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# PROCYANIDINS FROM MEDICINAL BIRCH: BONDING PATTERNS AND SEQUENCE OF UNITS IN TRIFLAVANOIDS OF MIXED STEREOCHEMISTRY

HERBERT KOLODZIEJ

Institut für Pharmazeutische Biologie und Phytochemie der Westfälischen Wilhelms-Universität, D-4400 Münster, F.R.G.

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**Key Word Index**— *Betula* spp.; Betulaceae; flavan-3-ol glycoside; procyanidins; linkage isomerism; $^1$H parameters.

**Abstract**—Six triflavanoid procyanidins have been isolated from medicinal birch bark and their structures unequivocally established as epicatechin-(4$\beta$→8)-epicatechin-(4$\beta$→8)-epicatechin ($C_1$), catechin-(4$\alpha$→8)-catechin-(4$\alpha$→8)-catechin ($C_2$), catechin-(4$\alpha$→8)-epicatechin-(4$\beta$→8)-epicatechin, epicatechin-(4$\beta$→8)-epicatechin-(4$\beta$→8)-catechin, epicatechin-(4$\beta$→8)-epicatechin-(4$\beta$→6)-catechin and epicatechin-(4$\beta$→6)-epicatechin-(4$\beta$→8)-catechin. The bonding patterns in the last three regioisomers, and also the sequence of units in these representatives of mixed stereochemistry, were resolved by $^1$H NMR spectroscopy through application of diagnostic chemical-shift data obtained from high-temperature spectra of their methyl ether acetate derivatives. Additionally, the occurrence of (+)-catechin and its 7-*O*-$\beta$-D-xylopyranoside, (−)-epicatechin and procyanidins $B_1$–$B_3$, $B_5$–$B_7$ was demonstrated.

## INTRODUCTION

Previous studies of *Betula* spp. revealed the presence of a variety of flavonoids, biphenylheptanoids, phenols, phenolic glycosides, triterpenoids, essential oil and indications of the presence of procyanidins [1]. The bark of birch (*Betula* spp.) is used in folk medicine for treatments of various skin diseases, as an antifebrile and diuretic [2]. Continued extension of our phytochemical survey of condensed tannins of medicinal plants includes a more detailed examination of the composition of oligomeric procyanidins of medicinal birch bark (*Betula* spp.). The recent communication on the isolation of a flavan-3-ol glycoside from the same source [3] thus prompted the present investigation of bark metabolites related to this class of natural products.

## RESULTS AND DISCUSSION

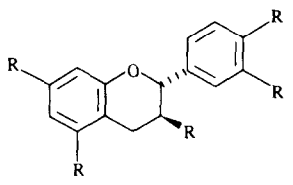
The ethyl acetate soluble portion, obtained from the methanol extract of medicinal birch bark, was chromatographed on Sephadex LH-20 to afford the putative competing nucleophiles (+)-catechin (**1**) and (−)-epicatechin (**3**) in approximately equal proportions. These monomeric precursors were accompanied by (+)-catechin 7-*O*-$\beta$-D-xylopyranoside (**4**), the structure and stereochemistry of which was established on the basis of hydrolytic studies and by $^1$H and $^{13}$C NMR as well as mass spectrometry of its acetate (**5**). The $\beta$-configuration was assigned to the anomeric centre based upon the coupling constant ($J = 6.5$ Hz) of the anomeric proton resonance ($\delta$ 5.17).

