delta$ 0.27) in comparison with the respective proton in **5a**, being also identical with that observed for 3-*O*-galloyl epigallocatechin-($4\beta\rightarrow8$)-catechin peracetate (**6a**) ($\Delta\delta$ 0.25) (see above), confirmed the location of the galloyl group. By analogy with the prodelphinidin peracetates described above and the respective procyanidin derivatives with a 2,3-*cis*-3,4-*trans*-configuration [14], the ^{1}H shift parameters involving H-6(A), H-8(A) and H-2(F) indicated the ($4\rightarrow8$)-interflavonid linkage. These features collectively defined the structure of **7** to be that described only recently from *C. incanus* [24].

The proanthocyanidin oligomer **8** from the ethylacetate phase was purified by multiple chromatographic



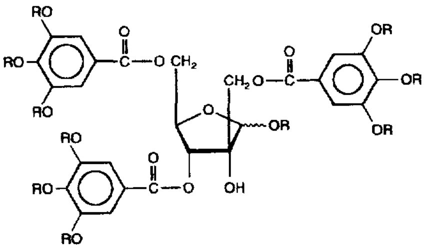
8 R = H/OH; n = 2 - 4

concluded from its chromatographic mobility when compared to that of a reference sample [28]. Evidence for the presence of 3-*O*-gallate units was available from acid hydrolysis, which afforded gallic acid. A closer definition of the flavan units in the oligomer was obtained from chemical degradation. The heterogeneity of the flavan B ring, i.e. di- and tri-hydroxylation patterns, was indicated by the treatment of the oligomer with alcoholic hydrochloric acid, which yielded delphinidin and cyanidin in a ratio of *ca* 1 : 1. That catechin was the lower terminal unit followed from mild acid hydrolysis. Reaction of the oligomer with phloroglucinol under acid conditions unexpectedly failed to produce detectable amounts of cleavage products within 72 hr, contrasting with the chemical behaviour of an epicatechin oligomer [28] concurrently subjected to similar degradation studies. The apparent absence of any phloroglucinol addition products in the reaction mixture strongly indicated steric hindrance to acid-catalysed cleavage of the interflavanoid linkages. This may be rationalized by the presence of the bulky galloyl group on the C-3 of extension units, the 3-*O* substituent being in close proximity to the interflavanoid bonds. A similar observation was made for the dimer 3-*O*-galloyl epigallocatechin-(4β,8)-catechin and a related polymer [27]. Accordingly, the extension units were eventually suggested to be galloylated throughout and, in addition, to possess the 2,3-*cis*-configuration.

Following demonstration of the presence of a series of galloylhamameloses, hitherto unique to *H. virginiana* respect to their chromatographic properties. Repetitive column chromatography of these fractions has led to enriched mixtures from which three additional galloylhamameloses were obtained upon acetylation and subsequent purification by preparative TLC.

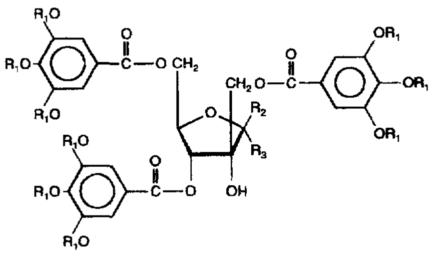
The galloylhamamelose constitution of **9** was apparent from the ^{1}H NMR spectrum of its acetate **9a**, which showed signal and coupling patterns in the δ 4.30–6.70 region reminiscent of a hamamelose moiety together with aromatic resonances [δ 7.68, 7.75, 7.83, 7.84 ($\times 2$) and 7.90] consistent with the presence of three galloyl groups [8]. Duplication of the absorptions due to the galloyl and carbohydrate moieties implied that the anomeric centre was not acylated and, hence, this compound existed in solution as an equilibrium mixture of α- and β-forms. Owing to unequal intensities (relative ratio *ca* 1:2, respectively) the signals arising from each anomer could be allocated. Another feature of diagnostic value included broad OH-absorptions at δ 2.64 and 3.11, being indicative of the non-acetylated tertiary 2-OH functionality in the sugar residue. Positive ion FAB-mass spectrometry of **9a** gave a $[M+H]^+$ peak at m/z 1055, which was also fully consistent with the proposed chemical constitution. These spectroscopic features facilitated definition of the structure of **9** as 2′,3,5-tri-*O*-galloyl-D-hamamelofuranose. This compound, previously isolated from *Castanea crenata* and *Sanguisorba officinalis* [29], was obtained from *Hamamelis* for the first time.

