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Structure and synthesis of the first procassinidin dimers based on epicatechin, and gallo- and epigallo-catechin ☆

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Abstract

The range of natural dimeric procassinidins is extended by identification of cassiaflavan-(4α → 8)-epicatechin, cassiaflavan-(4α → 8)-epigallocatechin, cassiaflavan-(4β → 8)-epicatechin, cassiaflavan-(4β → 8)-epigallocatechin, cassiaflavan-(4β → 8)-gallocatechin, *ent*-cassiaflavan-(4β → 8)-epicatechin and cassiaflavan-(4α → 6)-epicatechin in the bark of *Cassia petersiana*. Their structures and absolute configuration were confirmed by synthesis.

Keywords: *Cassia petersiana*; Leguminosae; Flavanoids; Proanthocyanidins; Procassinidins; Synthesis

1. Introduction

The occurrence of flavan containing dimeric proanthocyanidins in nature is rare (Porter, 1988, 1994). Only four sources of these compounds were reported, viz cassiaflavan-epiafzelechin analogues from *Cassia fistula*, (Morimoto, Nonaka, Chen & Nishioka, 1988), (2*S*)-3′,4′,7-trihydroxyflavan-catechin analogues with lipase inhibitory activity from *Cassia nomame* (Hatano et al., 1997), (2*S*)-4′,7,8-trihydroxyflavan-(4β → 6)-epioritin-4α-ol from *Acacia caffra* (Malan, Sireeparsad, Swinny & Ferreira, 1997) and butiniflavan-epicatechin and -epigallocatechin probutinidins from *Cassia petersiana* (Coetzee, Mciteka, Malan & Ferreira, 1999). We now report the structure and synthesis of the first procassinidins based on epicatechin and gallo- and epigallo-catechin from the bark of *C. petersiana*, an aqueous extract which is used in traditional African medicine to treat fevers, gonorrhoea and skin infections (Palgrave, 1983).

2. Results and discussion

The bark of *C. petersiana* was extracted with acetone and yielded a complex mixture of phenolic monomers and dimeric proanthocyanidins. Catechin, epicatechin, gallocatechin, epigallocatechin and the three novel butiniflavan-epicatechin and -epigallocatechin probutinidins (Coetzee et al., 1999) were accompanied by seven dimeric procassinidins **1**, **3**, **5**, **7**, **9**, **11** and **13**. The mixture could be resolved by gel column chromatography and the compounds purified by TLC as their permethylaryl ether acetate derivatives **2**, **4**, **6**, **8**, **10**, **12** and **14**, respectively.

The structures and relative configuration of these procassinidin derivatives were determined by ^{1}H NMR (Tables 1 and 2). Owing to the adverse effects of rotational isomerism about the C-4(C) → C-8(D) interflavanyl bond the spectra of compounds **6**, **8**, and **10**

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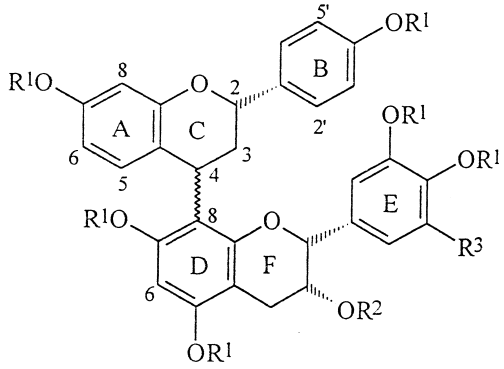
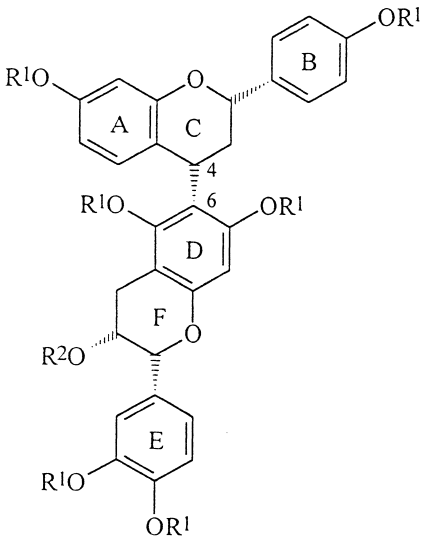
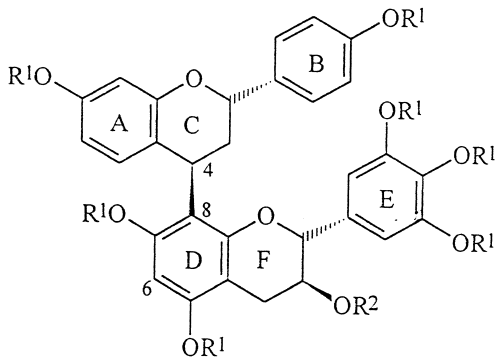


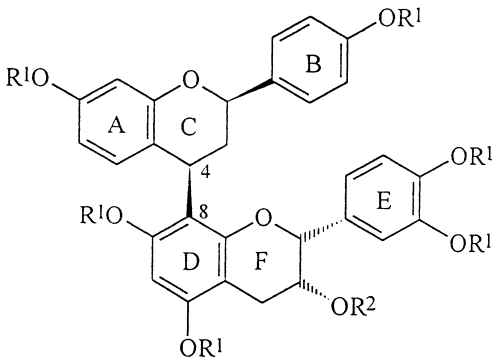
1 ≡ , $R^1=R^2=R^3=H$
2 ≡ , $R^1=Me$, $R^2=Ac$, $R^3=H$
3 ≡ , $R^1=R^2=H$, $R^3=OH$
4 ≡ , $R^1=Me$, $R^2=Ac$, $R^3=OMe$
5 ≡ , $R^1=R^2=R^3=H$
6 ≡ , $R^1=Me$, $R^2=Ac$, $R^3=H$
7 ≡ , $R^1=R^2=H$, $R^3=OH$
8 ≡ , $R^1=Me$, $R^2=Ac$, $R^3=OMe$



13 $R^1=R^2=H$
14 $R^1=Me$, $R^2=Ac$



9 $R^1=R^2=H$
10 $R^1=Me$, $R^2=Ac$



11 $R^1=R^2=H$
12 $R^1=Me$, $R^2=Ac$

were broadened and had to be recorded at elevated temperatures (70°C, C_6D_6) to obtain sharp sets of resonances. The spectra of compounds **2**, **4**, **12** and **14** showed two distinct rotamers at room temperature (20°C) for **2**, **12** (in $CDCl_3$) and **14** (C_6D_6), and at 40°C for **4** (C_6D_6). The close proximity of the 4-H(C) resonances, however, precluded definition of the two rotamers since observation of NOE effects between these protons and 7-OMe(D) is a prerequisite for rotamer differentiation.

