Synthesis of Condensed Tannins. Part 13. The First 2,3-*trans*-3,4-*cis*-Procyanidins: Sequence of Units in a 'Trimer' of Mixed Stereochemistry

Jan A. Delcour and Edward J. Serneels
Laboratorium voor Toegepaste Organische Scheikunde, Katholieke Universiteit Leuven, B-3030 Heverlee, Belgium
Daneel Ferreira and David G. Roux *
Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa

The condensation of (+)-leucocyanidin with (−)-epicatechin initiates a succession of substitutions leading mainly to the introduction of [4,8]-2,3-*trans*-3,4-*trans*-procyanidin units, but also to the incoporation of 'terminal' moieties which possess the hitherto unique 3,4-*cis*-procyanidin configuration. The bonding patterns in the products, and also the sequence of units in one of the 'trimers' of mixed stereochemistry, are resolved by ^{1}H n.m.r. spectroscopy through selective use of solvents at elevated temperatures.

Natural[1] and synthetic[2,3] procyanidins (also prodelphinidins) of 2,3-*trans* configuration have hitherto been found to consist exclusively of constituent flavanyl units with 3,4-*trans* stereochemistry. Such stereospecificity, contrasting with a relatively low degree of stereoselectivity inferred from the natural abundance of isomeric profisetinidins and prorobinetinidins and also observed during their synthesis,[4,5] was rationalized by invoking the stability–selectivity relationship for each type of 4-carbenium ion generated from phloroglucinol-type leucocyanidins/delphinidins and resorcinol-type leucofisetinidins/robinetinidins[6] respectively. The present synthetic study, however, provides the first evidence of the incorporation as terminal units of procyanidin moieties of 2,3-*trans*-3,4-*cis* configuration, albeit in low proportion, during those condensation sequences which are initiated by the reaction between (+)-leucocyanidin (**1**) and (−)-epicatechin (**2**) at pH 5 under ambient conditions. The work also illustrates simple means of overcoming problems connected with their recognition in oligomers, and with sequencing in procyanidin tannins of 'mixed' stereochemistry.

Condensation of (+)-leucocyanidin [(2*R*,3*S*,4*R* or 4*S*) (**1**) with (−)-epicatechin [(2*R*,3*R*)] (**2**) gives the anticipated (*cf.* ref.[5]) positional isomers [4,8]- and [4,6]-2,3-*trans*-3,4-*trans*:2′,3′-*cis*-(+)-catechin-(−)-epicatechin [B_4 and B_8[1,2] respectively; (**3**) and (**9**)] and in addition the novel [4,8]-2,3-*trans*-3,4-*cis*:2′,3′-*cis* diastereoisomer (**7**); the novel [4,8:4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-3,4-*trans*:2,3-*cis*-bi-[(+)-catechin]-(−)-epicatechin triflavanoid (**11**) and its C-4 (C-ring) epimer (**13**), together with evidence of an [all 4,8]-all-*trans*-tri-[(+)-catechin]-(−)-epicatechin tetramer (**15**) in the ratio of 22 : 4 : 0.2 : 12 : 1 : *ca.* 1 when using a 1:1 molar ratio of reactants.

The natural free-phenolic procyanidins B_4 and B_8 (**3**) and (**9**) were previously characterized by Haslam *et al.*[1,2] on a degradative and synthetic basis only (carbenium ion generated from polymeric tannins), while the deca-acetate of the predominant [4,8]-linked isomer (B_4) although derived from a similar condensation[7] was not subject to rigorous analysis. The 2,3-*trans*-3,4-*trans* stereochemistry of their 'upper' units was confirmed by ^{1}H n.m.r. spectroscopy of their octamethyl ether diacetates at 100 °C in $CDCl_3$ at 80 MHz, whence the large coupling constants (AMX-systems; $J_{2,3}$ 10.1, $J_{3,4}$ 9.5 Hz for B_4, and $J_{2,3}$ 10.1, $J_{3,4}$ 9.0 Hz for B_8) evident from sharp resonances, free from the effects of rotational isomerism (*cf.* Figure 1), are indicative of the *trans*-diaxial orientation of heterocyclic protons on the assumption of a half-chair conformation. The chemical shifts of residual D-ring protons (δ 6.10, 6.28 respectively) in $CDCl_3$ are characteristic[6,8] of 6- and 8-H of substituted (−)-epicatechin moieties and thus of [4,8] and [4,6] linkages respectively. These parameters coupled with mass fragmentation spectrometry (see later) and circular dichroism (negative Cotton effects[9,10] at 225, 218 nm, *cf.* Figure 2) reaffirm the structures, stereochemistry, and absolute configurations (2*R*,3*S*,4*S*:2*R*,3*R*) of procyanidins B_4 and B_8.

Methylation of the predominant [4,8]-procyanidin B_4 product (**3**) with diazomethane produces, in addition to the expected octamethyl ether, two nonamethyl ethers in low yield. These more fully methylated ethers result from additional methylation of the 3-hydroxy functions of the C- and F-rings respectively. Proof was provided by conversion into their respective monoacetates (**5**) and (**6**) when the 3-protons of the respective C- (δ 3.89) and F-rings (δ 3.68—3.83, underlying methoxy proton resonances) occupy high-field positions. While selective methylation of the 3-hydroxy function of the C-ring under these conditions is a known phenomenon,[10] similar substitution of the F-ring functionality has not been recorded hitherto. These monoacetates assist in confirming the allocations of acetoxy resonances of the octamethyl ether diacetate (**4**) of the parent compound.

Of further interest is that mass spectra of the respective nonamethyl ether acetates (**5**) and (**6**) are differentiated by the greater stability (abundance) of the molecular ion of the former, *m/z* 746 (M^+, 12.8, 0.9% respectively) associated with concomitant reversed abundance of the *m/z* 686 ion (M − 60, 2.0, 96% respectively) representing facile acetic acid loss only from the 'upper' C-ring of the latter (*cf.* refs. 11, 12). For compound (**6**) such acetic acid loss is followed by retro-Diels–Alder (RDA) fragmentation of the 'lower' F-ring to give the base peak *m/z* 492 (M − 60 − 194, 100%), whereas for its isomer (**5**) RDA-fragmentation of the 'upper' C-ring induces methoxy radical loss on recyclization (*cf.* refs. 13, 14) and instead provides the corresponding peak at *m/z* 521 (M − 194 − 31, 100%). These results extend the recent observation by one of us[15] that the 'lower' heterocyclic F-rings of the methyl ether diacetates of biflavanoids are more prone to RDA-fragmentation than their C-ring counterparts.