The series of the unique 1-*O*-(4-hydroxybenzoyl)-galloylhamameloses [8] is extended by the recognition of novel C-1 acylated analogues, the 1-*O*-(4-hydroxybenzoyl)-2′,3,5-tri-*O*-galloyl-α-D-hamamelofuranose (**10**) and its 1-*O*-β-anomer (**11**). These metabolites were obtained upon acetylation as an inseparable mixture (**10a** and **11a**, relative ratio 1:2) which again proved to be highly unstable. Thus, mass spectral analysis failed to detect the expected $[M]^+$. Structural assessment was effected by comparing the ^{1}H NMR data with those for the corresponding derivatives of the recently characterized C-1 acylated analogues of hamamelitannin [8]. Key features again include the presence of an AA′BB′ spin system at δ 7.18 and 7.26 (*d*, $J = 7.3$ Hz; overlapping for the mixture of **10a** and **11a**), characteristic of a 1,4-disubstituted benzene moiety, and the resonance of one aromatic acetoxy function located to lower fields (δ 2.36). The chemical shift of the latter signal agreed well with that for the acetoxy functionality of a reference sample of 4-acetoxybenzoic acid. Subsequent TLC analysis indicated degradation of the mixture by detection of 'liberated' *p*-hydroxybenzoic acid in the sample, a behaviour which matched our previous observation. The identification of **10** and **11**, in conjunction with our recent results, suggests that all the galloylhamameloses in *H. virginiana* may occur as their unstable 1-*O*-*p*-hydroxybenzoyl derivatives.



9 R = H

9a R = Ac



10 $R_1 = R_2 = H$; R_3 = O-(4-hydroxy)-benzoyl

10a $R_1 = Ac$; $R_2 = H$; R_3 = O-(4-hydroxy)-benzoylperacetate

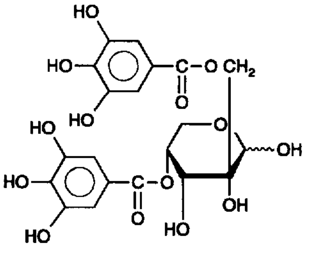
11 $R_1 = R_3 = H$; R_2 = O-(4-hydroxy)-benzoyl

11a $R_1 = Ac$; $R_3 = H$; R_2 = O-(4-hydroxy)-benzoylperacetate

In our recent paper, we reported on the structural

drate moiety is now unambiguously defined in the acetate **12a** by a long-range ^{1}H–^{13}C correlation experiment. The galloyl proton resonance (δ 7.82) was correlated by three-bond couplings with an ester carbonyl carbon signal (δ 163.8), which also showed a correlation with the H-2′ methylene protons (δ 4.41 and 4.66) of the hamamelose portion. Similarly, the remaining galloyl absorption (δ 7.75) was shown to be correlated with the lowfield ester carbonyl at δ 164.4, which in turn was coupled with the H-4 proton (δ 4.50) of the hamamelose entity. These allocations were additionally confirmed by long-range ^{1}H–^{13}C HETCOR connectivities between the aliphatic acetoxy groups and the anomeric proton (δ 6.21) and H-3 (δ 5.44), thus establishing the structure of **12** as 2′,4-di-*O*-galloyl-D-hamamelopyranose. The configuration of the anomeric centre still remains to be determined.