The ^1H NMR spectral data (Tables 1 and 2) for compounds **2**, **4**, **6**, **8**, **10**, **12** and **14** showed similarly substituted ABC-units comprising AA′BB′- and ABX-systems for the aromatic protons and an AMNX-coupled pattern, reminiscent of the heterocyclic protons of a C-4 substituted flavan moiety (Hatano et al., 1997; Malan et al., 1997; Coetzee et al., 1999). The spectra of all the compounds additionally displayed a one-proton singlet in the high field aromatic region and an AMXY-system for protons in the heterocyclic

Table 1
^{1}H NMR peaks (δ_H) for compounds **2**, **4**, **6** and **8** at 300 MHz. Splitting patterns and *J*-values (Hz) are given in parentheses

Ring	H	**2** ($CDCl_3$, 20°C)	**4** (C_6D_6, 40°C)	**6** (C_6D_6, 70°C)	**8** (C_6D_6, 70°C)
A	5	6.77, 6.66[a] (*d*, 8.5)	7.18, 7.20[a] (*d*, 8.5)	7.05(*d*, 8.5)	7.04 (*d*, 8.5)
	6	6.40, 6.34[a] (*dd*, 8.5, 2.5)	6.66, 6.73[a] (*dd*, 8.5, 2.5)	6.56(*dd*, 8.5, 2.5)	6.54 (*dd*, 8.5, 2.5)
	8	6.49, 6.47[a] (*d*, 2.5)	6.77, 6.91[a] (*d*, 2.5)	6.73(*d*, 2.5)	6.65 (*d*, 2.5)
B	2′, 6′	7.20, 7.45[a] (*d*, 9.0)	7.27, 7.46[a] (*d*, 9.0)	7.46(*d*, 9.0)	7.46 (*d*, 9.0)
	3′, 5′	6.82, 6.96[a] (*d*, 9.0)	6.80, 6.93[a] (*d*, 9.0)	6.85(*d*, 9.0)	6.86 (*d*, 9.0)
C	2	5.17, 5.10[a] (*dd*, 12.0, 2.0)	5.27, 5.16[a] (*dd*, 11.5, 2.0)	5.78(*dd*, 7.0, 3.5)	5.78 (*dd*, 7.0, 3.5)
	3	2.00, 2.20[a] (*ddd*, 13.0, 2.0, 5.5)	2.28, 2.41[a] (*ddd*, 13.0, 5.0, 2.5)	2.55(*dd*d, 13.5, 7.0, 6.0)	2.56 (*ddd*, 13.5, 7.0, 6.0)
	3	2.72, 2.78[a] (*m*)	3.32, 3.13[a] (*m*)	2.97 (*ddd*, 13.5, 7.0, 3.5)	2.94 (*ddd*, 13.5, 7.0, 3.5)
	4	4.95, 5.01[a] (*dd*, 12.0, 5.5)	5.43, 5.51[a] (*dd*, 12.0, 5.0)	5.07 (*dd*, 7.0, 6.0)	5.06 (*t*, 6.0)
D	6	6.25, 6.15[a] (*s*)	6.18, 6.06[a] (*s*)	6.11 (*s*)	6.12 (*s*)
	8	–	–	–	–
E	2′	6.53, 7.01[a] (*d*, 2.0)	6.46, 6.90[a] (*s*)	7.06 (*d*, 2.0)	6.68 (*s*)
	5′	6.65, 6.88[a] (*d*, 8.5)	–	6.75 (*d*, 8.5)	–
	6′	6.20, 6.99[a] (*dd*, 8.5, 2.0)	6.46, 6.90[a] (*s*)	6.83 (*dd*, 8.5, 2.0)	6.68 (*s*)
F	2	4.89, 5.13[a] (*br.s*)	4.65, 5.02[a] (*br.s*)	4.68 (*br.s*)	4.60 (*br.s*)
	3	5.31, 5.56[a] (*m*)	5.51, 5.78[a] (*m*)	5.62 (*m*)	5.59 (*m*)
	4	2.94, 2.98[a] (*m*)	3.06, 3.19[a] (*dd*, 18.0, 5.0)	3.06 (*dd*, 18.0, 5.0)	3.06 (*dd*, 18.0, 5.0)
	4	2.94, 2.98[a] (*m*)	3.31, 3.46[a] (*dd*, 18.0, 2.5)	3.32 (*dd*, 18.0, 2.5)	3.33 (*d*, 18.0, 2.5)
	OMe	3.51[a], 3.75[a], 3.76, 3.78, 3.79, 3.84[a], 3.85[a] (×2), 3.87, 3.89, 3.90[a], 3.91 (all *s*)	3.27[a], 3.36, 3.43[a], 3.48[a], 3.49[a], 3.50, 3.55, 3.61[a] (×2), 3.62, 3.70 (×2), 3.92, 3.96[a] (*s*)	3.41, 3.46, 3.49, 3.53, 3.60, 3.71 (all *s*)	3.43, 3.47, 3.49, 3.53, 3.68 (×2), 3.94 (*s*)
	OAc	1.79, 1.91[a] (*s*)	1.51, 1.53[a] (*s*)	1.63 (*s*)	1.64 (*s*)

[a] Signals of minor rotamer.

Table 2
^{1}H NMR peaks (δ_H) for compounds **10**, **12** and **14** at 300 MHz. Splitting patterns and *J*-values (Hz) are given in parentheses

