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Structure and synthesis of butiniflavan-epicatechin and -epigallocatechin probutinidins[☆]

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Abstract

The rare series of dimeric proanthocyanidins with flavan chain extender units is extended by characterization of butiniflavan-(4α → 8)- and (4β → 8)-epicatechins and butiniflavan-(4β → 8)-epigallocatechin from the bark of *Cassia petersiana*. The structure and absolute configuration of the dimers were confirmed by synthesis via reduction of the racemic flavanone, (±)-7,3′,4′-tri-*O*-methylbutin, to the diastereomeric flavan-4-ols and condensation with 5,7,3′,4′-tetra-*O*-methylepicatechin and 5,7,3′,4′,5′-penta-*O*-methylepigallocatechin using titanium tetrachloride as Lewis acid. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Cassia petersiana; Leguminosae; Flavan–flavan-3-ol dimers; Proanthocyanidins; Probutinidins; Synthesis

1. Introduction

The dimeric flavanoids possessing a flavan constituent unit as top and/or bottom moiety represent a rare group of naturally occurring polyphenols (Porter, 1988, 1994). Two additional sources of flavan–flavan-3-ol dimers were recently reported, viz. *Cassia nomame* containing (2*S*)-7,3′,4′-trihydroxyflavan-(4 → 8)-catechin analogues (Hatano et al., 1997) and *Acacia caffra* producing (2*S*)-7,8,4′-trihydroxyflavan-(4 → 6)-epioritin-4α-ol (Malan, Sireeparsad, Swinny & Ferreira, 1997), the first example with a flavan-3,4-diol bottom unit. Some of these biflavanoids show lipase-inhibiting activity (Hatano et al., 1997) while simple flavan glycosides may act as insect growth inhibitors (Kubo & Kim, 1987). We now report on the structure and synthesis of three 7,3′,4′-trihydroxyflavan-(4 → 8)-flavan-3-ol dimers **1**, **3** and **5** from the bark of *Cassia petersiana*, which is used in traditional African medicine as a purgative and to treat fevers, gonorrhoea and skin infections (Palgrave, 1983).

2. Results and discussion

The identification of dimeric proanthocyanidins with a flavan chain extender unit resulted in the creation of appropriate trivial names for both the monomer unit and the proanthocyanidin class, e.g. cassiaflavan designating the (2*S*)-7,4′-dihydroxyflavan top unit of the procassinidins (Porter, 1988, 1994). Owing to the close structural relationship of the ABC-unit in dimer **1** to the (2*S*)-7,3′,4′-trihydroxyflavanone, butin, we propose the trivial name butiniflavan for this moiety and *ent*-butiniflavan for a (2*R*)-7,3′,4′-trihydroxyflavan ABC-unit. The natural products are then butiniflavan-(4α → 8)-epicatechin **1**, butiniflavan-(4β → 8)-epicatechin **3** and butiniflavan-(4β → 8)-epigallocatechin **5**

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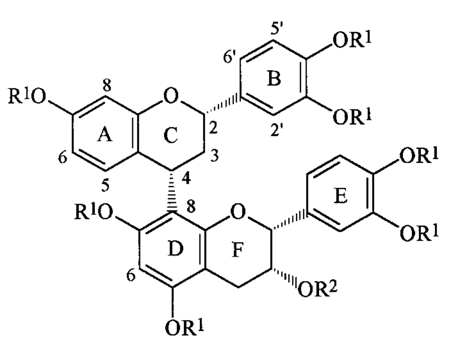

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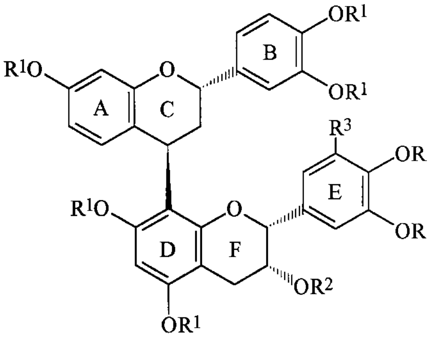
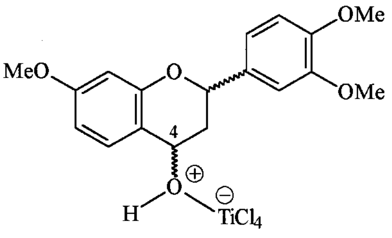


3 $R^1=R^2=R^3=H$
4 $R^1=Me, R^2=Ac, R^3=H$
5 $R^1=R^2=H, R^3=OH$
6 $R^1=Me, R^2=Ac, R^3=OMe$



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and they belong to the probutinidin class of proanthocyanidins.