The novel [4,8]-2,3-*trans*-3,4-*cis*:2′,3′-*cis* diastereoisomer (**7**) represents the first procyanidin unit with 2,3-*trans*-3,4-*cis* stereochemistry. However, differentiation of units of this configuration by n.m.r. spectroscopy presents a considerable degree of difficulty considering the increased temperatures required to overcome the effects of an enhanced rotational

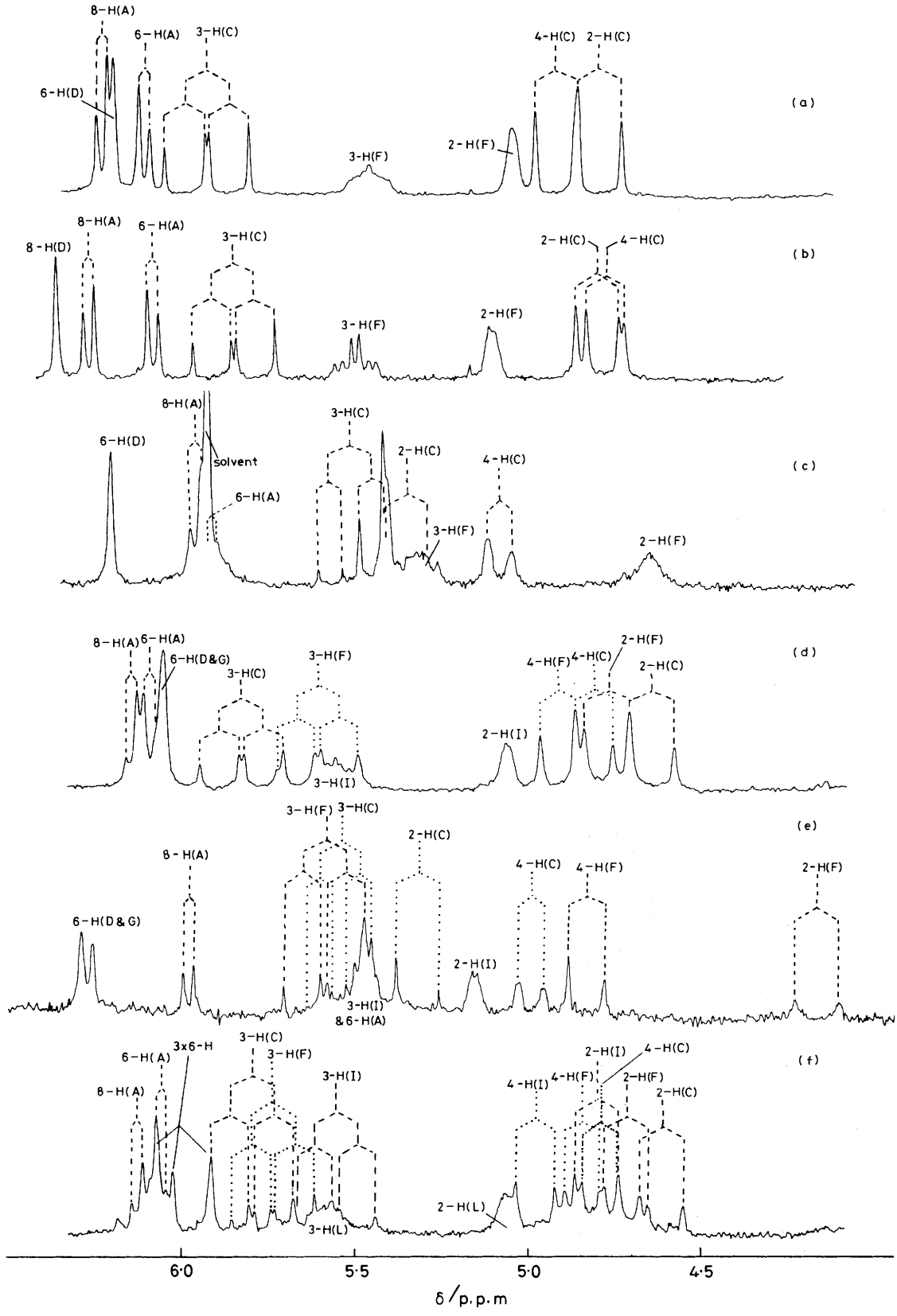


Figure 1. Diagnostic heterocyclic and high-field aromatic regions from the high temperature ^1H n.m.r. spectra of the methyl ether acetates of (a) the 'dimeric' procyanidin B_4 (**4**) ($CDCl_3$; 100 °C); (b) dimeric procyanidin B_8 (**10**) ($CDCl_3$; 100 °C); (c) the 3,4-*cis* isomer of B_4, (**8**) [$(CDCl_2)_2$; 170 °C]; (d) 'trimeric' [4,8:4,8]-all-*trans*:2,3-*cis* (**12**) ($CDCl_3$; 100 °C); (e) the 3,4-*cis* isomer (**14**) ([$^2H_6$]-DMSO; 150 °C); and (f) tetrameric [4,8:4,8:4,8]-all-*trans*:2,3-*cis* homologue (**16**) ($CDCl_3$; 100 °C) (tentative analysis)

Table 1. Chemical shifts of 'residual' A-ring equivalent protons of the methyl ether acetates of 'dimeric' and 'trimeric' procyanidins in $CDCl_3$ and $(CDCl_2)_2$ solutions

Compound	Proton	δ/p.p.m. $CDCl_3$	δ/p.p.m. $(CDCl_2)_2$
[4,8]-all-*trans*-Bi-(+)catechin (procyanidin B_3)	6-H(D)	6.16	6.12
[4,8]-2,3-*trans*-3,4-*trans*:2,3-*cis*-(+)-Catechin-(−)-epicatechin (**4**) (procyanidin B_4)	6-H(D)	6.10	6.11
[4,8]-2,3-*trans*-3,4-*cis*:2,3-*cis*-(+)-Catechin-(−)-epicatechin (**8**)	6-H(D)	6.16*	6.13
[4,8:4,8]-all-*trans*-Tri-(+)-catechin (procyanidin C_2)	6-H(D) 6-H(G)	6.06 6.06	5.91 6.02
[4,8:4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-3,4-*trans*:2,3-*cis*-(+)-Catechin-(+)-catechin-(−)-epicatechin (**12**)	6-H(D) 6-H(G)	6.00 6.00	6.00 6.00
[4,8:4,8]-2,3-*trans*-3,4-*cis*:2,3-*trans*-3,4-*trans*:2,3-*cis*-(+)-Catechin-(+)-catechin-(−)-epicatechin (**14**)	6-H(D) 6-H(G)	6.09* 6.06*	6.09 6.06
[4,6]-all-*trans*-Bi-(+)-catechin (procyanidin B_6)	8-H(D)	6.29	6.24
[4,6]-2,3-*trans*-3,4-*trans*:2,3-*cis*-(+)-Catchin-(−)-epicatechin (**10**) (procyanidin B_8)	8-H(D)	6.28	6.28

* The spectral lines are poorly resolved at 100 °C.

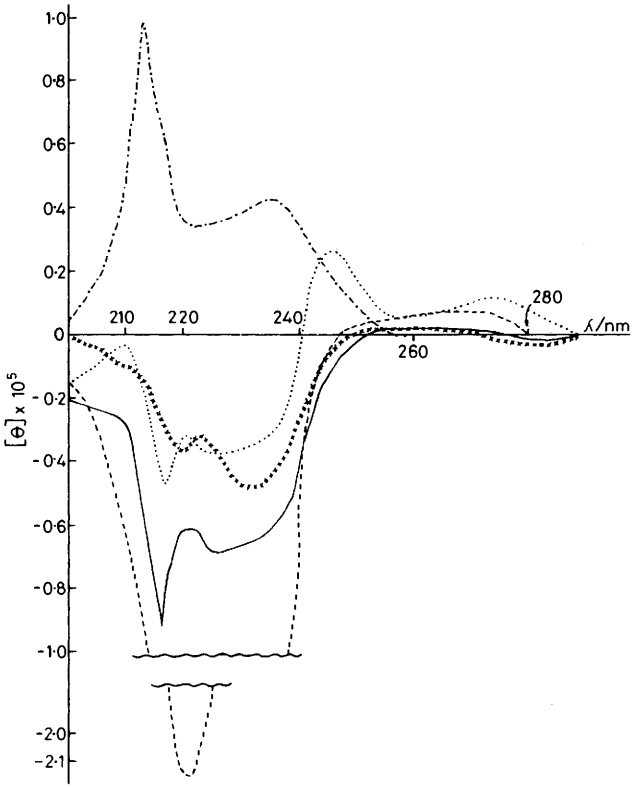


Figure 2. C.d. spectra of the methyl ether acetates of procyanidins B_4 (**4**) (——) and B_8 (**10**) (xxx), [4,8]-2,3-*trans*-3,4-*cis*:2′,3′-*cis* isomer (**8**) (— · — · —), [4,8:4,8]-all-*trans*:2′,3′-*cis* 'trimer' (**12**) (– – –) and its 3,4-*cis* isomer (**14**) (· · · ·)