Ring	H	**10** (C_6D_6, 70°C)	**12** ($CDCl_3$, 20°C)	**14** (C_6D_6, 20°C)
A	5	7.02 (*d*, 8.5)	6.61, 6.81[a] (*d*, 8.5)	7.43 (*d*, 8.5)
	6	6.56 (*dd*, 8.5, 2.5)	6.38, 6.23[a] (*dd*, 8.5, 2.5)	6.70 (*dd*, 8.5, 2.5)
	8	6.63 (*d*, 2.5)	6.50, 6.15[a] (*d*, 2.5)	6.92 (*d*, 2.5)
B	2′, 6′	7.41 (*d*, 9.0)	7.41, 7.43[a] (*d*, 9.0)	7.30 (*d*, 9.0)
	3′, 5′	6.85 (*d*, 9.0)	6.92, 6.94[a] (*d*, 9.0)	6.77 (*d*, 9.0)
C	2	5.67 (*dd*, 8.0, 3.5)	5.14, 5.08[a] (*dd*, 12.0, 2.0)	5.30 (*dd*, 12.0, 2.0)
	3	2.56 (*ddd*, 13.5, 7.0, 6.5)	2.22, 2.06[a] (*ddd*, 13.0, 5.5, 2.0)	2.44 (*dd*, 13.0, 5.5, 2.0)
	3	2.80 (*ddd*, 13.5, 6.5, 3.5)	2.77, 2.81[a] (*m*)	3.23 (*m*)
	4	5.01 (*t*, 6.0)	4.92, 5.00[a] (*dd*, 12.0, 5.5)	4.87 (*dd*, 12.0, 5.5)
D	6	6.12 (*s*)	6.25, 6.13[a] (*s*)	–
	8	–	–	6.38 (*s*)
E	2′	6.67 (*s*)	6.71, 6.99[a] (*d*, 2.0)	6.64 (*d*, 2.0)
	5′	–	6.77, 6.87[a] (*d*, 8.5)	6.78 (*d*, 8.5)
	6′	6.67 (*s*)	6.69, 7.01[a] (*dd*, 8.5, 2.0)	6.46 (*dd*, 8.5, 2.0)
F	2	4.73 (*d*, 7.5)	0.44, 5.12[a] (*br.s*)	4.58 (*br.s*)
	3	5.58 (*m*)	5.40, 5.49[a] (*m*)	5.45 (*m*)
	4	2.99 (*dd*, 17.0, 7.5)	2.87, 3.04[a] (*dd*, 18.0, 2.0)	2.94 (*dd*, 18.0, 4.5)
	4	3.40 (*dd*, 17.0, 6.0)	2.98, 3.04[a] (*dd*, 18.0, 5.0)	3.39 (*m*)
	OMe	3.45, 3.46, 3.52, 3.53, 3.63 (×2), 3.91 (all *s*)	3.54[a], 3.56, 3.76[a], 3.77[a], 3.78, 3.83[a], 3.84[a], 3.85, 3.87, 3.88, 3.90[a], 3.91 (all *s*)	3.31, 3.39, 3.40, 3.43, 3.49, 3.66 (all *s*)
	OAc	1.64 (*s*)	1.88, 1.94[a] (*s*)	2.00 (*s*)

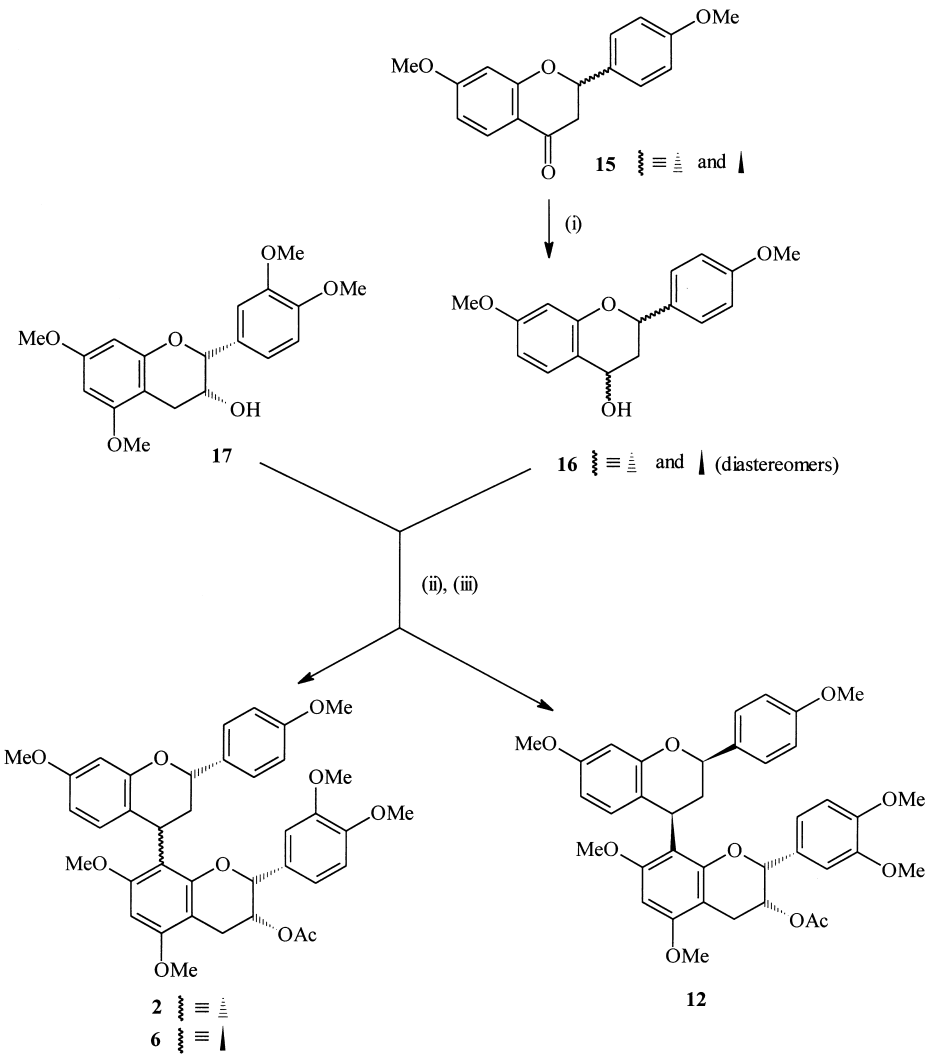
[a] Signals of minor rotamer.