Although the structure of the conventional hamamelitannin **13** is placed beyond doubt [30–32], definition of the α- and β-form of the distinct acetate derivatives (**13a** and **13b**) has received our special interest, since assessment of the configuration at the anomeric centre is excluded by a respective ^{1}H–^{1}H coupling constant due to the presence of a quaternary carbon at C-2. This problem has apparently been ignored in the literature, being hitherto exclusively



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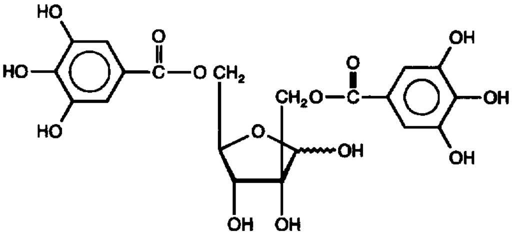

13

associated with the configuration of either acetate. In the NOESY spectra, NOE interactions of the anomeric proton (δ 6.56 and 6.62) with the methylene functionality at C-2′ was observed in each instance, differing only in intensity with the individual protons of this group. The NOESY spectrum, however, of the acetate of the α-form according to the literature [5] showed, in addition, a selective NOE correlation with 4-H (δ 4.49), indicating that these two protons are on the same side of the furanose ring and, hence, suggesting revision of previous assignments of NMR data [5].

EXPERIMENTAL

General experimental and extraction procedures are described in ref. [8].

Conversion of proanthocyanidins into anthocyanidins. Proanthocyanidins (*ca* 1 mg) were refluxed with 5% HCl in EtOH for 1 hr. The reaction mixt. was subsequently chromatographed on cellulose with HOAc–HCl–H_2O (30:3:10) and pigment zones compared with those of ref. anthocyanidins.

Acid hydrolysis. Gallic acid was liberated after acid hydrolysis and identified by comparison with an authentic sample (TLC analysis).

Identification of 'lower' terminal flavan-3-ol units. Treatment of proanthocyanidin samples (*ca* 1 mg) with 0.1 M HCl in EtOH (2 ml) at 60° for 15 min liberated the 'lower' terminal flavan-3-ol unit, which was de-(90 × 5.5 cm) with EtOH as eluent. After emergence of phenolic material, 16 ml frs were collected. Subsequent fractionation of the content of tubes 166–855 (57.4 g) by MPLC (RP-18; 5–20 μm; 30 × 2.5 cm; flow rate 1 ml/min^{-1}, 17 bar pressure) using a linear gradient system of H_2O–MeOH containing increasing amounts of MeOH (MeOH, 0–30%: 120 min, MeOH, 30–100%: 10 min) afforded 3 subfrs (I: 1–60 ml, 11.25 g; II: 61–180 ml, 27.45 g; III: 181–210 ml, 2.55 g). Fr. II was further resolved by CC (90 × 5.5 cm) on Sephadex LH-20 in EtOH to give 5 frs (II-a: 1–3000 ml, 1.3 g; II-b: 3001–6400 ml, 16.2 g; II-c: 6401–10200 ml, 3.8 g; II-d: 10 201–14 000 ml, 2.78 g; II-e: 14 001–22 000 ml, 1.23 g).

Catechin-(4α→8)-catechin (**1**). A portion (2.1 g) of fr. II-b was subjected to MPLC (RP-18; 5–20 μm; 30 × 2.5 cm; flow rate 1 ml/min^{-1}, 14 bar pressure) using a linear gradient system of H_2O–MeOH containing increasing amounts of MeOH (MeOH 12.5%, 360 min; MeOH 12.5–13.5%, 180 min; MeOH, 13.5–17%, 180 min; MeOH, 17–30%, 60 min; MeOH, 30–100%, 10 min). Acetylation of a portion (37 mg) of the content of subfr. 0–240 ml (64 mg) followed by prep. TLC in $CHCl_3$–EtOAc (3:2) yielded **1a** (12 mg) at R_f 0.40. EI-MS: $[M]^+$ m/z 998. ^1H NMR data: see Table 1. CD data: see ref. [10].