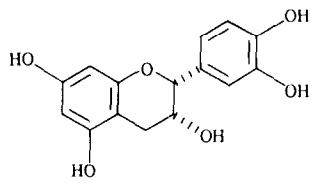
The $^1$H NMR spectrum of the acetate **5** compared with that of (+)-catechin penta-acetate (**2**) showed shielding of aromatic A-ring protons. The significant upfield shift ($\Delta\delta$ 0.22) of the aromatic 6-H ($\delta$ 6.42) and 8-H resonance ($\delta$ 6.54) in **5** suggested that the sugar could be placed on the C-7 position, consistent with previous results [3]. Further evidence regarding $\beta$-linkage and bonding position was available from $^{13}$C NMR data, showing deshielding of the C-7 resonance ($\Delta\delta$ +6.3) and shielding of the signals of C-6 ($\Delta\delta$ −4.37), C-8 ($\Delta\delta$ −5.16) and C-4a ($\Delta\delta$ −2.9) relative to those of **2**. The optical rotation $[\alpha]_D^{20}$ −13.2° ($CHCl_3$; *c* 0.57) (lit. [4] $[\alpha]_D$ −13.9°), taken in conjunction with calculated $[M]_D$ values for the $\alpha$- and $\beta$-xylopyranoside +285.5°, and −80.5°, respectively, strongly supported the presence of the $\beta$-anomer. Elemental analysis of **5** corresponded to the molecular formula $C_{34}H_{36}O_{17}$ in accordance with the proposed structure. The occurrence of the flavan-3-ol glycoside **4** as major metabolite is significant in that such secondary products are rarely found in Nature in constrast to the ubiquitous flavonoid glycosides.

On further chromatographic fractionation of the ethyl acetate soluble portion on Sephadex LH-20 a complex mixture of oligomeric flavan-3-ols was obtained. The complexity of the metabolic pool represented in the bark of the birch is due to participation of precursors of both 2,3-*trans* and 2,3-*cis* configuration in the formation of condensed tannins, as demonstrated by the characterization of the following products of mixed stereochemistry.

The biflavanoid fraction was represented by the (4→8)-procyanidins $B_1$ (**6**) $B_2$ (**10**), $B_3$ (**14**) and their (4→6)-isomers $B_7$ (**8**), $B_5$ (**12**) and $B_6$ (**16**) respectively. These compounds were identified by means of spectroscopic data of their octamethyl ether diacetates (**7**, **11**, **15**, **9**, **13** and **17**), respectively, exhibiting spectral properties ($^1$H NMR, CD) identical to those of products obtained by synthesis [5].

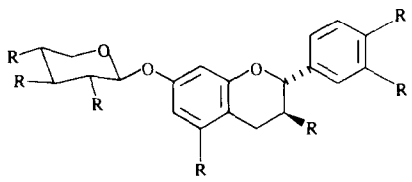
Choice of the methyl ether acetate derivatives is based, amongst others, on both the convenient recognition of





1 R = OH
2 R = OAc

3



4 R = OH
5 R = OAc

the degree of condensation of oligomers as judged by the number of resonances due to aliphatic acetoxy functions, and definition of the points of bonding to flavan units from chemical-shift data obtained from high-temperature spectra ($CDCl_3$, 100°) [5, 6]. The sharply defined spectra also provided proof of the stereochemical purity of the products under investigation and hence the absence of diastereoisomers.

In addition, the metabolic pool was populated by a variety of triflavanoid procyanidins, including the structurally homogeneous epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin* ($C_1$) (**18**) and catechin-(4α→8)-catechin-(4α→8)-catechin ($C_2$) (**20**) as well as representatives of mixed stereochemistry (**22**, **24**, **26** and **28**). The catechin-(4α→8)-epicatechin-(4β→8)-*epicatechin* (**22**), the natural existence of which was very recently demonstrated by its isolation from willow bark [Kolodziej, H., unpublished results], and the epicatechin-(4β→8)-epicatechin-(4β→8)-catechin **24** were characterized as the methyl ether acetates (**23** and **25**) by comparison of their spectroscopic data with those of authentic samples [5; Kolodziej, H., unpublished results]. Structural assessment of compounds **18** and **20** was similarly effected by comparing the spectral properties ($^1$H NMR, CD) of their dodecamethyl ether triacetates (**19** and **21**) with those of their synthetic counterparts [7, 8].