Table 3
^{13}C NMR peaks (δ_C) for compounds **2**, **4**, **6**, **8**, **10** and **12**

Ring	C	**2** ($CDCl_3$, 20°C)	**4** (C_6D_6, 40°C)	**6** (C_6D_6, 70°C)	**8** (C_6D_6, 70°C)	**10** (C_6D_6, 70°C)	**12** ($CDCl_3$, 20°C)
A	5	128.7	128.7	128.7	128.7	128.9	128.1
	6	107.3	107.7	107.7	107.8	107.7	107.9
	8	101.5	101.9	101.9	102.0	101.9	101.5
B	2′, 6′	127.9	127.5	127.2	127.2	127.8	127.9
	3′, 5′	114.2	114.4	114.4	114.4	114.4	114.2
C	2	79.4	78.7	75.8	75.8	75.6	78.8
	3	35.0	36.5	36.2	36.3	36.7	35.8
	4	32.0	29.1	28.9	29.1	29.2	32.0
D	6	88.6	89.3	90.3	90.3	90.3	88.7
E	2′	109.6	105.8	104.3	105.7	105.8	109.9
	5′	110.8	–	106.7	–	–	110.7
	6′	119.4	105.8	114.2	105.7	105.8	119.2
F	2	77.3	77.8	78.1	78.3	79.7	77.6
	3	68.0	68.9	68.1	68.1	70.0	67.9
	4	26.6	27.0	27.1	27.0	26.0	26.7
	$O\underline{C}H_3$	55.7, 55.8, 56.1, 56.3, 56.4, 56.9	55.0 (×2), 55.2, 56.1, 56.4 (×2), 60.5	55.0, 55.3, 56.2, 56.4, 56.7, 56.9	55.0 (×2), 55.1, 56.1, 56.4 (×2), 60.5	54.9, 55.0, 55.2, 56.1, 56.3 (×2), 60.5	55.5, 55.7 (×2), 56.2, 56.3, 56.8
	$CH_3\underline{C}OO$–	170.5	169.5	169.4	169.4	169.5	170.9

region. When taken in conjunction with the presence of an aromatic ABX-system for derivatives **2**, **6**, **12** and **14**, an aromatic A_2-spin system for derivatives **4**, **8** and **10** and the relevant number of aromatic *O*-methyl and aliphatic *O*-acetyl resonances, the data collectively indicated 5,7,3′,4′-tetrahydroxyflavan-3-ol DEF units for compounds **1**, **5**, **11** and **13**, and 5,7,3′,4′,5′-pentahydroxyflavan-3-ol units for **3**, **7** and **9**. The dimeric structures were supported by FAB-MS data which indicated molecular ions *m/z* 656 ($C_{38}H_{40}O_{10}$) and 686 ($C_{39}H_{42}O_{11}$), for derivatives **2**, **6**, **12** and **14**, and **4**, **8** and **10**, respectively.

COSY experiments indicated coupling of 2-H(C) with 2′,6′-H(B) and 2-H(F) with 2′- and 6′-H(E) for derivates **2**, **4**, **6**, **8**, **10**, **12** and **14**. The same experiment revealed $^4J_{HH}$ coupling between 4-H(C) and 5-H(A) which defined the ABC- and DEF-units for all the compounds. Phase sensitive NOESY experiments of derivatives **2**, **4**, **12** and **14** showed association between 2- and 4-H(C), indicating a 2,4-*cis* relative configuration of the C-ring. The conspicuous absence of any NOE association between 2- and 4-H(C) in compounds **6**, **8** and **10** strongly suggested 2,4-*trans* relative configuration of their C-rings. These allocations of relative configuration were corroborated by the multiplicity and coupling constants of 2- and 4-H(C) for the 2,4-*cis* configuration, e.g. **2**, where both 2- and 4-H(C) resonate as double doublets ($^3J_{2,3} = 2.0$, 12.0 Hz; $^3J_{3,4} = 5.5$, 12.0 Hz). Analogues with a 2,4-*trans* relative configuration, e.g. **6**, show a double doublet for both 2- ($^3J_{2,3} = 3.5$, ca. 7.0 Hz) and 4-H(C) ($^3J_{3,4} = 6.0$, 7.0 Hz) and in some instances a triplet for 4-H(C) ($^3J_{3,4} = 6.0$ Hz) (Coetzee et al., 1999). The chemical shifts of the C-2 (C-ring) resonances in the ^{13}C NMR spectra of derivatives **2**, **4**, **6**, **8**, **10** and **12** fully supported these relative configurations. Those compounds with a 2,4-*trans* configuration (**6**, **8** and **10**) possess shielded 2-C(C) signals (ca. 4 ppm) com-



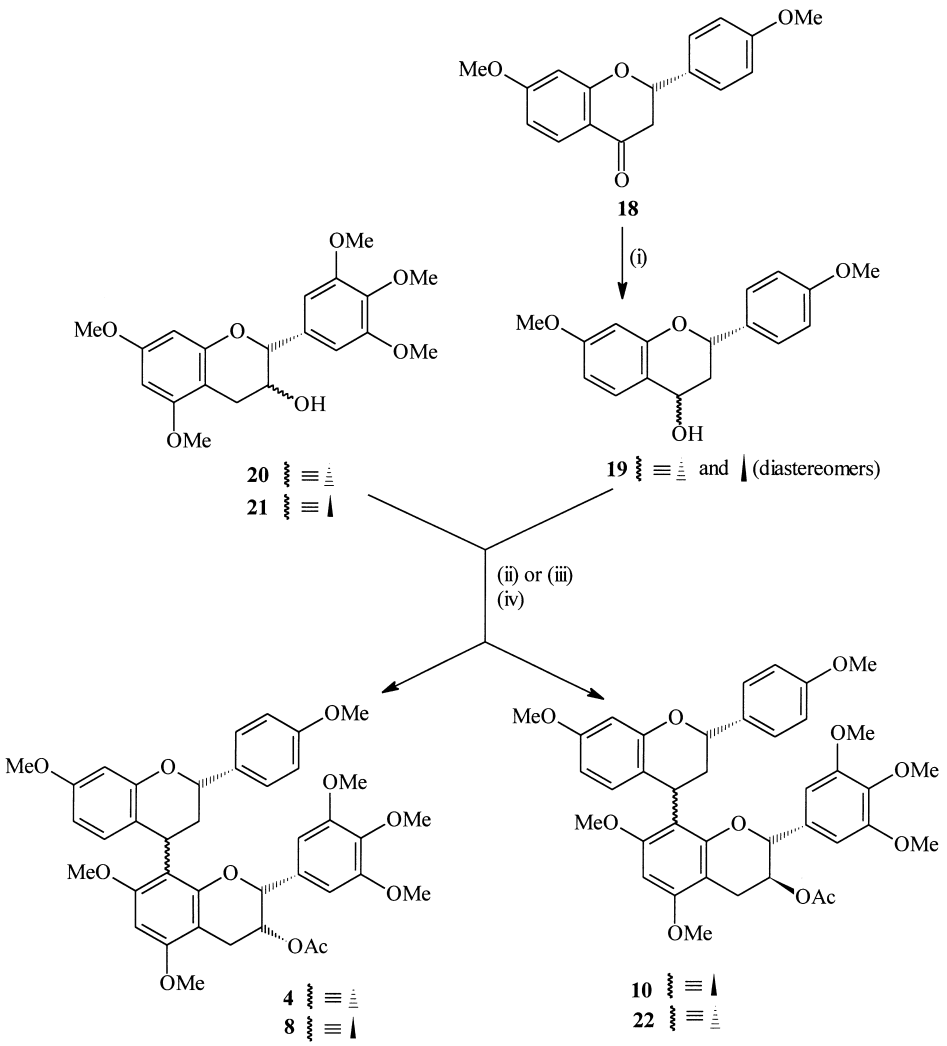
Scheme 1. Reagents and conditions: (i) $NaBH_4$, EtOH; (ii) **17** then, $TiCl_4/CH_2Cl_2$; (iii) Ac_2O, pyridine.