3-O-*Galloyl-epicatechin-(4β→8)-catechin* (**2**). A portion (2.1 g) of fr. II-b was subjected to MPLC on MCI-gel CHP 20 P (37–75 μm; 40 × 2.5 cm; flow rate 1 ml/min^{-1}, 14 bar pressure) using a linear gradient system of H_2O–MeOH containing increasing amounts of MeOH (MeOH 12.5%, 360 min; MeOH 12.5–13.5%, 180 min; MeOH 13.5–17%, 180 min; MeOH 17–30%, 60 min; MeOH 30–100%, 10 min) to afford **2** (601–1100 ml, 80 mg). Spectral measurements were carried out after acetylation (R_f 0.49 in $CHCl_3$–EtOAc, 3:2). DCI-MS: $[M + 18]^+$ m/z 1252. ^1H NMR data: see Table 1. CD data: $[\Theta]_{300}$ − 725, $[\Theta]_{271}$ 0, $[\Theta]_{241}$ + 28620, $[\Theta]_{230}$ 0.

*Epicatechin-(4β→8)-catechin-*3-O-*(4-hydroxy) benzoate* (**3**). Fr. II-c (3 g) from the Sephadex LH-20 column was subjected to MCI-gel chromatography (37–75 μm; 40 × 2.5 cm, flow rate 1 ml/min^{-1}, 17 bar pressure) with a H_2O–MeOH gradient (MeOH 35%, 480 min; MeOH 35–40%, 480 min; MeOH 40–100%, 10 min). The content (65 mg) of the subfr. 1–105 ml was acetylated and purified by prep. TLC in $CHCl_3$–EtOAc (3:2) to afford **3a** at R_f 0.54 (35 mg). FAB-MS: $[M + H - 43]^+$ m/z 1059. ^1H NMR data: see Table 1. CD data: $[\Theta]_{318}$ − 705, $[\Theta]_{284}$ − 14335, $[\Theta]_{248}$ − 22935, $[\Theta]_{228}$ 0.

3-O-*Galloyl-epigallocatechin-(4β→8)-catechin* (**6**). Fr. II-b (2.1 g) was resolved by MPLC on MCI-gel CHP 20 P using the following linear H_2O–MeOH gradient system: MeOH 15%, 360 min; MeOH 15–23%, 60 min; MeOH 23–25%, 120 min; MeOH 25–35%, 360 min; MeOH 35–40%, 240 min; MeOH

Table 1. ^{1}H NMR data for proanthocyanidin peracetates **1a**–**7a** ($CDCl_3$, 300 MHz)