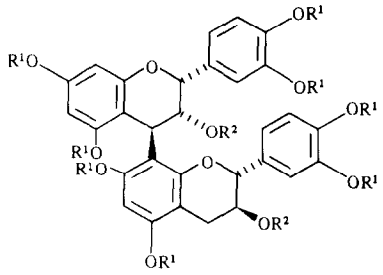
Although the positional isomers **24**, **26** and **28** with the epicatechin-epicatechin-catechin sequence of constituent units have recently been reported from several plant sources [9–18], their characterization was, however, mainly based on an indirect method involving acid catalysed degradation with toluene-α-thiol [19] coupled with spectroscopic evidence of cleavage products. By contrast, various chemical-shift criteria and other NMR-parameters [5, 6, 8, 20, 21] which have proved to be helpful in the structure elucidation of procyanidin methyl ether acetates under specific conditions ($CDCl_3$, 100°), permit direct determination of both bonding patterns and sequence of units in procyanidins of higher complexity. The relatively small quantities required for spectral analysis are advantageous, as is the avoidance of degradative techniques. The latter preclude assessment of the stereochemistry at the points of bonding; a limitation which is critical, considering recent reports of the existence of synthetic and natural 3,4-*cis* procyanidins [22–24], contrasting with their hitherto generally accepted 3,4-*trans* configurations.

With the detailed analysis of the synthetic epicatechin-(4β→8)-epicatechin-(4β→8)-catechin methyl ether acetate (**24**) as a basis [5], the structures of the isomeric triflavanoids (**27** and **29**) were unequivocally assigned as the (4β→8, 4β→6)- and (4β→6, 4β→8)-regioisomers, respectively. The mode of linkages was clearly indicated by the chemical shifts of residual A-ring protons. While the successive (4→8, 4→8)-coupling of the predominant triflavanoid **25** was confirmed by two high-field aromatic singlets at δ6.05 and 6.11, the chemical shifts of aromatic singlets of the isomeric (4→8, 4→6)- [δ6.11 and 6.34] and (4→6, 4→8)-triflavanoids [δ6.18 and 6.26] **27** and **29**, respectively, signified (4→8)- and (4→6)-linkages of different flavan units in each instance.

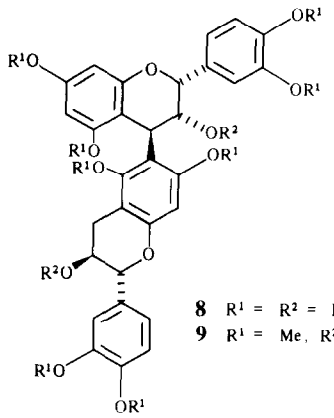
The proposed sequence of the constituent units of **27** and **29** followed from decoupling experiments of the methylene protons, revealing association with signals of large coupling constants ($J = 8.0$–$10.0$ Hz) thus defining catechin as the 'lower' terminal unit, in line with similar experiments for **25**. The broadened singlets and the small couplings of remaining signals in the heterocyclic region indicated a 2,3-*cis*-3,4-*trans* configuration of constituent epicatechin units.

Chemical shifts of the aromatic A-ring proton resonances (δ5.84, 5.94 for **25**; δ5.87, 5.92 for **27**) were significant in that the (4→8)-linkage of the 'upper' biflavanoid unit of **25** and **27** was confirmed [Kolodziej, H. unpublished results] supported by the line-broadening of 6-H doublets (A-ring) compared with sharp absorptions exhibited by their *meta*-coupled 8-H (A) counterparts in

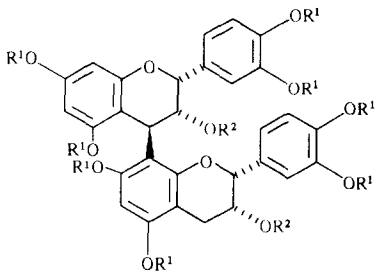
* Nomenclature based on the proposals by Hemingway, R. W., Foo, L. Y. and Porter, L. J. (1982) *J. Chem. Soc. Perkin Trans. I*, 1209.