pared to the chemical shifts of these carbons in derivatives with a 2,4-*cis* configuration (Table 3) as a result of the γ-*gauche* effect (Fletcher, Porter, Haslam & Gupta, 1977) operating in the former group of compounds. The same shielding phenomena are also evident from the results of Hatano et al. (1997). The chemical shifts of the hydrogen-bearing carbons in compounds **2**, **4**, **6**, **8**, **10** and **12** are collated in Table 3. Sample size precluded the collection of ^{13}C NMR data for the 4,6-linked analogue **14**.

Bonding positions to the D-ring were established for all the derivatives by the familiar NOE associations exhibited by 'residual' D-ring protons with methoxyl(s) i.e. the D-ring singlet showing strong association with the 5- and 7-methoxy groups of the D-ring in derivatives **2**, **4**, **6**, **8**, **10** and **12** and selectively with 7-OMe in **14** (Young, Brandt, Young, Ferreira & Roux, 1986).

The CD spectra of compounds **2**, **4** and **14** exhibited high amplitude negative Cotton effects at ca. 240 nm, indicating a 4α-flavanyl C-ring substituent (De Angelis & Wildman, 1969; Van der Westhuizen, Ferreira & Roux, 1981). Derivatives **6**, **8**, **10** and **12** showed positive Cotton effects near 240 nm which is reminiscent of a 4β C-ring substituent. When taken in conjunction with the relative 2,4-*cis* configuration of derivatives **2**, **4**, **12** and **14**, the negative Cotton effects for **2**, **4** and **14** indicated their 2*S*,4*S* absolute configuration and the positive Cotton effect for **12** its 2*R*,4*R* absolute stereochemistry. The positive Cotton effects similarly supported the 2*S*,4*R* absolute configuration of derivatives **6**, **8** and **10** with their 2,4-*trans* relative configurations.

Derivatives **2**, **4**, **6**, **8**, **12** and **14** showed 2-H(F) as a broad singlet [$^3J_{2,\ 3(F)}$ ca 1.0 Hz] while **10** exhibited a coupling constant of 7.5 Hz. This indicated a 2,3-*cis* relative configuration of the F-rings (Steynberg, Burger, Malan, Cronjé, Young & Ferreira, 1990) of the former group and 2,3-*trans* for derivative **10**. Thus, we opted to synthesize these derivatives in order to estab-



Scheme 2. Reagents and conditions: (i) $NaBH_4$, EtOH; (ii) **20** then, $TiCl_4/CH_2Cl_2$; (iii) **21** then, $TiCl_4/CH_2Cl_2$; (iv) Ac_2O/pyridine.

lish the absolute configuration (Nonaka, Miwa & Nishioka, 1982) and constitution of the natural products **1**, **3**, **5**, **7**, **9**, **11** and **13**.

Racemic 4′,7-dimethoxyflavanone **15** was reduced by sodium borohydride (Hatano et al., 1997; Malan et al., 1997) to give the diastereomeric mixture of the flavan-4-ol **16** in 96% yield (Scheme 1). Treatment of this mixture with optically pure tetra-*O*-methylepicatechin **17** in dichloromethane containing titanium tetrachloride ($TiCl_4$) as Lewis acid (Kawamoto, Nakatsubo & Murakami, 1991) afforded a mixture of compounds, which were purified by PLC to give three procassinidin-type dimers in fair yields. Acetylation yielded the permethylaryl ether acetates **2**, **6** and **12** which had identical ^{1}H NMR and CD spectral data when compared to derivatives obtained from the natural compounds **1**, **5** and **11**. We could not detect any of the expected permethyl aryl ether of the (4α → 6)-analogue **13**, an observation which is supported by previous reports of absence (Hatano et al., 1997; Coetzee et al., 1999) or formation in low yields (Young, Cronjé, Botes, Ferreira & Roux, 1985) of the (4 → 6)-linked dimers. Compounds **1**, **5** and **11** were hence unequivocally identified as cassiaflavan-(4α → 8)-epicatechin, cassiaflavan-(4β → 8)-epicatechin, and *ent*-cassiaflavan-(4β → 8)-epicatechin, respectively, and **13** tentatively as cassiaflavan-(4α → 6)-epicatechin.

In contrast to the above and our previous syntheses of dimeric proanthocyanidins with flavan constituent units (Malan et al., 1997; Coetzee et al., 1999) where racemic flavanones were utilized as source of the flavan molecular backbone, compounds **4** and **8** were synthesized from optically pure (2*S*)-di-*O*-methylliquiritigenin **18** and penta-*O*-methylepigallocatechin **20** (Scheme 2). Initial $NaBH_4$-reduction of **18** gave the intermediate 4′,7-dimethoxyflavan-4-ol **19** (diastereomers, quantitative yield) which was subsequently treated with penta-*O*-methylepigallocatechin **20** in dichloromethane containing $TiCl_4$. The ^{1}H NMR and CD spectra of the *O*-acetyl derivatives of the synthetic dimer derivatives **4** and **8** corresponded with those of the same derivatives of the procassinidins from *C. petersiana*. The structures and absolute stereochemistry of the natural products **3** and **7** could thus be defined as cassiaflavan-(4α → 8)-epigallocatechin and cassiaflavan-(4β → 8)-epigallocatechin, respectively.

A similar procedure (Scheme 2) was employed to synthesize **10** by using (2*S*)-4′,7-di-*O*-methylliquiritigenin **18** and penta-*O*-methylgallocatechin **21**. Subsequent acetylation afforded the cassiaflavan-(4β → 8)-gallocatechin and cassiaflavan-(4α → 8)-gallocatechin derivatives **10** and **22**. The lower yields of **4** and **22** compared to those of **8** and **10** indicated that the stereochemical course of coupling is directed by the C-2 *eq*. aryl group.