Ring	H	**1a**	**2a**	**3a**	**4a***	**5a**	**6a**	**7a**
A	6	6.48 *d* (2.3)	6.06 *d* (2.2)	5.99 *d* (2.2)	6.00 *d* (2.2)	5.99 *d* (2.3)	6.06 *d* (2.2)	6.16 *d* (2.2)
	8	6.51 *d* (2.3)	6.33 *d* (2.2)	6.29 *d* (2.2)	6.31 *d* (2.2)	6.29 *d* (2.3)	6.34 *d* (2.2)	6.32 *d* (2.2)
B†	2	7.00 *d* (2.0)	—‡	—‡	—‡	7.17 *s*	7.19 *s*	7.19 *s*
	5	7.10 *d* (8.0)	—‡	—‡	—‡	—	—	—
	6	6.97 *dd* (2.0, 8.0)	—‡	—‡	—‡	7.17 *s*	7.19 *s*	7.19 *s*
C	2	4.76 *d* (10.0)	5.56 s	5.42 s	5.45 s	5.42 s	5.54 s	5.57 s
	3	5.63 *t* (Σ 19.4)	5.40 *br s*	5.13 *br s*	5.14 *br s*	5.16 *br s*	5.41 *br s*	5.40 *br s*
	4	4.48 *d* (9.4)	4.55 *d* (2.0)	4.43 *d* (2.0)	4.41 *d* (1.5)	4.43 *d* (1.7)	4.53 *d* (2.0)	4.53 *d* (1.9)
D	6	6.64 *s*	6.71 s	6.68 s	6.67 s	6.68 s	6.71 s	6.71 s
E†	2	6.90 *d* (2.0)	6.88 *d* (2.0)	6.88 *d* (2.0)	6.88 *d* (2.0)	6.87 *d* (2.0)	6.91 *d* (1.8)	6.86 *s*
	5	7.11 *d* (8.5)	7.16 *d* (8.7)	7.18 *d* (8.5)	7.16 *d* (8.5)	7.11 *d* (8.3)	7.12 *d* (8.4)	—
	6	6.70 *dd* (2.0, 8.5)	6.96 *dd* (2.0, 8.7)	6.95 *dd* (2.0, 8.5)	6.94 *dd* (2.0, 8.5)	6.95 *dd* (2.0, 8.3)	6.97 *dd* (1.8, 8.4)	6.86 *s*
F	2	4.95 *d* (7.9)	4.36 *d* (9.9)	4.32 *d* (9.9)	4.37 *d* (10.0)	4.32 *d* (9.7)	4.35 *d* (9.7)	4.32 *d* (10.0)
	3	5.03 *m*	5.05 *m*	5.10 *m*	5.05 *m*	5.06 *m*	5.05 *m*	5.02 *m*
	4_{ax}	2.66 *dd* (7.6, 16.6)	2.56 *dd* (9.0, 16.7)	2.56 *dd* (9.0, 16.7)	2.55 *dd* (9.0, 16.5)	2.656 *dd* (9.3, 16.6)	2.56 *dd* (9.0, 16.7)	2.56 *dd* (9.0, 16.7)
	4_{eq}	2.93 *dd* (5.6, 16.6)	3.23 *dd* (6.7, 16.7)	3.24 *dd* (6.7, 16.7)	3.18 *dd* (6.5, 16.7)	3.24 *dd* (6.7, 16.6)	3.25 *dd* (6.7, 16.7)	3.25 *dd* (6.7, 16.7)
	Galloyl	—	7.54 *s*	—	—	—	7.54 *s*	7.55 *s*
	Benzoyl	—	—	7.21 *d* (8.6) 8.13 *d* (8.6)	—	—	—	—

J values (Hz) given in parentheses.

*Data taken from ref. [12].

†These allocations may be reversed.

‡Signals at δ 7.1–7.25 overlapping with other signals.

0.36 (250 mg). DCI-MS: $[M + 18]^+$ *m/z* 1310. ^{1}H NMR data: see Table 1. CD data: $[\Theta]_{320} - 325$, $[\Theta]_{282} - 16065$, $[\Theta]_{265} - 1075$, $[\Theta]_{258}$ 0, $[\Theta]_{240} + 23875$, $[\Theta]_{225}$ 0.

3-O-*Galloyl-epigallocatechin*-(4β → 8)-*gallocatechin* (**7**). Fr. II-d (1.5 g) was subjected to MPLC on MCI-gel CHP 20 P with the same gradient system described for the isolation of **3** fr. II-c. The content of subfr. 1–180 ml was acetylated and purified by prep. TLC in toluene–Me_2CO (3:2) to give a band at R_f 0.48 (8 mg) consisting of **7a**. DCI-MS: $[M + 18]^+$ *m/z* 1368. ^{1}H NMR data: see Table 1. CD data: $[\Theta]_{291}$ 0, $[\Theta]_{280} - 3315$, $[\Theta]_{272}$ 0, $[\Theta]_{242} + 14210$, $[\Theta]_{230} + 560$, $[\Theta]_{214} + 42575$, $[\Theta]_{215}$ 0.