The acetone extract of the bark of *C. petersiana* afforded the known flavan-3-ols, (+)-catechin, (−)-epicatechin, (+)-gallocatechin and (−)-epigallocatechin. They were identified by comparison of the ^{1}H NMR and CD data of the permethylaryl ether acetate derivatives with those of authentic samples from our collection of reference compounds. The flavan-3-ols were accompanied by a variety of dimeric compounds of which the probutinidins **1**, **3** and **5** will be discussed here. Owing to the complexity of the phenolic mixture the dimers were purified and identified as the heptamethyl ether acetate derivatives **2** and **4** and as the octamethyl ether acetate **6**, the additional chromatographic steps offered by derivatization being a prerequisite for sample purity.

The ^{1}H NMR data (Table 1) of derivatives **2**, **4** and **6** indicated similarly substituted ABC-units via the presence of two ABX-spin systems for aromatic protons as well as an AMNX-spin pattern, reminiscent of the heterocyclic protons of a C-4 substituted 7,3′,4′-trimethoxyflavan constituent unit (Hatano et al., 1997; Malan et al., 1997). The remaining spin systems, i.e. a one-proton aromatic singlet for derivatives **2**, **4** and **6**, aromatic ABX- and A_2-spin systems for **2** and **4** and **6**, respectively and heterocyclic AMXY-systems for **2**, **4** and **6**, indicated D-ring substituted 5,7,3′,4′-tetramethoxy-3-*O*-acetylflavan-3-ol DEF-units for derivatives **2** and **4** and a 5,7,3′,4′,5′-pentamethoxy-3-*O*-acetylflavan-3-ol unit for derivative **6**, hence indicating the dimeric nature of all three compounds. Differentiation of the spin systems and the connectivities between aromatic and heterocyclic protons were effected with NOESY and COSY experiments. FAB-MS data indicated molecular ions at *m/z* 686 for **2** and **4** and 716 for **6** reminiscent of a molecular formula of $C_{39}H_{42}O_{11}$ for **2** and **4** and $C_{40}H_{44}O_{12}$ for **6**. When taken in conjunction with the aforementioned aromatic oxygenation patterns these molecular ions strongly supported 3-deoxy (C-ring) flavanyl (≡flavan) constituent ABC-units for **2**, **4** and **6**.

The ^{1}H NMR spectra of all three derivatives showed the adverse effects of dynamic rotational isomerism about the interflavanyl bonds at 20°. At this temperature derivative **2** showed two rotamers in a ratio approximating 99:1. Owing to line broadening and overlap of the protons crucial for definition of rota-

Table 1
^{1}H NMR (300 MHz) data of compounds **2**, **4**, **6**, **12**, **13** and **14**