energy barrier for 'fast' rotation relative to procyanidin units with 2,3-*trans*-3,4-*trans* stereochemistry.[6] Limitations imposed by the relatively low stability of procyanidins at high temperatures (> 160 °C), were accordingly met by maintaining low magnetic field strengths (1.88 T, 80 MHz), while using suitable higher boiling point solvents, *e.g.* $[^2H_6]$-1,1,2,2-tetrachloroethane $(CDCl_2)_2$.* Under these limiting conditions the resolution of the heterocyclic protons was incomplete (*cf.* Figure 1), but the 3-H (C-ring) quartet ($J_{2,3}$ 9.5, $J_{3,4}$ 5.5 Hz) and 4-H (C-ring) doublet were discernible; the coupling constants differ from those of the [4,8]-2,3-*trans*-3,4-*trans*:2′,3′-*cis* isomer ($J_{2,3}$ 10.1, $J_{3,4}$ 9.5 Hz) and accordingly define the 2,3-*trans*-3,4-*cis* stereochemistry of the 'upper' unit. The chemical shift of the residual D-ring proton, δ 6.16, is in agreement with those of 6-H resonances also in this solvent* (*cf.* Table 1) and hence indicates a [4,8]-linkage.

Confirmation of the 2,3-*trans*-3,4-*cis* stereochemistry of the 'upper' procyanidin unit was also obtained from circular dichroism (positive Cotton effect at low wavelength,[9,10] *cf.* Figure 2), and from mass spectrometry whence the relatively stable molecular ion [*m/z* 774 (53%)] and relatively low intensity (*M* − 60) peak [*m/z* 714 (32%)] indicate stability towards acetic acid loss in direct contrast to reverse phenomena associated with the [4,8]-2,3-*trans*-3,4-*trans* stereochemistry of the isomer (**4**) [*m/z* 774 (0.0%) and 714 (100%)] (*cf.* refs. 11, 12). Taken in conjunction, these physical methods combine to define the stereochemistry of the novel compound.

The application of similar methods also permits differentiation between the two novel triflavanoid procyanidins (**11**) and (**13**) as reaction products. The dodecamethyl ether triacetate of the dominant [4,8:4,8]-triflavanoid of 2,3-*trans*-3,4-trans:2,3-*trans*-3,4-*trans*:2,3-*cis* configuration (**12**) gives a ^1H n.m.r. spectrum at 100 °C in $CDCl_3$ which defines both [4,8] modes of linkage [6-H (D) and (G), δ 6.00, *cf.* Figure 1] and the all-*trans* stereochemistry of the biflavanoid procyanidin substituent on (−)-epicatechin [$J_{2,3}$ 10.1, 10.0; $J_{3,4}$ 9.1, 8.8 Hz respectively for the C- and F-rings]. The two AMX systems for these heterocyclic rings were correlated by spin-tickling experiments, and differentiated by comparison of the chemical shifts of 3-H(C) and 3-H(F) resonances (δ 5.81, 5.59 respectively) with those of the [4,8]-2,3-*trans*-3,4-*trans*:2,3-*cis* 'dimeric' homologue (**4**) [3-H(C), δ 5.89] and [4,8:4,8]-3,4-*cis* 'trimeric' analogue (**14**) [3-H(F), δ 5.61] respectively (*cf.* below). The exclusive 3,4-*trans* allocation is supported by the low wavelength (225 nm) negative Cotton effect of exceptionally high intensity (Figure 2), and also the absence of the molecular ion (*m/z* 1 160) in the mass fragmentation spectrum; however the *m/z* 1 100 (*M* − 60, 81%) and *m/z* 1 040 (*M* − 120, 79%) peaks are of high abundance, reflecting the ease of acetic acid loss through the McLafferty rearrangement associated with the 2,3-*trans*-3,4-*trans* stereochemistry of 4-arylflavan-3-ol derivatives.[12]

Interpretation of the ^1H n.m.r. spectra of the dodecamethyl ether triacetate of the [4,8:4,8]-2,3-*trans*-3,4-*cis*:2′,3′-*trans*-3′,4′-trans:2″,3″-*cis* isomer (**14**), as in the case of the 'dimeric' analogue (**8**), presented considerable difficulty in terms of higher temperature requirements and particularly the enhanced overlap of heterocyclic resonances due to increased structural complexity. The compound was accordingly examined at 100 °C in $CDCl_3$ and at higher temperatures in $(CDCl_2)_2$ (150 °C) and in $[^2H_6]$-DMSO (170 °C), with a low magnetic field strength (1.88 T, 80 MHz) to take advantage of solvent-induced shifts and also to ensure coalescence with adequate

* The use of $(CDCl_2)_2$ (b.p. 146 °C) permits higher temperatures (up to 170 °C) than $CDCl_3$ (max. temperature 100 °C under pressure), while maintaining similar (*cf.* refs.[6,8]) chemical shift differences for 'residual' aromatic D- and G-ring protons (*cf.* Table 1). The solvent may be removed with relative ease. These properties, apart from permitting high temperatures (up to 170 °C) are not shared by $[^2H_6]$-DMSO.