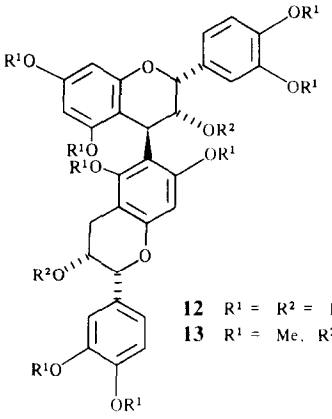
**6** $R^1 = R^2 = H$
**7** $R^1 = Me$, $R^2 = Ac$



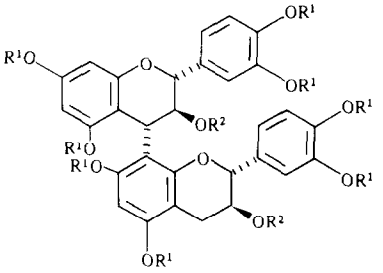
**8** $R^1 = R^2 = H$
**9** $R^1 = Me$, $R^2 = Ac$



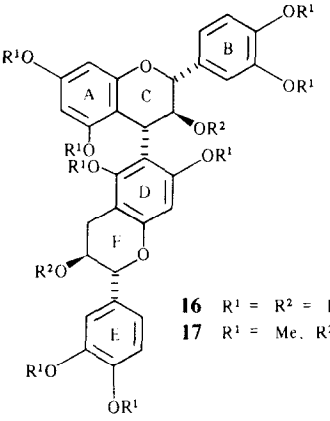
**10** $R^1 = R^2 = H$
**11** $R^1 = Me$, $R^2 = Ac$



**12** $R^1 = R^2 = H$
**13** $R^1 = Me$, $R^2 = Ac$



**14** $R^1 = R^2 = H$
**15** $R^1 = Me$, $R^2 = Ac$



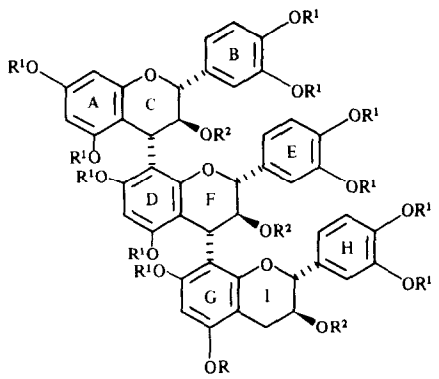
**16** $R^1 = R^2 = H$
**17** $R^1 = Me$, $R^2 = Ac$

each instance [7]. By contrast, the sharply defined aromatic A-ring doublets of **29**, when taken in conjunction with the relative down-field position of the AB-system [$\delta$6.04, 6-H (A); $\delta$6.19, 8-H (A)], attributable to relief from shielding induced by the aromatic E-ring of the next 2,3-*cis*-3,4-*trans* unit (Dreiding models), indicated (4→6)-bonding of the 'upper' biflavanoid unit of **29**.

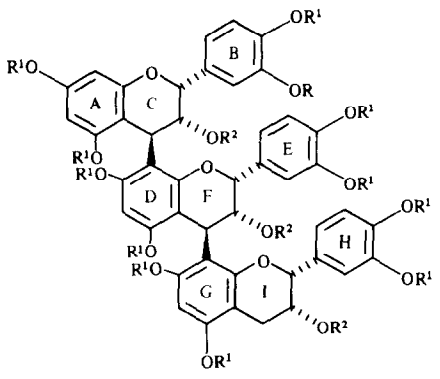
Further evidence regarding bonding patterns in the regioisomers **25**, **27** and **29** was available from chemical shifts of 2-H resonances of ring F. Although the values for 2-H (F) of **25** ($\delta$5.09), **27** ($\delta$5.15) and **29** ($\delta$5.37) do not fall within in the range of (4→8)-[$\delta$4.50-4.98] and (4→6)-biflavanoid procyanidin methyl ether acetates [$\delta$4.97-5.11] [5], presumably owing to increased molecular complexity, deshielding of 2-H (F) of **29** relative to those of **25** and **27** was in agreement with the anticipated (4→6)-linkage of the former and hence (4→8)-linkages of the latter.