Ring	H	**2** (CDCl$_3$, 20°C)	**4** (C$_6$D$_6$, 70°C)	**6** (C$_6$D$_6$, 70°C)	**12** (CDCl$_3$, 20°C)	**13** (CDCl$_3$, 20°C)	**14** (CDCl$_3$, 20°C)
A	5	6.75 (d, 8.5)	7.07 (d, 8.5)	7.05 (d, 8.5)	6.64, 6.82[a] (d, 8.5)	6.70, 6.61[a] (d, 8.5)	6.61, 6.81[a] (d, 8.5)
	6	6.41 (dd, 8.5, 2.5)	6.57 (dd, 8.5, 2.5)	6.55 (dd, 8.5, 2.5)	6.25, 6.40[a] (dd, 8.5, 2.5)	6.38, 6.35[a] (dd, 8.5, 2.5)	6.23, 6.37[a] (dd, 8.5, 2.5)
	8	6.51 (d, 2.5)	6.75 (d, 2.5)	6.67 (d, 2.5)	6.20, 6.52[a] (d, 2.5)	6.43, 6.49[a] (d, 2.5)	6.12, 6.50[a] (d, 2.5)
B	2′	6.70 (d, 2.5)	7.17 (d, 2.5)	7.17 (d, 2.5)	6.69, 6.91[a] (d, 2.5)	6.73, 7.06[a] (d, 2.5)	7.02, 7.03[a] (d, 2.5)
	5′	6.77 (d, 8.5)	6.73 (d, 8.5)	6.75 (d, 8.5)	7.01, 6.90[a] (d, 8.5)	6.77, 6.91[a] (d, 8.5)	6.89, 6.94[a] (d, 8.5)
	6′	6.84 (dd, 8.5, 2.5)	7.13 (dd, 8.5, 2.5)	7.13 (dd, 8.5, 2.5)	7.05, 6.89[a] (dd, 8.5, 2.5)	6.84, 7.02[a] (dd, 8.5, 2.5)	7.05, 6.91[a] (dd, 8.5, 2.5)
C	2	5.17 (dd, 12.0, 2.0)	5.81 (dd, 6.5, 3.0)	5.81 (dd, 6.5, 3.0)	5.15, 5.08[a] (dd, 11.5, 1.5)	5.20, 5.09[a] (dd, 11.5, 2.0)	5.15, 5.09[a] (dd, 12.0, 1.5)
	3	1.98 (ddd, 13.0, 5.5, 2.0)	2.59 (ddd, 13.0, 7.0, 6.0)	2.61 (ddd, 13.0, 7.0, 6.0)	2.22, 2.09[a] (ddd, 12.5, 5.5, 1.5)	1.97, 2.22[a] (ddd, 13.0, 5.0, 2.5)	2.31, 2.10[a] (ddd, 13.0, 6.0, 2.0)
	3	2.79 (m)	3.00 (ddd, 13.0, 7.0, 3.5)	2.97 (ddd, 13.0, 7.0, 3.5)	2.82, 2.84[a] (m)	2.78, 2.91[a] (m)	2.75, 2.85[a] (m)
	4	4.94 (dd, 12.0, 5.5)	5.11 (t, 6.0)	5.10 (t, 6.0)	4.94, 5.01[a] (dd, 12.0, 6.0)	4.95, 5.52[a] (dd, 12.5, 5.0)	4.93, 5.01[a] (dd, 12.5, 6.0)
D	6	6.25 (s)	6.12 (s)	6.12 (s)	6.26, 6.15[a] (s)	6.27, 6.10[a] (s)	6.27, 6.15[a] (s)
E	2′	6.54 (d, 2.5)	7.06 (d, 2.5)	6.69 (s)	6.74, 7.02[a] (d, 2.5)	6.26, 6.69[a] (s)	6.36, 6.69[a] (s)
	5′	6.67 (d, 8.5)	6.77 (d, 8.5)	–	6.77, 6.87[a] (d, 8.5)	–	–
	6′	6.32 (dd, 8.5, 2.5)	6.84 (dd, 8.5, 2.5)	6.69 (s)	6.70, 7.02[a] (dd, 8.5, 2.5)	6.26, 6.69[a] (s)	6.36, 6.69[a] (s)
F	2	4.89 (br.s)	4.68 (br.s)	4.60 (br.s)	5.13, 4.49[a] (br.s)	4.84, 5.11[a] (br.s)	4.39, 5.12[a] (br.s)
	3	5.30 (m)	5.62 (m)	5.59 (m)	5.46, 5.50[a] (m)	5.27, 5.59[a] (m)	5.37, 5.51[a] (m)
	4	2.94 (m)	3.32 (dd, 17.0, 2.5)	3.33 (dd, 18.0, 2.5)	3.05, 3.05[a] (m)	2.99, 3.08[a] (dd, 18.0, 4.5)	3.01, 3.09[a] (dd, 18.0, 2.5)
	4	2.94 (m)	3.05 (dd, 17.0, 4.5)	3.06 (dd, 18.0, 5.0)	2.98, 2.87[a] (dd, 18.0, 5.0)	2.95, 2.95[a] (m)	2.87, 2.98[a] (dd, 18.0, 4.5)
	OMe	3.55, 3.75, 3.79, 3.85, 3.86, 3.89, 3.90 (7 × s)	3.42, 3.49, 3.53, 3.55, 3.57, 3.60, 3.71 (7 × s)	3.44, 3.49, 3.53, 3.56, 3.58, 3.93 (6 × s), 3.69 (2 × s)	3.55, 3.58, 3.78, 3.84, 3.85, 3.87, 3.88, 3.91, 3.92, 3.93, 3.78 (×2), 3.90 (×2) (s)	3.52[a], 3.57, 3.74, 3.76 (×2), 3.77[a], 3.82, 3.83[a], 3.84[a], 3.85, 3.87[a], 3.89, 3.90[a], 3.91, 3.92[a], 3.95[a] (s)	3.56[a], 3.57[a], 3.75 (×2), 3.76 (×2), 3.77[a], 3.84, 3.85[a], 3.86, 3.88[a], 3.89[a], 3.90, 3.91, 3.92[a], 3.93[a] (s)
	OAc	1.77 (s)	1.64 (s)	1.65 (s)	1.87, 1.95 (s)	1.81, 1.93 (s)	1.96, 1.89 (s)