sharpening of all resonances. Chemical shifts of the two high-field aromatic singlets in $CDCl_3$ (δ 6.11, 6.13) and $(CDCl_2)_2$ (δ 6.09, 6.06) indicate [5] [4,8:4,8] linkages as for the all-*trans*:2,3-*cis* isomer (**11**). The spectrum in [$^2H_6$]-DMSO taken in conjunction with that in $(CDCl_2)_2$ establishes the presence of both 2,3-*trans*-3,4-*cis* ($J_{2,3}$ 9.9; $J_{3,4}$ 5.7 Hz) and 2,3-*trans*-3,4-*trans* ($J_{2,3}$ 10.0; $J_{3,4}$ 8.5 Hz) heterocyclic systems. The sequence of these units follows from the uniquely high-field position of 2-H(F) (δ 4.13, 4.17) in two solvent systems, correlating with shielding induced by the 3-acetoxy function of a (2*R*,3*S*,4*S*)*-[4,8]-2,3-trans-3,4-*cis* unit (*cf.* ref. 16). Apart from this, the chemical shifts of the heterocyclic protons of the upper 2,3-*trans*-3,4-*cis* procyanidin units in the corresponding derivatives of both the 'dimer' (**8**) {δ[$(CDCl_2)_2$; 170 °C] *ca.* 5.3 (2-H), 5.45 (3-H), 5.03 (4-H)} and the 'trimer' (**14**) {δ *ca.* 5.4, 5.33 (2-H), *ca.* 5.5, 5.55 (3-H), 5.03, 5.00 (4-H), in $(CDCl_2)_2$ at 150 °C and [$^2H_6$]-DMSO at 170 °C respectively} are in agreement, as are their coupling constants ($J_{2,3}$ 9.5, 9.9; $J_{3,4}$ 5.5, 5.7 Hz respectively) and the relative broadening of their 4-H(C) resonances. Similarly the chemical shifts of the 2-H and 3-H resonances of the 'lower' (−)-epicatechin unit in the derivative of this 'trimer' (**14**) is in agreement with those of the unsubstituted parent compound and of all other (−)-epicatechin-based tannin units which possess a [4,8]-(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-procyanidin substituent (*cf.* Table 2 and discussion below). Independent support for the presence of a 2,3-*trans*-3,4-*cis* unit in the triflavanoid (**14**) was available from mass spectrometry as evidenced by the presence of the molecular ion *m/z* 1 160 (M^+, 2.9%) and the reduced relative abundance of all ions involving acetic acid loss compared with those of the all-*trans*:2,3-*cis* isomer (**12**) (*cf.* refs. 11, 12 and above), *e.g.* *m/z* 1 114 (*M* − 60 + 14,† 7.9, 31 respectively), 1 100 (*M* − 60, 77, 81), 1 069 (*M* − 60 − 31, 0.0, 13.5), 1 054 (*M* − 120 + 14,† 0.0, 13.9), 1 040 (*M* − 120, 34, 79), and 1 009 (*M* − 120 − 31, 17.8, 58); and also from comparison of their c.d. spectra (*i.e.* reduced amplitude of the negative Cotton effect at 220 nm, with introduction of a positive effect at 245 nm) (*cf.* Figure 2).

The remaining higher procyanidin oligomer appears to be a [4,8:4,8:4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-3,4-*trans*:2,3-*trans*-3,4-*trans*:2,3-*cis*-tetraflavanoid as adjudged by four acetoxy resonances, three 2,3-*trans*-3,4-*trans* heterocyclic systems (two with $J_{2,3}$ 10.2; $J_{3,4}$ 9.0 Hz each and one $J_{2,3}$ 10.0; $J_{3,4}$ 8.5 Hz), and the grouping of all high-field aromatic singlet resonances in the region δ 5.9—6.1 ($CDCl_3$ at 100 °C) indicative of residual 6-H in the D-, G-, and J-rings.

At high temperatures the chemical shifts of 2-H and 3-H resonances of the methyl ether acetates of (−)-epicatechin and of (−)-epicatechin moieties substituted in the 8- (also 6-) position with (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*trans*-procyanidin units are consistent (2-H, δ 5.03—5.05; 3-H, 5.44—5.56) in $CDCl_3$ and $(CDCl_2)_2$ solutions and chemical shift differences between them are, therefore, reasonably uniform (Δδ 0.39—0.51) (*cf.* Table 2). By contrast, with the (2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*cis*-procyanidin as the 8-substituent, both the 2-H and 3-H resonances of (−)-epicatechin are shielded (Δδ −0.44 and −0.22 respectively), strong shielding of the 2-H (axial) resonance and also the resultant chemical shift difference between them (Δδ 0.67) being in agreement with similar effects for (+)-catechin units.[16] Previously established parameters of this type for (2*R*)- and (2*S*)-flavanyl substituents[16] should, therefore, also be applicable to procyanidins, and of direct value in sequencing as in the case of the 'trimer' of mixed stereochemistry (**13**) containing (−)-epicatechin as the 'terminal' moiety.

* Defined as (2*R*,3*S*,4*R*) for procyanidins on the basis of increased 'A-ring' functionality.

† Intermolecular transfer of methylene function under conditions of mass spectrometry is characteristic of highly methoxylated flavanoids.

Table 2. Chemical shifts of 2- and 3-protons of (−)-epicatechin units present in the methyl ether acetates of procyanidin oligomers*

Derivative of	δ/p.p.m. 3-H	2-H	Δδ
(−)-Epicatechin	5.47	5.03	0.44
[4,8]-(+)-Catechin-(−)-epicatechin (**4**)	5.44	5.05	0.39
[4,6]-Isomer (**8**)	5.47	5.05	0.42
[4,8]-3,4-*cis*-Isomer (**10**)	5.28	4.61	0.67
[4,8:4,8]-all-*trans*-Bi-[(+)-catechin]-(−)-epicatechin (**12**)	*ca.* 5.53	5.05	0.48
[4,8:4,8]-3,4-*cis*-Isomer (**14**)	*ca.* 5.50	5.05	0.45
[4,8:4,8:4,8]-all-*trans*Tri-[(+)-catechin[-(−)-epicatechin (**16**)	*ca.* 5.56	5.05	0.51

* Shifts are in $CDCl_3$, with the exception of 3,4-*cis*-isomers (**10**) and (**14**) in $(CDCl_2)_2$.

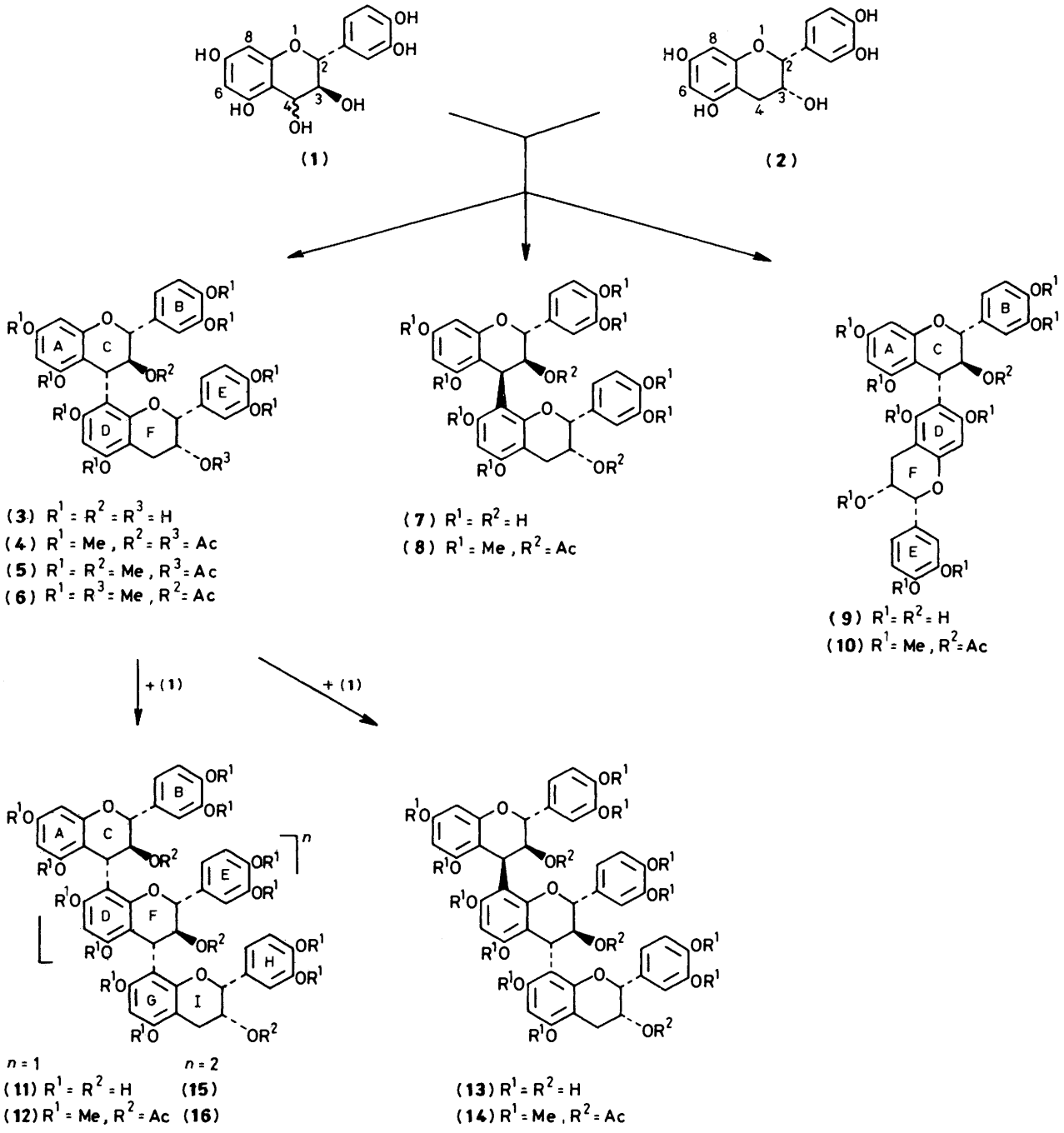
Consistent coupling constants at high temperatures of the methyl ether acetate derivatives of the introduced procyanidin units of 2,3-*trans*-3,4-*trans* configuration ($J_{2,3}$ 10.0—10.1; $J_{3,4}$ 8.5—9.5 Hz) and also for those available for the 2,3-*trans*-3,4-*cis* configuration ($J_{2,3}$ 9.5, 9.9; $J_{3,4}$ 5.5, 5.7 Hz) (Table 3), differ from similarly self-consistent values for 2,3-*trans*-3,4-*trans* ($J_{2,3}$ = $J_{3,4}$ = 9.5 Hz) and 2,3-*trans*-3,4-*cis* ($J_{2,3}$ = $J_{3,4}$ = 7.3—8.0 Hz) profisetinidins and prorobinetinidins.[16] These differences cannot be rationalized at present.