Chemical-shift difference ($\Delta\delta$2-H, 3-H) between resonances which were allocated to 2-H and 3-H of 'lower' terminal catechin units similarly permitted assignment of the (4→8, 4→6)- and (4→6, 4→8)-sequence of **27** ($\Delta\delta$0.28) and **29** ($\Delta\delta$0.71), respectively, when compared with those of the analogous biflavanoid derivatives of $B_7$ ($\Delta\delta$0.27) and $B_1$ ($\Delta\delta$0.69) [5]. However, this parameter appeared somewhat critical considering the value $\Delta\delta$0.19 for the (4→8, 4→8)-triflavanoid **25** indicating (4→6)-coupling in contrast to the proposed bonding pattern. With chemical shift differences ($\Delta\delta$2-H, 3-H) of 'lower' terminal catechin units in oligomeric 2,3-*trans* procyanidins as a basis [8] that of **25** falls into line.

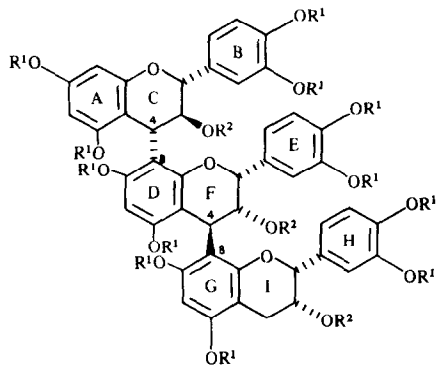
Finally, the chemical shifts of the aliphatic 3-acetoxy



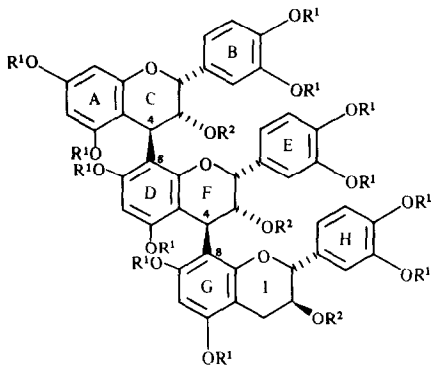
**20** $R^1 = R^2 = H$
**21** $R^1$ = Me, $R^2$ = Ac



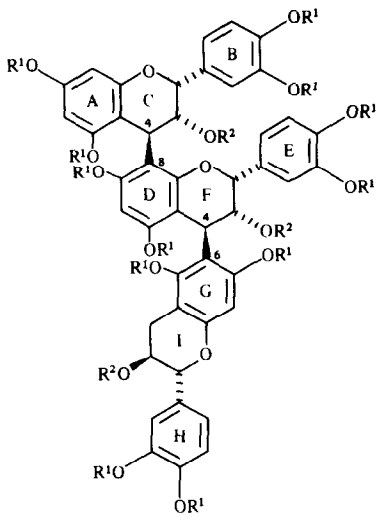
**18** $R^1 = R^2 = H$
**19** $R^1$ = Me, $R^2$ = Ac



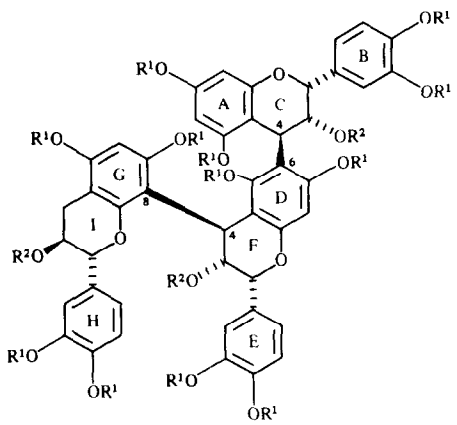
**22** $R^1 = R^2 = H$
**23** $R^1$ = Me, $R^2$ = Ac