[a] Signals of the minor rotamer.

mers, i.e. 2-H(C) and 4-H(C), 3-H_{ax}(C) and 3-H_{eq}(C) and 7-OMe(D) (Steynberg et al., 1995), the observation of NOE enhancements was too risky to permit unequivocal differentiation of the rotamers at this temperature. The spectra of derivatives **4** and **6** were thus recorded at 70° in deuteriobenzene where a single set of resonances was evident for each derivative.

Prominent $^4J_{HH}$ couplings, evident in the COSY spectra of **2** and **4**, between 2-H(C) (δ 5.17, 5.81 for **2** and **4**, resp.) and 2′- and 6′-H(B), as well as between 2-H(F) (δ 4.89, 4.68 for **2** and **4**, resp.) and 2′- and 6′-H(E) differentiated the ABX-spin systems of the B- and E-rings. The A/C-ring junction in all three derivatives was connected via the observed benzylic coupling of 5-H(A) (δ 6.75, 7.07, 7.05 for **2**, **4** and **6**, resp.) with 4-H(C) (δ 4.94, 5.11, 5.10 for **2**, **4** and **6**, resp.). A (4 → 8)-interflavanyl linkage was evident via observation of prominent NOE associations of 6-H(D) (δ 6.25, 6.12, 6.12 for **2**, **4** and **6**, resp.) with both 5- and 7-OMe(D) (Young, Brandt, Young, Ferreira & Roux, 1986).

A phase sensitive NOESY experiment of derivative **2** showed association between 2- and 4-H(C), hence indicating 2,4-*cis* relative configuration of the C-ring of this compound. By the same token the conspicuous absence of NOE association between 2- and 4-H(C) in derivatives **4** and **6** was interpreted as confirmation of the 2,4-*trans* relative configuration of their C-rings. The CD spectrum of compound **2** exhibited a high-amplitude negative Cotton effect ($\Delta\epsilon_{max}$ -1.664×10^4) at 244.7 nm while those of derivatives **4** and **6** showed intense positive Cotton effects ($\Delta\epsilon_{max}$ $+1.405\times10^4$ and $+4.798\times10^4$, respectively) at 244.6 nm. The signs of these Cotton effects are in accordance with a 4α-flavanyl substituent for **2** and with 4β-substituents for both **4** and **6**, hence indicating 4*S* absolute configuration for **2** and 4*R* configuration for **4** and **6** by application of the aromatic quadrant rule (De Angelis & Wildman, 1969; Van der Westhuizen, Ferreira & Roux, 1981). When taken in conjunction with the above NOE observations, the CD data then permitted definition of 2*S* absolute configuration for all three derivatives **2**, **4** and **6**.