A likely sequence in the formation of the procyanidin oligomers of 'mixed' stereochemistry from (+)-leucocyanidin and (−)-epicatechin is shown in the Scheme. While the sequence resembles that obtained under identical conditions, but with (+)-catechin as nucleophile (*cf.* ref. 3), the most significant difference lies in the generation of 'upper terminal' 2,3-*trans*-3,4-*cis* units in bi- and tri-flavanoids [(**7**) and (**13**) respectively]. The reason for such stereoselectivity (*vs.* anticipated [3] stereospecificity) is obscure. However, our previous observation [4,17] that the occurrence of 'upper terminal' units of identical configuration in a variety of profisetinidins apparently inhibits their continued 'linear' condensation to higher oligomers appears relevant in the present instance.

Although no natural procyanidin oligomers constituted of 2,3-*trans*-3,4-*cis* flavanyl units have been isolated hitherto, their presence at very low concentrations in plant extracts in association with tannin analogues of 'mixed' stereochemistry may be anticipated from the present study.

Experimental

^1H N.m.r. spectra were recorded on a Bruker WP-80 FT (Fourier transform) spectrometer with $CDCl_3$, $(CDCl_2)_2$, and $(CD_3)_2SO$ as solvents and Me_4Si as internal standard. Tubes were firmly stoppered to avoid loss where spectra were recorded above the boiling points of solvents. All spectra were recorded at high temperatures (100, 150, or 170 °C) in order to overcome the effects of rotational isomerism. The identity of heterocyclic systems was established by extensive spin-tickling experiments. Mass spectra were obtained by electron impact under similar conditions (70 eV, 220—230 °C) with a Varian CH-5 instrument, and c.d. data in methanol on a Jasco J-20 spectropolarimeter. T.l.c. of the free phenols was performed on precoated Merck t.l.c. plastic sheets (silica gel 60 PF_{254}, 0.25 mm) in ethyl acetate–formic acid–water (90:5:5 v/v) unless stated otherwise. Plates were sprayed with H_2SO_4–HCHO (40:1) after development. Preparative plates (p.l.c.) (20 × 20 cm; Kieselgel PF_{254}, 1.0 mm) were air-dried and used without prior activation. Methylations were performed with an excess of



Scheme

diazomethane in methanol–diethyl ether during 48 h at −15 °C, while acetylations were in acetic anhydride–pyridine at room temperature. Evaporations were carried out under reduced pressure at 50 °C in a rotary evaporator.

(+)-Leucocyanidin–(−)-Epicatechin Condensation (ca. 1:5 *Molar Ratio*).—(+)-Dihydroquercetin (1.93 g) and (−)-epicatechin (9.67 g) were dissolved in ethanol (200 ml) and a solution of sodium borohydride (1.54 g) in ethanol (50 ml) then added dropwise under nitrogen during 30 min. Water (250 ml) was then added and the pH adjusted to 5.0 with acetic acid solution (0.15M). After 1 h at room temperature (*ca.* 20 °C) under nitrogen, the solution was further diluted with water to a final volume of 800 ml. The mixture was extracted with ethyl acetate (1 × 500 ml and 5 × 250 ml). After evaporation of the solvent the product was separated on three Sephadex LH-20 columns (53 × 2.5 cm) in ethanol using 3.9 g of phenolics per separation. Fractions (16.9 ml) were collected. Fractions 31—44 contained unchanged (−)-epicatechin (R_F on silica 0.82, recovery 7.4 g). The columns were subsequently eluted with methanol (5 l per column), and the solids recovered (4.2 g) were re-separated on a Sephadex LH-20 column (57 × 2.5 cm) as above; 20-ml fractions were collected.

Fractions 27—44 contained (−)-epicatechin (690 mg); fractions 45—65 (200 mg) (R_F 0.74 and 0.63 components); fractions 66—111 (1.75 g) (R_F 0.63). The column was subsequently eluted with methanol (5 l) and gave a fraction with R_F 0.57 (840 mg).

Table 3. Coupling constants of heterocyclic protons of 2,3-*trans*-procyanidin substituents on (−)-epicatechin at elevated temperatures: methyl ether acetate derivatives

Compound	3,4-Configuration *	Temperature (°C)	Solution	$J_{2,3}$ (Hz)	$J_{3,4}$
B_4 (**4**)	*trans*	100	$CDCl_3$	10.1	9.5
B_8 (**10**)	*trans*	100	$CDCl_3$	10.1	9.0
B_8 (**10**)	*trans*	100	$(CDCl_2)_2$	10.1	8.8
'Dimer' (**8**)	*cis*	170	$(CDCl_2)_2$	9.5	5.5
'Trimer' (**12**)	*trans*	100	$CDCl_3$	10.1	9.1
	trans			10.0	8.8
'Trimer' (**14**)	*cis*	150	$(CDCl_2)_2$		6.0
		170	$[^2H_6]$-DMSO	9.9	5.7
	trans	150	$(CDCl_2)_2$	10.0	8.6
		170	$[^2H_6]$-DMSO	10.0	8.5
'Tetramer' (**16**)	*trans*	100	$CDCl_3$	10.2	9.0
	trans			10.0	8.5
	trans			10.2	9.0

* 'Upper' units.