The *ent*-cassiaflavan-(4β → 8)-epicatechin **11** is the first compound in the class of flavan → flavan-3-ol proanthocyanidins possessing a flavan top unit with 2*R* absolute configuration, hence presumably also indicating the involvement of a 2*R*-flavanone in their biosynthesis (Stich & Forkmann, 1988).

3. Experimental

^{1}H NMR spectra were recorded at 300 MHz for solutions in $CDCl_3$ or C_6D_6, with TMS as int. standard. FAB-MS were recorded on a VG 70-70E instrument with a VG 11-250J data system and an iontech saddlefield FAB gun. CD data were obtained in MeOH. TLC was performed on precoated Merck plastic sheets (silica gel 60 PF_{254} 0.25 mm) and the plates were sprayed with H_2SO_4–HCHO (40:1, vol/vol) after development. Prep. TLC plates, Kieselgel PF_{254} (1.0 mm) were air dried and used without prior activation. Compounds were recovered from the absorbent with Me_2CO. CC was on Sephadex LH-20 in EtOH. Methylations were performed with an excess of CH_2N_2 in MeOH–Et_2O over a period of 48 h at −15°C, while acetylations were in Ac_2O-pyridine at ambient temperature. Evaporations were done under reduced pressure at ambient temperatures in a rotary evaporator, and freeze drying of aqueous solutions on a Virtis 12SL freezemobile.

3.1. General procedure for the synthesis of procassinidin derivatives

The reduction of flavanones **15** and **18** was done according to standard procedures (Hatano et al., 1997; Malan et al., 1997). To a dry solution of 4′,7-dimethoxyflavan-4-ol **16** (90.0 mg) in CH_2Cl_2 (20 ml) was added the permethylaryl ether **17** of epicatechin (296 mg) and $TiCl_4$ (0.04 ml, 1.2–1.4 equivalent). The mixture was stirred at 0°C under N_2 for 60 min and the temperature was allowed to rise to 40°C for a further 6 h. An excess of cold H_2O (40 ml) was added and the mixture was extracted with Et_2O (3 × 20 ml). After drying (Na_2SO_4) the Et_2O was removed under vacuum and the mixture was resolved by prep. TLC in hexane–C_6H_6–Me_2CO (5:2:3) to give three bands at R_f 0.53, 0.40 and 0.33. After acetylation of the R_f 0.33 band (42 mg) it was further separated in hexane–C_6H_6–Me_2CO (5:4:1) to give four bands at R_f 0.57 (5.5 mg), 0.37 (5.7 mg), 0.29 (8.2 mg) and 0.22 (14.1 mg). The R_f 0.57 band yielded starting material **17**. The R_f 0.37 band yielded 4′7-di-*O*-methyl-*ent*-cassiaflavan-(4β → 8)-3′,4′,5,7-tetra-*O*-methyl-3-*O*-acetyl-epicatechin **12** as a *light-brown amorphous solid*. The R_f 0.29 band gave 4′,7-di-*O*-methylcassiaflavan-(4β → 8)-3′,4′,5,7-tetra-*O*-methyl-3-*O*-acetylepicatechin **6** and

the R_f 0.22 band 4′,7-di-*O*-methylcassiaflavan-(4α → 8)-3′,4′,5,7-tetra-*O*-methyl-3-*O*-acetylepicatechin **2**.

(2*S*)-4′,7-dimethoxyflavan-4-ol **19** (90.0 mg) was dissolved in dry CH_2Cl_2 (20 ml), the $TiCl_4$ (0.04 ml) added to give a dark purple solution after which the epigallocatechin or gallocatechin permethylaryl ethers **20**/**21** (290 mg) was added during separate experiments. The mixture was treated and worked up as above, and the residue was separated by prep. TLC in C_6H_6–Me_2CO (8:2) to give two bands at R_f 0.39 (40 mg) and 0.26 (17.3 mg). The R_f 0.39 band yielded starting material **20**. Acetylation of the R_f 0.26 band followed by prep. TLC in hexane–C_6H_6–Me_2CO (5:4:1, ×4) gave two bands which comprised 4′,7-di-*O*-methylcassiaflavan-(4β → 8)-epigallocatechin penta-*O*-methylether acetate **8** and 4′,7-di-*O*-methylcassiaflavan-(4α → 8)-epigallocatechin-penta-*O*-methylether acetate **4** at R_f 0.29 (4.0 mg) and 0.39 (10 mg), respectively. Derivatives **4** and **8** showed identical NMR and CD spectral data as the same derivatives of the corresponding natural products **3** and **7**. The yield of compound **10** and very likely also that of **22** during a similar procedure using the gallocatechin permethylaryl ether **21** were low and after initial prep. TLC separation in hexane–C_6H_6–Me_2CO (5:2:3) only 6 mg of an inseparable mixture of **10** and **22** was obtained. However, it was possible to identify compound **10** by using the ^{1}H NMR spectral data of the natural compound to fingerprint the synthetic mixture.

3.2. Isolation of phenolic compounds

Milled bark (6.3 kg) of *C. petersiana* was repeatedly extracted with Me_2CO (3 × 7.5 l) for 48 h periods at 25°C. The Me_2CO was removed under vacuum at 35°C and the residue dissolved in H_2O and freeze dried to give a brown powder (370 g).

The extract (two batches of 25 g each) was subjected to CC on Sephadex LH-20 in EtOH (6 × 180 cm) column, 0.5 ml/min flow rate, 32 min fractions) to give the following fractions: B_1 (tubes 225–264, 82 mg), B_2 (265–279, 62 mg), B_3 (280–285, 10 mg), B_4 (286–319, 70 mg), B_5 (320–354, 110 mg), B_6 (355–364, 29 mg), B_7 (365–399, 260 mg) and B_8 (400–414, 117 mg). A portion (200 mg) of fraction B_8 was methylated and the mixture was separated by prep. TLC in C_6H_6–Me_2CO (9:1, ×3) to give four bands at R_f 0.64 ($B_{8.1}$, 33 mg), 0.40 ($B_{8.2}$, 24.5 mg), 0.37 ($B_{8.3}$, 12.5 mg) and 0.13 ($B_{8.4}$, 15.6 mg). All four fractions were separately acetylated and purified by prep. TLC in C_6H_6–Me_2CO (24:1, ×3) to yield the following compounds.