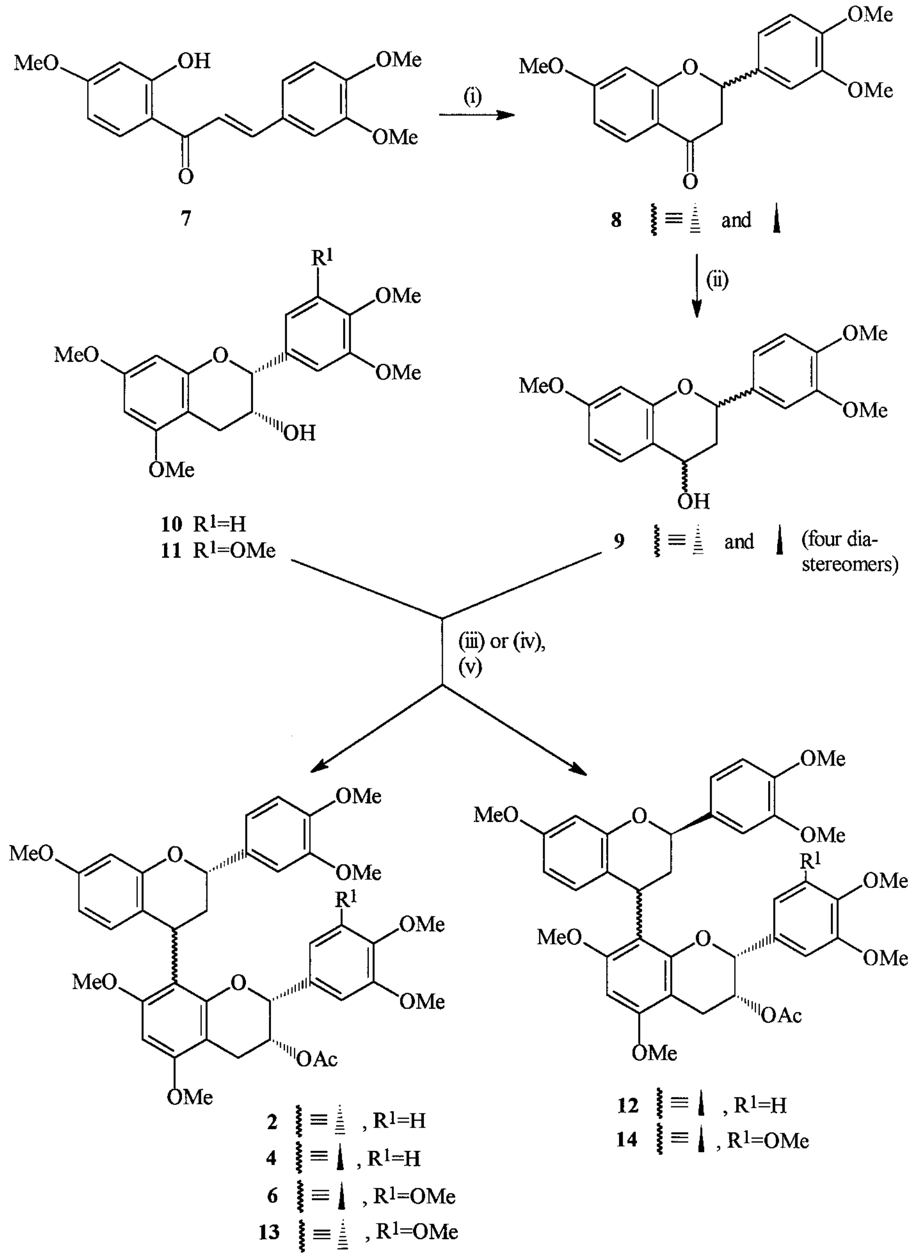
All three derivatives exhibited $^3J_{2,3(F)}$-values of ca. 1.0 Hz hence indicating 2,3-*cis* relative configuration of their F-rings. Since these may be compatible with either 2*R*,3*R*- or 2*S*,3*S*-absolute configuration (Nonaka, Miwa & Nishioka, 1982), we took recourse to synthesis of derivatives **2**, **4** and **6** in order to unequivocally establish absolute stereochemistry of the F-rings (Scheme 1).