The combined fractions 45—65 were re-separated on a Sephadex LH-20 column (57 × 2.5 cm) in ethanol. Fractions (15.1 ml) were collected. Fractions 61—69 contained (+)-dihydroquercetin (10 mg) (R_F 0.88); fractions 70—113 contained the R_F 0.74 and 0.63 components (90 mg); fractions 114—160 contained more of the R_F 0.63 component (100 mg). The combined fractions 70—113 were further separated on a Fractogel[18] TSK HW-40 (S) column (60 × 2.5 cm) in methanol. Fractions (16.5 ml) were collected. (+)-Dihydroquercetin (10 mg) was present in the fractions 46—54; the R_F (silica) 0.74 fraction was recovered from the fractions 56—61 (40 mg). Test tubes 62—65 contained both the R_F 0.74 and 0.63 components (31 mg). More of the R_F component was collected from the fractions 66—74 (30 mg).

Reseparation of the mixture of the R_F 0.74 and 0.63 components (31 mg) yielded 13 mg of the R_F 0.74 and 21 mg of the R_F 0.63 compound.

The above-cited methanol eluate (840 mg) was separated on a Fractogel TSK HW-40 (S) column (58 × 2.5 cm) in methanol. 9.4-ml Fractions were collected. (−)-Epicatechin (60 mg) was found in the fractions 60—73. The R_F 0.63 compound (200 mg) was recovered from the fractions 101—120. Fractions 121—150 yielded a compound with R_F 0.75 (88 mg).

[4,8]-2,3-trans-3,4-trans:2′,3′-cis-(+)-*Catechin*-(−)-*epicatechin* (*Procyanidin* B_4) (**3**). A portion of the R_F 0.63 fraction (800 mg) was methylated and resolved by p.l.c. [benzene–acetone (8:2 v/v)]. Acetylation of the octamethyl ether, R_F 0.33 (241 mg) followed by p.l.c. [hexane–acetone–ethyl acetate (×4) (60:25:15 v/v)] afforded the *octamethyl ether diacetate* (**4**), R_F 0.49 as a solid (126 mg) (Found: C, 65.0; H, 6.1. $C_{42}H_{46}O_{14}$ requires C, 65.1; H, 6.0%); δ (80 MHz; $CDCl_3$; 100 °C) 6.98—6.59 [m, 6 × ArH(B- and E-rings)], 6.15 [d, *J* 2.4 Hz, 8-H(A)], 6.10 [s, 6-H(D)], 6.03 [d, *J* 2.4 Hz, 6-H(A)], 5.89 [dd, Σ*J* 19.5 Hz, 3-H(C)], 5.44 [m, 3-H(F)], 5.05 [br s, *J* < 1, 2-H(F)], 4.86 [d, *J* 9.5 Hz, 4-H(C)], 4.73 [d, *J* 10.1 Hz, 2-H(C)], 3.83, 3.81, 3.78, 3.75, 3.70 (×2), 3.69, 3.39 (8 × s, 8 × OMe), 2.89 [m, CH_2(F)], 1.72 [s, 3-$COCH_3$(F)], and 1.60 [s, 3-$COCH_3$(C)]; c.d. spectrum (see Figure 2).

Methylation of the R_F 0.63 fraction also yielded two nonamethyl ethers, R_F 0.50 (31 mg) and 0.58 (42 mg). The R_F 0.58 fraction was acetylated and purified by p.l.c. [hexane–acetone–ethyl acetate (×2) (60:25:15 v/v]. The resulting *nonamethyl ether monoacetate* [3-*O*-methyl (C-ring)], (**5**), R_F 0.34, was recovered as a solid (26 mg); δ (80 MHz; $CDCl_3$; 100 °C) 7.06—6.67 [m, 6 × ArH(B- and E-rings)], 6.14 [s, 6-H(D)], 6.11 [d, *J* 2.2 Hz, 8-H(A)], 5.97 [d, *J* 2.2 Hz, 6-H(A)], 5.37 [m, 3-H(F)], 4.96 [br s, *J* < 1 Hz, 2-H(F)], 4.78 [d, $J_{3,4}$ 8.0 Hz, 4-H(C)], 4.52 [d, $J_{2,3}$ 9.6 Hz, 2-H(C)], 3.89 [dd, 3-H(C)], 3.82 (×3), 3.77, 3.73, 3.69, 3.66, 3.36 (8 × s, 8 × OMe), 2.89 [m, CH_2(F)], 2.84 [s, 3-OCH_3(C)], and 1.70 [s, 3-$COCH_3$(F)].

Acetylation of the R_F 0.54 fraction and purification by p.l.c. [hexane–acetone–ethyl acetate (×4) (60:25:15 v/v)] gave the *nonamethyl ether monoacetate* (**6**) [3-*O*-methyl (F-ring)], R_F 0.53, as a solid (12 mg); δ (80 MHz; $CDCl_3$; 100 °C) 7.05—6.66 [m, 6 × ArH(B-and E-rings)], 6.10 [d, *J* 2.4 Hz, 8-H(A)], 6.06 [s, 6-H(D)], 5.99 [d, *J* 2.4 Hz, 6-H(A)], 5.87 [dd, Σ*J* 19.5 Hz, 3-H(C)], 4.93 [br s, *J* < 1 Hz, 2-H(F)], 4.84 [d, $J_{3,4}$ 9.25 Hz, 4-H(C)], 4.73 [d, $J_{2,3}$ 10.2 Hz, 2-H(C)], 3.83, 3.82, 3.78, 3.77, 3.73, 3.68, 3.66, 3.34 (8 × s, 8 × OMe), 3.10 [s, 3-OMe(F)], 2.83 [m, CH_2(F)], and 1.59 [s, 3-$COCH_3$(C)].

Significant ions in the mass fragmentation spectra of the nonamethyl ether monoacetates (**5**) and (**6**) of procyanidin B_4 and their respective relative abundances are *m/z* 746 (12.8, 0.9%), 687 (0.9, 43), 686 (2.0, 96), 522 (35, 1.4), 521 (100, 4.9), 492 (2.4, 100), 461 (6.8, 35), 299 (44, 45), 194 (2.1, 35.5), 222 (2.1, 1.5), and 151 (26, 66).

[4,6]-2,3-trans-3,4-trans:2′,3′-cis-(+)-*Catechin*-(−)-*epicatechin* (*Procyanidin* B_8) (**9**). The R_F 0.75 fraction was methylated and the mixture resolved by p.l.c. [hexane–acetone–ethyl acetate (×3) (65:20:15 v/v)]. Acetylation of the resulting methyl ether, R_F 0.13 (23 mg), followed by p.l.c. [1,2-dichloroethane–acetone (95:5 v/v)] afforded the *octamethyl ether diacetate* (**10**), R_F 0.20, as a solid (16 mg) (Found: C, 64.9; H, 6.1. $C_{42}H_{46}O_{14}$ requires C, 65.1; H, 6.0%); δ (80 MHz; $CDCl_3$; 100 °C) 7.09—6.70 [m, 6 × ArH(B-and E-rings)], 6.28 [s, 8-H(D)], 6.19 [d, *J* 2.2 Hz, 8-H(A)], 6.00 [d, *J* 2.2 Hz, 6-H(A)], 5.77 [dd, Σ*J* 18.75, 3-H(C)], 5.47 [m, 3-H(F)], 5.05 [br s, *J* < 1 Hz, 2-H(F)], 4.72 [d, $J_{2,3}$ 10.1 Hz, 2-H(C)], 4.70 [d, $J_{3,4}$ 9.0 Hz, 4-H(C)], 3.84, 3.83 (×3), 3.72, 3.64, 3.47, 3.37 (8 × s, 8 × OMe), 3.00 [d, CH_2(F)], 1.84 [s, 3-$COCH_3$ (F)], and 1.64 [s, 3-$COCH_3$(C)]; c.d. spectrum (see Figure 2).