4′,7-Di-O-methylcassiaflavan-(4α → 8)-3′,4′,5,7-tetra-O-methyl-3-O-acetylepicatechin **2** (R_f 0.64, 15 mg) from $B_{8.1}$ as a *white amorphous solid*. (Found: M^+, 656.2623. $C_{38}H_{40}O_{10}$ requires M^+, 656.2620; δ_H (Table 1); ^{13}C NMR ($CDCl_3$, 20°C): δ (major rotamer) 21.2 [$\underline{C}H_3COO$–], 26.6 [4-C(F)], 32.0 [4-C(C)], 35.0 [3-C(C)], 55.7 [–O$\underline{C}H_3$], 55.8 [–O$\underline{C}H_3$], 56.1 [–O$\underline{C}H_3$], 56.3 [–O$\underline{C}H_3$], 56.4 [–O$\underline{C}H_3$], 56.9 [–O$\underline{C}H_3$], 68.0 [3-C(F)], 77.3 [2-C(F)], 79.4 [2-C(C)], 88.6 [6-C(D)], 101.5 [8-C(A)], 107.3 [6-C(A)], 109.6 [2′-C(E)], 110.8 [5′-C(E)], 114.2 (×2) [3′,5′-C(B)], 119.1, 119.4 [6′-C(E)], 119.5, 127.9 (×2) [2′,6′-C(B)], 128.2, 128.5, 128.7 [5-C(A)], 130.6, 134.4, 148.8, 156.3 [$\underline{Ar}$-OCH_3], 157.7 [$\underline{Ar}$-OCH_3], 158.0 [$\underline{Ar}$-OCH_3], 158.6 [$\underline{Ar}$-OCH_3], 158.9 [$\underline{Ar}$-OCH_3], 159.6 [$\underline{Ar}$-OCH_3], 170.5 [$CH_3\underline{C}OO$–]; CD [θ]$_{276.1}$ 2704, [θ]$_{243.9}$ −16710, [θ]$_{234.7}$ 1016.

4′,7-Di-O-methyl-ent-cassiaflavan-(4β → 8)-3′,4′,5,7-tetra-O-methyl-3-O-acetylepicatechin **12** (R_f 0.61, 4.5 mg) from $B_{8.1}$ as a *white-yellowish amorphous solid*. (Found: M^+, 656.2621. $C_{38}H_{40}O_{10}$ requires M^+, 656.2620. δ_H (Table 2); ^{13}C NMR ($CDCl_3$, 20°C): δ (major rotamer) 21.5 [$\underline{C}H_3COO$–], 26.7 [4-C(F)], 32.0 [4-C(C)], 35.8 [3-C(C)], 55.5 [–O$\underline{C}H_3$], 55.7 [–O$\underline{C}H_3$], 55.7 [–O$\underline{C}H_3$], 56.2 [–O$\underline{C}H_3$], 56.3 [–O$\underline{C}H_3$], 56.8 [–O$\underline{C}H_3$], 67.9 [3-C(F)], 77.6 [2-C(F)], 78.8 [2-C(C)], 88.7 [6-C(D)], 101.5 [8-C(A)], 107.9 [6-C(A)], 109.9 [2′-C(E)], 110.7 [5′-C(E)], 114.2 (×2) [3′,5′-C(B)], 119.0, 119.2 [6′-C(E)], 119.5, 127.86, 127.9 (×2) [2′,6′-C(B)], 128.1 [5-C(A)], 128.5, 130.4, 134.4, 148.7, 156.3 [$\underline{Ar}$-OCH_3], 157.5 [$\underline{Ar}$-OCH_3], 157.8 [$\underline{Ar}$-OCH_3], 158.6 [$\underline{Ar}$-OCH_3], 158.7 [$\underline{Ar}$-OCH_3], 159.6 [$\underline{Ar}$-OCH_3], 170.9 [$CH_3\underline{C}OO$–]; CD [θ]$_{286.5}$ 4178, [θ]$_{273.3}$ −4770, [θ]$_{246.5}$ 6630.

4′,7-Di-O-methylcassiaflavan-(4α → 6)-3′,4′,5,7-tetra-O-methyl-3-O-acetylepicatechin **14** (R_f 0.36, 8.3 mg) from $B_{8.2}$ as a *white amorphous solid*. (Found: M^+, 656.2620. $C_{38}H_{40}O_{10}$ requires M^+, 656.2620. δ_H (Table 2); CD [θ]$_{286.8}$ 4591, [θ]$_{244.1}$ −15230, [θ]$_{230.3}$ 3321.

4′,7-Di-O-methylcassiaflavan-(4β → 8)-3′,4′,5,7-tetra-O-methyl-3-O-acetylepicatechin **6** (R_f 0.47, 2.5 mg) from $B_{8.3}$ as a *white amorphous solid*. (Found: M^+, 656.2621. $C_{38}H_{40}O_{10}$ requires M^+, 656.2620. δ_H (Table 1); ^{13}C NMR (C_6D_6, 70°C): δ 20.5 [$\underline{C}H_3COO$–], 27.1 [4-C(F)], 28.9 [4-C(C)], 36.2 [3-C(C)], 55.0 [–O$\underline{C}H_3$], 55.3 [–O$\underline{C}H_3$], 56.2 [–O$\underline{C}H_3$], 56.4 [–O$\underline{C}H_3$], 56.7 [–O$\underline{C}H_3$], 56.9 [–O$\underline{C}H_3$], 68.1 [3-C(F)], 75.8 [2-C(C)], 78.1 [2-C(F)], 90.3 [6-C(D)], 101.9 [8-C(A)], 102.6, 104.3 [2′-C(E)], 106.7 [5′-C(E)], 107.7 [6-C(A)], 111.4, 114.2 [6′-C(E)], 114.4 (×2) [3′,5′-C(B)], 118.4, 119.4, 127.2 (×2) [2′,6′-C(B)], 128.7 [5-C(A)], 133.4, 136.8, 140.2, 154.6 [$\underline{Ar}$-OCH_3], 156.6 [$\underline{Ar}$-OCH_3], 157.9 [$\underline{Ar}$-OCH_3], 158.4 [$\underline{Ar}$-OCH_3], 159.8 [$\underline{Ar}$-OCH_3], 159.8 [$\underline{Ar}$-OCH_3], 169.4 [$CH_3\underline{C}OO$–]; CD [θ]$_{275.8}$ −7354, [θ]$_{244.0}$ 15780, [θ]$_{230.6}$ 1358.

Fraction $B_{8.4}$ (R_f 0.29, 8.5 mg) comprised the heptamethyl ether diacetate of fisetinidol-(4β → 8)-epicatechin (Steynberg et al., 1990).