**24** $R^1 = R^2 = H$
**25** $R^1$ = Me, $R^2$ = Ac



**26** $R^1 = R^2 = H$
**27** $R^1$ = Me, $R^2$ = Ac



**28** $R^1 = R^2 = H$
**29** $R^1$ = Me, $R^2$ = Ac

functions were of note. These resonances have proved of diagnostic value, those of 'lower' units differentiating between (4→8)- and (4→6)-isomers and those of 'upper' units distinguishing between catechin and epicatechin moieties [5, 21]. The values for the 3-OAc resonances (C-ring) of **25**, **27** and **29** ($\delta$1.71, 1.72 and 1.75, respectively) were consistent with those of 'upper' terminal epicatechin units ($\delta$1.72–1.80). 3-Acetoxy proton resonances of 'lower' units of **27** ($\delta$1.94) and **29** ($\delta$1.80) were similarly significant, signifying interflavanoid links to the 6- and 8-position, respectively, when compared with (4→6)-[$\delta$1.88–1.91] and (4→8)-biflavanoid procyanidins

[δ1.80–1.88]. The (4→8, 4→8)-derivative **25** again appeared to be problematical in view of the chemical shift of the 3-OAc (**I**) resonance at δ1.90.

Collectively these $^1$H parameters then defined the sequence of units and the bonding patterns of **27** and **29** as epicatechin-(4β→8)-epicatechin-(4β→6)-catechin and epicatechin-(4β→6)-epicatechin-(4β→8)-catechin, respectively. Thus, $^1$H-parameters have also proved to be reliable for higher oligomers of mixed stereochemistry, provided that they were used in conjunction and applied circumspectly. The absolute stereochemistry at C-4 of flavanoid units of **25**, **27** and **29** was confirmed by circular dichroism as evident from positive Cotton effects at low wavelengths in each instance (see Experimental) [25–27]. Considering that condensed tannins have significant affinity for proteins, the medicinal use of birch bark in skin diseases might well be attributed to presence of the identified oligomers.

## EXPERIMENTAL

NMR spectra were recorded ($CDCl_3$ 100°) with TMS as internal standard, unless stated otherwise. NMR tubes were firmly stoppered to avoid loss owing to pressure at temperatures above the boiling point of $CDCl_3$. CD data were obtained in MeOH. Analyses (C and H) were performed by the Department of Organic Chemistry, Westfälische Wilhelms-Universität, Münster. Preparative plates (20 × 20 cm; Kieselgel $PF_{254}$, 0.5 mm) were air-dried and used without prior activation. Methylations were performed with an excess $CH_2N_2$ in MeOH–$Et_2O$ for 48 hr at −15°, while acetylations were in $Ac_2O$–pyridine at room temp.

*Extraction and isolation of compounds.* Plant material (1 kg) (Fa. Caesar & Loretz, Hilden) was exhaustively extracted with MeOH and the combined extracts (5 l) evapd *in vacuo* to 500 ml, diluted with $H_2O$ (2 l) and defatted with hexane (5 × 500 ml). Extraction with EtOAc (15 × 500 ml) gave, on evapn of the solvent, a brown solid. A portion (15 g) of this material was chromatographed on Sephadex LH-20 (2.5 × 90 cm) using EtOH as eluant. After the emergence of phenolic material, 15 ml fractions were collected: test tubes 1–20 afforded a mixture (7.50 g) of flavonoids; tubes 21–50 (670 mg) contained (+)-catechin (**1**), (−)-epicatechin (**3**) and (+)-catechin 7-*O*-β-D-glycoside (**4**).