[4,8]-2,3-trans-3,4-cis:2′,3′-cis-(+)-*Catechin*-(−)-*epicatechin* (**7**). The R_F 0.74 fraction was methylated and the octamethyl ether resolved by p.l.c. [benzene–acetone (8:2 v/v)]. Acetylation of the methyl ether, R_F 0.29 (30 mg), followed by p.l.c. [hexane–acetone–ethyl acetate (×3) (65:20:15 v/v)] afforded the *octamethyl ether diacetate* (**8**), R_F 0.51, as a solid (24 mg) (Found: C, 65.0; H, 6.0. $C_{42}H_{46}O_{14}$ requires C, 65.1; H, 6.0%); δ [80 MHz; $(CDCl_2)_2$; 170 °C] 6.97—6.64 [m, 6 × ArH(B- and E-rings)], 6.16 [s, 6-H(D)], 5.88 [br s, overlap * of 8-H(A) + 6-H(A)], 5.45 [dd, Σ*J* 15.0 Hz, 3-H(C)], 5.29 [d, $J_{2,3}$ *ca.* 9.5 Hz, 2-

* These resonances are resolved in $[^2H_6]$-DMSO at 170 °C with 8-H(A) at δ 6.02 and 6-H(A) at δ 5.83 as doublets (*J ca.* 2.4 Hz).

H(C)], 5.28 [m, 3-H(F)], 5.03 [d, $J_{3,4}$ 5.5 Hz, 4-H(C)], 4.61 [br s, J < 1 Hz, 2-H(F)], 3.77 (× 3), 3.75, 3.70, 3.56 (× 2), 3.44 (8 × s, 8 × OMe), 2.89 [m, CH_2(F)], 1.84 [s, 3-$COCH_3$(F)], and 1.63 [s, 3-$COCH_3$(C)]; c.d. spectrum (see Figure 2).

Significant ions in the mass fragmentation spectra of the octamethyl ether diacetates of the procyanidins B_4 (**4**), B_8 (**10**) and the [4,8]-3,4-*cis* isomer (**8**) and their respective relative abundances are *m/z* 774 (M^+, 0.0, 7.3, 53%), 714 (M − 60, 100, 100, 32), 683 (M − 60 − 31, 4.4, 43, 22), 654 (40, 38, 55), 492 (21, 35, 30), 343 (26, 21, 93), 328 (6.9, 66, 14.3), 327 (24, 50, 41), 299 (42, 43, 99), 180 (27, 42, 37), and 151 (70, 67, 100).

[4,8:4,8]-all-trans:2,3-cis-*Bi*-[(+)-*catechin*]-(−)-*epicatechin* (**11**). The R_F 0.57 fraction was methylated and resolved by p.l.c. [benzene–acetone (7:3 v/v)]. Acetylation of the dodecamethyl ether, R_F 0.21 (40 mg), followed by p.l.c. [1,2-dichloroethane–acetone (× 2) (9:1 v/v)] yielded the *dodecamethyl ether triacetate* (**12**) as a solid, R_F 0.39 (32 mg) (Found: C, 65.1; H, 6.0. $C_{63}H_{86}O_{21}$ requires C, 65.2; H, 5.9%); δ (80 MHz; $CDCl_3$; 100 °C) 7.16—6.69 [m, 9 × ArH(B-, E- and H-rings)], 6.09 [d, J *ca.* 2.5 Hz, 8-H(A)], 6.05 [d, J *ca.* 2.5 Hz, 6-H(A)], 6.00 [s, 6-H(D) + 6-H(G)], 5.81 [dd, ΣJ 19.3 Hz, 3-H(C)], 5.59 [dd, ΣJ 18.5 Hz, 3-H(F)], *ca.* 5.53 [m, 3-H(I)], 5.05 [br s, J < 1 Hz, 2-H(I)], 4.91 [d, J 8.8 Hz 4-H(F)], 4.78 [d, J 9.1 Hz, 4-H(C)], 4.77 [d, J 10.0 Hz, 2-H(F)], 4.63 [d, J 10.1 Hz, 2-H(C)], 3.85, 3.83 (× 2), 3.81, 3.78, 3.72 (× 2), 3.68, 3.42 (× 2), 3.34 (12 × s, 12 × OMe), 2.93 [m, CH_2(I)], 1.77 [s, 3-$COCH_3$(I)], 1.67 [s, 3-$COCH_3$(C)], and 1.63 [s, 3-$COCH_3$(F)]; c.d. spectrum (Figure 2).

(+)-*Leucocyanidin*-(−)-*Epicatechin Condensation* (ca. 1:1 *Molar Ratio*.—(+)-Dihydroquercetin (3.99 g) and (−)-epicatechin (3.99 g) were dissolved in ethanol (80 ml). A solution of sodium borohydride (3.2 g) in ethanol (80 ml) was added dropwise during 30 min under N_2. Water (400 ml) was added to the solution and the pH adjusted to 5.0 with aqueous acetic acid (0.15M). After 1 h at room temperature (*ca.* 20 °C) under N_2, the solution was extracted with ethyl acetate (6 × 320 ml). Evaporation of the solvent gave a product which was subjected to primary separation on two Sephadex LH-20 columns (56 × 2.5 cm) in ethanol using 4 g of phenolics per separation. Fractions (20 ml each) were collected and combined as follows: fractions 33—49 [(−)-epicatechin (1.27 g)], 50—68 (430 mg), 69—80 [procyanidin B_4 (**3**) (1.03 g)], 81—100 (1.07 g), and 101—190 (1.29 g). The columns were finally eluted with methanol (5 l) to yield a methanol fraction (2.62 g).

The combined fractions 50—68 (430 mg) from the primary separation were subjected to secondary separation on a Sephadex LH-20 column (53 × 2.5 cm) in ethanol. Fractions (16.5 ml each) were collected and combined as follows: 40—51 [(−)-epicatechin (165 mg)], 52—80 (210 mg), and 81—95 [procyanidin B_4 (**3**) (66 mg)]. The above 52—80 (210 mg) fraction was reseparated on a Fractogel TSK HW-40 (S) column (80 × 2.5 cm) in methanol with collection of 15-ml fractions: tubes 41—48 [(+)-dihydroquercetin (17 mg)], 49—58 [3,4-*cis* isomer of B_4 (**7**) (75 mg)], and 61—72 [procyanidin B_4 (58 mg)].

The combined fractions 81—100 (1.07 g) from the primary separation were separated on two Fractogel TSK WH-40 (S) columns (57 × 2.5 cm) in methanol (9.9 ml fractions), yielding (−)-epicatechin (20 mg, fractions 57—63), procyanidin B_4 (**3**) (873 mg, 86—117), and procyanidin B_8 (**9**) (20 mg).

Also reseparated on two Fractogel TSK WH-40 (S) columns (57 × 2.5 cm) in methanol was the product recovered from tubes 101—190 (1.2 g) of the primary separation. Fractions (10 ml) obtained were as follows: tubes 57—70 [(−)-epicatechin (130 mg)], 95—104 [procyanidin B_4 (**3**) (213 mg)], 105—124 [procyanidin B_8 (**9**) (126 mg)], 125—163 ['trimeric' fraction with R_F 0.56, 0.66, and 0.71 components (163 mg)], and 164—200 {[4,8:4,8]-all-*trans*:2,3-*cis*-procyanidin (**11**)} (210 mg).