A portion of fraction B_7 (200 mg) was methylated and the mixture was separated by PLC in C_6H_6–Me_2CO (8:2), to give a band at R_f 0.67 (47 mg) which was subsequently acetylated. Further separation in

hexane–Me_2CO–EtOAc (12:5:3, vol/vol) afforded two bands at R_f 0.51 (5.2 mg) and 0.45 (6.0 mg) comprising compounds **10** and **8**, respectively. 4′,7-Di-*O*-methylcassiaflavan-(4β → 8)-3′,4′,5′,5,7-penta-*O*-methyl-3-*O*-acetylgallocatechin **10** was obtained as *colorless amorphous flakes*. (Found: M^+, 686.2723. $C_{39}H_{42}O_{11}$ requires M, 686.2724; δ_H (Table 2); ^{13}C NMR (C_6D_6, 70°C): δ 20.5 [$\underline{C}H_3COO$–], 26.0 [4-C(F)], 29.2 [4-C(C)], 36.7 [3-C(C)], 54.9 [–O$\underline{C}H_3$], 55.0 [–O$\underline{C}H_3$], 55.2 [–O$\underline{C}H_3$], 56.1 [–O$\underline{C}H_3$], 56.3 (×2) [–O$\underline{C}H_3$], 60.5 [–O$\underline{C}H_3$], 70.0 [3-C(F)], 75.6 [2-C(C)], 79.7 [2-C(F)], 90.3 [6-C(D)], 101.9 [8-C(A)], 103.7, 105.8 (×2) [2′,6′-C(E)], 107.7 [6-C(A)], 110.2, 114.4 (×2) [3′,5′-C(B)], 114.9, 119.4, 127.8 (×2) [2′,6′-C(B)], 128.9 [5-C(A)], 133.4, 135.4, 140.1, 154.1 [$\underline{Ar}$-OCH_3], 154.2 [$\underline{Ar}$-OCH_3], 156.5 [$\underline{Ar}$-OCH_3], 157.9 [$\underline{Ar}$-OCH_3], 158.5 [$\underline{Ar}$-OCH_3], 159.7 [$\underline{Ar}$-OCH_3], 160.0 [$\underline{Ar}$-OCH_3], 169.5 [$CH_3\underline{C}OO$–]; CD $[\theta]_{275.8}$ −4174, $[\theta]_{238.9}$ 14440 and $[\theta]_{227.2}$ −313. 4′,7-Di-*O*-methylcassiaflavan-(4β → 8)-3′,4′,5′,5,7-penta-*O*-methyl-3-*O*-acetylepigallocatechin **8** was isolated as *colorless amorphous platelets*. (Found: M^+, 686.2722. $C_{39}H_{42}O_{11}$ requires M, 686.2724); δ_H (Table 1); ^{13}C NMR (C_6D_6, 70°C): (20.5 [$\underline{C}H_3COO$–], 27.0 [4-C(F)], 29.1 [4-C(C)], 36.3 [3-C(C)], 55.0 (×2) [–O$\underline{C}H_3$], 55.1 [–O$\underline{C}H_3$], 56.1 [–O$\underline{C}H_3$], 56.4 (×2) [–O$\underline{C}H_3$], 60.5 [–O$\underline{C}H_3$], 68.1 [3-C(F)], 75.8 [2-C(C)], 78.3 [2-C(F)], 90.3 [6-C(D)], 102.0 [8-C(A)], 103.6, 105.7 (×2) [2′,6′-C(E)], 107.8 [6-C(A)], 110.2, 114.4 (×2) [3′,5′-C(B)], 114.8, 119.3, 127.2 (×2) [2′,6′-C(B)], 128.7 [5-C(A)], 133.4, 135.3, 140.1, 154.1 [$\underline{Ar}$-OCH_3], 154.6 [$\underline{Ar}$-OCH_3], 156.4 [$\underline{Ar}$-OCH_3], 157.9 [$\underline{Ar}$-OCH_3], 158.4 [$\underline{Ar}$-OCH_3], 159.7 [$\underline{Ar}$-OCH_3], 159.8 [$\underline{Ar}$-OCH_3], 169.4 [$CH_3\underline{C}OO$–]; CD $[\theta]_{275.0}$ −1254, $[\theta]_{248.1}$ 12180 and $[\theta]_{239.7}$ −804.

A portion (200 mg) of fraction C_4 (Coetzee et al., 1999) was methylated and subjected to the same procedure of separation and purification as for compounds **8** and **10**. The band at R_f 0.64 (22 mg) obtained after the first separation was acetylated and purified to yield 4′,7-Di-*O*-methylcassiaflavan-(4α → 8)-3′,4′,5′,5,7-penta-*O*-methyl-3-*O*-acetylepigallocatechin **4** (R_f 0.54, 6.0 mg) as a *white amorphous solid*. (Found: M^+, 686.2724. $C_{39}H_{42}O_{11}$ requires M, 686.2724); δ_H (Table 1); ^{13}C NMR (C_6D_6, 40°C): δ (major rotamer) 20.4 [$\underline{C}H_3COO$–], 27.0 [4-C(F)], 29.1 [4-C(C)], 36.5 [3-C(C)], 55.0 (×2) [–O$\underline{C}H_3$], 55.2 [–O$\underline{C}H_3$], 56.1 [–O$\underline{C}H_3$], 56.4 (×2) [–O$\underline{C}H_3$], 60.5 [–O$\underline{C}H_3$], 68.9 [3-C(F)], 77.8 [2-C(F)], 78.7 [2-C(C)], 89.3 [6-C(D)], 101.9 [8-C(A)], 103.7, 105.8 (×2) [2′,6′-C(E)], 107.7 [6-C(A)], 110.2, 114.4 (×2) [3′,5′-C(B)], 114.8, 119.4, 127.5 (×2) [2′,6′-C(B)], 128.7 [5-C(A)], 133.4, 135.3, 140.0, 154.1 [$\underline{Ar}$-OCH_3], 154.3 [$\underline{Ar}$-OCH_3], 156.4 [$\underline{Ar}$-OCH_3], 157.8 [$\underline{Ar}$-OCH_3], 158.5 [$\underline{Ar}$-OCH_3], 159.7 [$\underline{Ar}$-OCH_3], 159.8 [$\underline{Ar}$-OCH_3], 169.5 [CH3$\underline{C}$OO–]; CD $[\theta]_{275.5}$ 2535, $[\theta]_{243.7}$ −11860 and $[\theta]_{222.9}$ 2205.

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