(+)-*Catechin* 7-O-β-D-*glycoside hepta-acetate* (**5**). Acetylation of fractions 21–50 (150 mg) and subsequent prep. TLC purification in $C_6H_6$–$Me_2CO$ (9:1; × 2) afforded peracetate **5** at $R_f$ 0.40 (75 mg) as a solid. (Found: C, 56.9; H, 5.2. Calc. for $C_{34}H_{36}O_{17}$: C, 57.0; H, 5.1%). $[\alpha]_D^{20}$ −13.2° ($CHCl_3$; *c* 0.5); mp 181–182° (uncorr.). $^1$H NMR (300 MHz, $CDCl_3$): δ2.00 and 2.08 (× 3) (s, 4 × aliph. OAc), 2.28–2.30 (s, 3 × phenol. OAc), 3.45 [*dd*, *J* = 7.5 and 12.5 Hz, 5″-$H_{ax}$], 4.20 [*dd*, *J* = 4.5 and 12.5 Hz, 5″-$H_{eq}$], 5.12–5.26 [*m*, 2″-H, 3″-H and 4″-H], 5.17 [*d*, *J* = 6.5 Hz. 1″-H], 5.00 [*m*, 3-H (C)], 5.18 [*d*, *J* = 6.5 Hz, 2-H (C)], 6.42 [*d*, *J* = 2.2 Hz, 6-H (A)], 6.54 [*d*, *J* = 2.2 Hz, 8-H (A)], 7.14–7.26 [*m*, 3 × H(B)]. $^{13}$C NMR ($CDCl_3$): δ23.78 (C-4), 61.84 (C-5″), 68.28 (C-3), 68.35 (C-4″), 69.88 (C-2″), 70.54 (C-3″), 77.56 (C-2), 98.23 (C-1″), 102.39 (C-8), 104.27 (C-6), 107.17 (C-4a), 121.65 (C-2′), 123.64 (C-5′), 124.29 (C-6′), 136.15 (C-1′), 142.04 (C-3′ + C-4′), 149.79 (C-5), 154.54 (C-8a), 156.04 (C-7). MS: $[M]^+$ 716 (2%).

*Dimeric procyanidins.* Frs 66–132 (717 mg) afforded procyanidin $B_1$ (**6**) and $B_3$ (**14**), frs 133–150 (160 mg) $B_3$ and $B_5$ (**12**), frs 151–190 (84 mg) $B_6$ (**16**) and $B_7$ (**8**) and frs 191–230 (194 mg) $B_2$ (**10**) and $B_6$. Methylation and subsequent acetylation of each fr. resolved by prep. TLC in $C_6H_6$–$Me_2CO$ (4:1), afforded their methyl ether acetate derivatives, the physical data ($^1$H NMR, CD, MS) of which were identical with those of authentic samples [5].

*Trimeric procyanidins.* Methylation of the contents of tubes 231–270 (213 mg) afforded a mixture of methyl ethers [toluene–$Me_2CO$ (7:3); $R_f$ 0.24, 0.17 and 0.15]. Acetylation of the $R_f$ 0.24 and 0.17 fractions and subsequent purification by prep. TLC in $C_6H_6$–$Me_2CO$ (4:1) yielded the methyl ether acetates of $C_1$ (**19**) [$R_f$ 0.51 (38 mg)] and $C_2$ (**21**) [$R_f$ 0.54 (28 mg)], the physical data of which ($^1$H NMR, CD) were identical with authentic samples [7, 8].

*Epicatechin-(4β→8)-epicatechin-(4β→8)-catechin* (**24**). The fraction $R_f$ 0.15 was subjected to prep. TLC using $C_6H_6$–$Me_2CO$ (3:1) to yield the methyl ether acetate (**25**) [$R_f$ 0.59 (19 mg)] which proved to be identical with that obtained by synthesis [5]. (Found: C, 65, 1; Calc. for $C_{63}H_{68}O_{21}$: C, 65.2; H, 5.9%).