Finally the 'methanol fraction' (2.62 g) from the primary fraction was separated in methanol on five Fractogel TSK HW-40 (S) columns (58 × 2.5 cm) using 0.5 g of phenolics per separation. Fractions collected (14.8 ml each) were as follows: tubes 74—86 [procyanidin B_4 (**3**) (156 mg)], 87—108 [procyanidin B_8 (**9**) (156 mg)], 109—126 ['trimeric' fraction with R_F 0.56, 0.66, and 0.71 components (266 mg)], 127—157 {[4,8:4,8]-all-*trans*:2,3-*cis*-procyanidin (**11**) (607 mg)}, and 230—265 [R_F 0.48 component (346 mg)].

The aforementioned 'trimeric' fractions (163 and 266 mg) were combined and separated on a Fractogel TSK HW-40 (S) (57 × 2.5 cm) column in methanol (9.6-ml fractions). Fractions 162—170 contained the R_F 0.56 component (70 mg).

[4,8:4,8]-2,3-trans-3,4-cis:2,3-trans-3,4-trans:2,3-cis-*Bi*-[(+)-*catechin*]-(−)-*epicatechin* (**13**). The R_F 0.56 fraction (70 mg) was methylated and resolved by p.l.c. [benzene–acetone (× 4) (4:1 v/v)]. Acetylation of the dodecamethyl ether, R_F 0.29 (12 mg), followed by p.l.c. [hexane–acetone–ethyl acetate (50:35:15 v/v)] yielded the *dodecamethyl ether triacetate* (**14**) as a solid, R_F 0.24 (8 mg) (Found: C, 65.2; H, 6.2. $C_{63}H_{86}O_{21}$ requires C, 65.2; H, 5.9%); δ (80 MHz; $CDCl_3$; 100 °C) 6.11, 6.13 [2 × s, 2 × 6-H(D and G)]; δ [80 MHz; $(CDCl_2)_2$; 150 °C] 7.08—6.58 [m, 9 × ArH(B-, E-, and H-rings], 6.09 [s, 6-H(D)], 6.06 [s, 6-H(G)], 5.95 [d, J *ca.* 2.5 Hz, 8-H(A)], 5.84 [d, J *ca.* 2.5 Hz, 6-H(A)], 5.61 [dd, ΣJ 18.6 Hz, 3-H(F)], *ca.* 5.5 [m, 2-H-(C) + 3-H(C) + 3(H(I)], 5.05 [br s, J < 1 Hz, 2-H(I)], 5.03 [d, J 6.0 Hz, 4-H(C)], 4.85 [d, J 8.6 Hz, 4-H(F)], 4.13 [d, J 10.0 Hz, 2-H(F)], 3.81, 3.80 (× 2), 3.78, 3.77, 3.75, 3.68, 3.66, 3.52, 3.50, 3.47, 3.34 (12 × s, 12 × OMe), 2.92 [m, CH_2(I)], 1.75 [s, 3-$COCH_3$(I)], and 1.59 (× 2) [s, 3-$COCH_3$ (C and F)]; δ (80 MHz; [$^2H_6$]-DMSO; 150 °C) 7.23—6.61 [m, 12 × ArH(B-, E-, and H-rings)], 6.28, 6.25 [2 × 2, 6-H (D and G)], 5.98 [d, J 2.5 Hz, 8-H(A)], 5.59 [dd, ΣJ 18.5 Hz, 3-H(F)], 5.55 [dd, ΣJ 15.2 Hz, 3-H(C)], *ca.* 5.50 [d, 6-H(A)], 5.47 [m, 3-H(I)], 5.33 [d, J 9.9 Hz, 2-H(C)], 5.16 [br s, J < 1 Hz, 2-H(I)], 5.00 [d, J 5.7 Hz, 4-H(C)], 4.83 [d, J 8.5 Hz, 4-H(F)], 4.17 [d, J 10.0 Hz, 2-H(F)], 3.83, 3.82, 3.75 (× 4), 3.69 (× 2), 3.59, 3.56, 3.50, 3.42 (12 × s, 12 × OMe), 3.01 [m, CH_2(F)], and 1.78, 1.64, 1.62 [3 × s, 3 × 3-$COCH_3$ (C, F, and I)]; c.d. spectrum (Figure 2).

Significant ions in the mass fragmentation spectra of the dodecamethyl ether triacetates of the trimeric procyanidins (**12**) and (**14**) and their respective relative abundances are *m/z* 1 160 (M^+, 0.0, 5.9%), 1 129 (3.1, 0.0), 1 114 (31, 7.9), 1 054 (13.9, 0.0), 1 100 (81, 77), 1 069 (13.5, 0.0), 1 040 (79, 34), 1 023 (12.4, 0.0), 1 009 (58, 17.8), 949 (18.6, 6.8), 714 (49, 16.0), 713 (80, 29), 654 (29, 7.6), 653 (25, 10.3), 628 (37, 9.9), 409 (61, 15.9), 328 (76, 36), 327 (74, 36), 222 (24, 8.1), 180 (85, 73), 179 (82, 30), 167 (81, 48), 165 (77, 44), and 151 (100, 100).

[4,8:4,8:4,8]-all-trans-*Tri*-[(+)-*catechin*]-(−)-*epicatechin* (**15**). The above cited R_F 0.48 fraction (346 mg) was methylated and the hexadecamethyl ether purified by p.l.c. [benzene–acetone–methanol (× 2) (70:29:1 v/v)], R_F 0.28 (15 mg). After acetylation the *hexadecamethyl ether tetra-acetate* (**16**) (tentative identification) was purified by p.l.c. [benzene–acetone (7:3 v/v)] and separated as solid, R_F 0.48 (10 mg), δ (80 MHz; $CDCl_3$; 100 °C) 7.25—6.63 [m, 12 × ArH (B-, E-, H-, and K-rings)], 6.13 [d, J *ca.* 2.5 Hz, 8-H(A)], 6.08 [d, 6-H(A)], 6.08, 6.02, 5.92 [3 × s, 3 × 6-H (D-, G-, and J-rings)], 5.80 [2 × dd, ΣJ 19.2 Hz, 3-H(C)], 5.74 [dd, ΣJ 18.9 Hz, 3-H(F)], 5.56 [dd, ΣJ 19.0 Hz, 3-H(I)], *ca.* 5.56 [m, 3-H(L)], 5.05 [br s, J < 1 Hz, 2-H(L)], 4.98 [d, J *ca.* 9.0 Hz, 4-H(I)], 4.83 [d, J *ca.* 8.5 Hz, 4-H(F)], 4.80 [d, J 10.2 Hz, 2-H(I)], 4.78 [d, J *ca.* 9.0 Hz, 4-H(C)], 4.72 [d, J 10.0 Hz, 2-H(F)], 4.60 [d, J 10.2 Hz, 2-H(C)], 3.91, 3.88 (× 5), 3.84, 3.83, 3.76 (× 2), 3.73, 3.53, 3.42, 3.39, 3.34, 3.28 (16 × s, 16 × OMe), 2.94 [m, CH_2(L)], and 1.84, 1.80, 1.73, 1.63 [4 × s, 3-$COCH_3$ (C-, F-, I-, and L-rings)].

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