Thus, base-catalyzed cyclization (Ferreira, Van der Merwe & Roux, 1974) of the (*E*)-chalcone **7** (Van der Westhuizen, Ferreira & Roux, 1980) afforded the racemic flavanone **8** (Van der Westhuizen, et al., 1980) which was reduced by sodium borohydride (Hatano, et al., 1997; Malan, et al., 1997) to give the flavan-4-ol **9** as a mixture of the two diastereomeric pairs. Treatment of this mixture with optically pure tetra-*O*-methylepicatechin **10** using titanium tetrachloride in dichloromethane as Lewis acid (Kawamoto, Nakatsubo & Murakami, 1991), afforded a mixture of dimeric compounds which was resolved by PLC to give three probutinin-type dimers. Acetylation afforded the permethylaryl ether acetates **2**, **4** and **12** of which compounds **2** and **4** were identical to the same derivatives of the natural products **1** and **3** by comparison of their ^{1}H NMR and CD data. These compounds are hence butiniflavan-(4α → 8)-epicatechin **1**, butiniflavan-(4β → 8)-epicatechin **3**, the first dimeric proanthocyanidins with a flavan top unit that is based on epicatechin as chain terminating moiety. The structure of the remaining diastereomer, i.e. 7,3′,4′-tri-*O*-methyl-*ent*-butiniflavan-(4β → 8)-5,7,3′,4′-tetra-*O*-methyl-3-*O*-acetylepicatechin **12** was established using the same ^{1}H NMR (Table 1) and CD protocol as was described above. Although the signals of the two rotamers in **12** could be assigned at 20°, the aforementioned overlap of C-ring protons again precluded differentiation of the two rotamers, i.e. assigning the absolute configuration of the interflavanyl bond.

Similar treatment of the diastereomeric mixture of flavan-4-ols **9** with penta-*O*-methylepigallocatechin **11** followed by purification and acetylation gave the three permethylaryl ether acetates **6**, **13** and **14**. Derivative **6** displayed identical ^{1}H NMR and CD data compared to those of the same derivative of the natural product **5** hence defining this compound as butiniflavan-(4β → 8)-epigallocatechin, the first dimer with a flavan chain extender unit that is based on epigallocatechin as the bottom unit. The remaining diastereomers, i.e. 7,3′,4′-tri-*O*-methylbutiniflavan-(4α → 8)-5,7,3′,4′,5′-penta-*O*-methyl-3-*O*-acetylepigallocatechin **13** and 7,3′,4′-tri-*O*-methyl-*ent*-butiniflavan-(4β → 8)-5,7,3′,4′,5′-penta-*O*-methyl-3-*O*-acetylepigallocatechin **14** were identified via the ^{1}H NMR (Table 1) and CD methods outlined above, signal overlap of C-ring protons again precluding assignment of absolute configuration of the two rotamers.

Analysis of the ^{1}H NMR data (Table 1) of the butiniflavan derivatives **2**, **4**, **6**, **12**, **13** and **14** and of related dimers (Hatano et al., 1997), indicates that for 2,4-*cis* configuration, e.g. **2**, both 2- and 4-H(C) resonate as double doublets ($^3J_{2,3}$ = ca. 2.0, 12.0 Hz; $^3J_{3,4}$ = ca. 6.0, 12.0 Hz). Analogues with 2,4-*trans* relative configuration, e.g. **4**, show a double doublet for 2-H(C) ($^3J_{2,3}$ = ca. 3.0, 7.0 Hz) and a triplet for 4-H(C) ($^3J_{3,4}$ = ca. 7.0 Hz).

We were unable to identify any (4 → 6)-linked dimers or 2*R*,4*S* (*trans*)-diastereomers in the coupling of the flavan-4-ol diastereomers **9** with the flavan-3-ol derivatives **10** and **11**. The 2*R*,4*S* (*trans*)-diastereomers



Scheme 1. Reagents and conditions: (i) NaOAc, EtOH/H_2O, reflux; (ii) $NaBH_4$, EtOH; (iii) tetra-*O*-methylepicatechin **10**, $TiCl_4$, CH_2Cl_2; (iv) penta-*O*-methylepigallocatechin **11**, $TiCl_4$, CH_2Cl_2; (v) Ac_2O, pyridine.

may have been overlooked due to low concentrations. The apparent preference for (4 → 8) bond formation was also observed in the synthesis of procassinidin-type biflavanoids (Hatano, et al., 1997) and may in our case presumably be attributed to the formation of a 'soft' intermediate electrophile **15** which would then permit regioselective substitution at C-8 of the flavan-3-ols **10** and **11**, i.e. the position where the HOMO displays maximum amplitude (Elliot, Sackwild & Richards, 1982).

3. Experimental

^{1}H NMR spectra were recorded at 300 MHz for solutions in $CDCl_3$ or deuteriobenzene, with TMS as int. standard. FAB-MS were recorded on a VG 70-70E instrument with a VG 11-250J data system and an ion-tech 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) 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 48h at −15°, while acetylations were in Ac_2O-pyridine at ambient temps. Evaporations were done under red. pres. at ambient temps. in a rotary evaporator and freeze drying of aqueous solutions on a Virtis 12SL freezemobile.