Epigallocatechin-(4β → 8)-*catechin* (**5**). Acetylation of fr. II-a in ref. [6] and subsequent purification of the mixt. by prep. TLC in $CHCl_3$–EtOAc (3:2) afforded the acetate **5a** at R_f 0.51 (6 mg). DCI-MS: $[M + 18]^+$ *m/z* 1074. ^{1}H NMR data: see Table 1. CD data: $[\Theta]_{300} - 455$, $[\Theta]_{283} - 2830$, $[\Theta]_{270}$ 0, $[\Theta]_{240} + 8030$, 40 × 2.5 cm; flow rate 1 ml/min^{-1}, 17 bar pressure) using a linear gradient system of H_2O–MeOH containing increasing amounts of MeOH (MeOH 35%, 480 min; MeOH 35–40%, 420 min; MeOH 40–100%, 10 min). The content of the eluate 481–528 ml afforded a chromatographically homogeneous oligomer (590 mg). Acid hydrolysis produced gallic acid, cyanidin and delphinidin, identified by co-chromatography with authentic samples. Reaction of **8** with phloroglucinol failed to produce phloroglucinol adducts within 72 hr.

2′,3,5-*Tri*-O-*galloyl*-D-*hamamelofuranose* (**9**). A portion (1.5 g) of fr. II-d was subjected to MPLC using the same experimental conditions as described for the isolation of **7**. The contents of the eluate 556–900 ml (83 mg) consisted of **9**. A portion was acetylated and purified by prep. TLC to yield the acetate **9a** (R_f 0.54 in $CHCl_3$–EtOAc, 3:2; 83 mg). FAB-MS: $[M + H]^+$ 1055; DCI-MS: $[M + 18]^+$ *m/z* 1073. ^{1}H NMR (300 MHz): δ 1.97–2.32 (10 × OAc), 3.11 (*br s*, 2-

(α-form); 2.64 (*br s*, 2-OH), 4.65, 5.08 (each *d*, J = 12.4 Hz, 2'-H_2), 5.76 (*d*, J = 3.5 Hz, 3-H), 6.71 (*s*, 1-H), 7.83, 7.84, 7.90 (each *s*, 3 × galloyl 2-H, 6-H) (β-form).

1-O-(4-*Hydroxybenzoyl*)-2',3,5-*tri*-O-*galloyl*-α/β-D-*hamamelofuranose* (**10**/**11**). A portion (1.2 g) of fr. II-c was further resolved by MPLC on RP-18 (5–20 μm, 30 × 2.5 cm; flow rate 1 ml/min^{-1}, 17 bar pressure) using a linear gradient system of H_2O–MeOH containing increasing amounts of MeOH (MeOH 12.5%, 240 min; MeOH 12.5–13.5%, 240 min; MeOH 13.5–15%, 120 min; MeOH 15–17%, 120 min; MeOH 17–100%, 20 min). The content of the eluate 251–600 ml was acetylated and purified by prep. TLC in $CHCl_3$–EtOAc (3:2) to give an inseparable mixt. of **9** and **10** at R_f 0.41 (15 mg). Owing to unequal intensities of signals the following assignments could be made. Acetate **10a**: ^{1}H NMR (300 MHz): δ 1.97–2.36 (10 × OAc), 3.83 (*br s*, 2-OH), 4.35–4.83 (*m*, 4-H, 5-H_2), 4.82, 5.28 (each *d*, J = 12.5 Hz, 2'-H_2), 5.85 (*d*, J = 7.6 Hz, 3-H), 6.65 (*s*, 1-H), 7.18, 7.26 (each *d*, J = 7.3 Hz, benzoyl group), 7.68, 7.75, 7.84 (each *s*, 3 × galloyl 2-H, 6-H). Acetate **11a**: ^{1}H NMR (300 MHz): δ 1.97–2.36 (10 × OAc), 2.63 (*br s*, 2-OH), 4.35–4.83 (*m*, 4-H, 5-H_2), 4.65, 5.08 (each *d*, J = 12.4 Hz, 2'-H_2), 5.76 (*d*, J = 3.5 Hz, 3-H), 6.71 (*s*, 1-H), 7.18, 7.26 (each *d*, J = 7.3 hz, benzoyl group), 7.83, 7.84, 7.90 (each *s*, 3 × galloyl 2-H, 6-H).

2'-4-*Di*-O-*galloyl*-D-*hamamelopyranose* (**12**). For ^{1}Hand ^{13}C NMR data see ref. [8].

Hamamelitannin (**13**). For ^{1}H and ^{13}C NMR data see ref. [8].

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