The content of frs 271–305 (104 mg) was methylated to give one band at $R_f$ 0.40 [toluene–$Me_2CO$ (7:3)]. Acetylation followed by prep. TLC [$C_6H_6$–$Me_2CO$ (17:3; × 2)] afforded two fractions at $R_f$ 0.39 and $R_f$ 0.36.

*Epicatechin-(4β→6)-epicatechin-(4β→8)-catechin* (**28**). The former fraction, $R_f$ 0.39 (9 mg), yielded the methyl ether acetate (**29**) as an amorphous powder from hexane. (Found: C, 65.2; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%). $^1$H NMR ($CDCl_3$, 100°): δ1.75, 1.76 and 1.80 (*s*, 3 × OAC), 3.06 [m, $CH_2$ (I)], 3.44-3.84 (m, 12 × OMe), 4.56 [*d*, *J* = 2.0 Hz, 4-H (F)], 4.72 [*d*, *J* = 2.0 Hz, 4-H (C)], 5.06 [*d*, *J* = 9.0 Hz, 2-H(I)], 5.19 [*t*, *J* = 1.5 and 2.0 Hz, 3-H (F)], 5.31 [*t*, *J* = 1.5 and 2.0 Hz, 3-H (C)], 5.37 *br* [*s*, 2-H (F)], 5.46 *br* [*s*, 2-H (C)], 5.79 [*m*, 3-H (I)], 6.04 [*d*, *J* = 2.5 Hz, 6-H (A)], 6.18 [*s*, 6-H (G)], 6.19 [*d*, *J* = 2.5 Hz, 8-H (A)], 6.26 [*s*, 8-H (D)], 6.69–7.00 [*m*, 9 × H (B, E and H)]. CD $[\theta]_{255}$O, $[\theta]_{243}$ + 70890. $[\theta]_{220}$ + 2150, $[\theta]_{216}$ + 42960, $[\theta]_{210}$O.

*Epicatechin-(4β→8)-epicatechin-(4β→6)-catechin* (**26**). The latter fraction, $R_f$ 0.36 (12 mg), yielded the methyl ether acetate (**27**) as an amorphous powder from hexane. (Found: C, 65.1; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%). $^1$H NMR ($CDCl_3$, 100°): δ1.72, 1.81 and 1.94 (s, 3 × OAc), 2.87 [*m*, $CH_2$ (I)], 3.56–3.84 (*m*, 12 × OMe), 4.66 *br* [*s*, 4-H (F)], 4.78 *br* [*s*, 4-H (C)], 5.03 [*d*, *J* = 6.5 Hz, 2-H (I)], 5.15 *br* [*s*, 2-H (F)], 5.19 [*m*, 3-H (F)], 5.31 [*m*, 3-H (I)], 5.35 [*t*, *J* = 1.5 and 2.0 Hz, 3-H (C)], 5.57 *br* [*s*, 2-H (C)], 5.84 *br* [*d*, 6-H (A)], 5.92 [*d*, *J* = 2.0 Hz, 8-H (A)], 6.11 [*s*, 6-H (D)], 6.34 [*s*, 8-H (G)], 6.66–7.03 [*m*, 9 × H (B, E and H)]. CD $[\theta]_{250}$ + 4140, $[\theta]_{230}$ + 134640, $[\theta]_{225}$ + 124285, $[\theta]_{220}$ + 147070.

*Catechin-(4α→8)-epicatechin-(4β→8)-epicatechin* (**22**). Methylation of the content of tubes 306–380 (184 mg), followed by acetylation of the $R_f$ 0.28 fraction [$C_6H_6$–$Me_2Co$ (7:3)] and subsequent purification by prep. TLC in the same solvent system afforded the methyl ether acetate (**23**) at $R_f$ 0.68 (14 mg), the physical data ($^1$H NMR, CD) of which were identical with those of an authentic sample [Kolodziej, H., unpublished results].

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