3.1. General procedure for the synthesis of probutinidin derivatives

To a dry solution of 7,3′,4′-trimethoxyflavan-4-ol **9** (90.0 mg) in CH_2Cl_2 (20 ml) was added the permethylaryl ethers **10**/**11** of epicatechin/-epigallocatechin (296 mg) and $TiCl_4$ (0.04 ml, 1.2–1.4 equiv.). The mixture was stirred at 0° under N_2 for 60 min and the temperature was allowed to rise to 40° for a further 6 h. An excess of cold H_2O (40 ml) was added and the mixture extracted with Et_2O (3×20 ml). After drying (Na_2SO_4) the ether was removed under vacuum and the mixture was resolved by prep. TLC in MeOH–benzene–Me_2CO (5:2:3).

3.2. Isolation of phenolic compounds

Milled bark (6.3 kg) was repeatedly extracted with Me_2CO (3×7.5 l) for 48 h periods at 25°. The Me_2CO was removed under vacuum at 35° and the residue dissolved in H_2O and freeze dried to give a brown powder (370 g). Two portions (2×25 g) were 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: C_1 (tubes 21–27, 1.571 g), C_2 (28–33, 1.293 g), C_3 (34–42, 0.61 g), C_4 (90–109, 2.394 g), C_5 (110–160, 1.186 g), C_6 (162–281, 1.989 g), C_7 (388–421, 1.980 g), C_8 (422–469, 1.504 g), C_9 (470–505, 1.207 g), C_{10} (506–579, 3.144 g) and C_{11} (580–683, 1.464 g).

3.3. 7,3′,4′-Tri-O-methylbutiniflavan-(4α → 8)-5,7,3′,4′-tetra-O-methyl-3-O-acetylepicatechin 2

A portion (200 mg) of fraction C_4 was methylated and the mixture was separated by prep. TLC in benzene–Me_2CO (8:2) to give five bands at R_f 0.64 (21.8 mg), 0.60 (17.3 mg), 0.51 (10.5 mg), 0.45 (25.2 mg) and 0.36 (13.7 mg). The R_f 0.51 band was acetylated and separated by prep. TLC in dichloroethane–Me_2CO (95:2, ×2) to give compound **2** (R_f 0.39, 5.2 mg) as a *brown amorphous solid*. (Found: M^+, 686.2724. $C_{39}H_{42}O_{11}$ requires M^+, 686.2727); δ_H (Table 1); CD $[\theta]_{284.8}$ −2190, $[\theta]_{273.1}$ 3877, $[\theta]_{244.7}$ −16640 and $[\theta]_{236.1}$ 1660.

The remaining bands contain related proanthocyanidin-type compounds which will be dealt with elsewhere.

3.4. 7,3′,4′-Tri-O-methylbutiniflavan-(4β → 8)-5,7,3′,4′-tetra-O-methyl-3-O-acetylepicatechin 4

Methylation of a portion (200 mg) of fraction C_3 followed by prep. TLC in benzene–Me_2CO (8:2) gave three bands at R_f 0.65 (47.3 mg), 0.48 (52.2 mg) and 0.32 (44.8 mg). Acetylation of the R_f 0.48 band followed by prep. TLC in benzene–Me_2CO (8:2) gave a prominent band at R_f 0.54 (26.7 mg) which was further purified by prep. TLC in benzene–EtOAc–Me_2CO (21:3:1, ×2) to give derivative **4** (R_f 0.29, 3.9 mg) as a *light-brown amorphous solid*. (Found M^+, 686.2725. $C_{39}H_{42}O_{11}$ requires M^+, 686.2727); δ_H (Table 1); CD $[\theta]_{275.4}$ −8029, $[\theta]_{244.6}$ 14050 and $[\theta]_{233.6}$ 2013.

The remaining bands contain related proanthocyanidin-type compounds which will be described elsewhere.

The mixture resulting from the $TiCl_4$ catalyzed coupling of **9** and **10** (Scheme 1) to acquire **2** was separated by prep. TLC in MeOH–benzene–Me_2CO (5:2:3) to give three bands at R_f 0.36 (128.0 mg), 0.31 (48.9 mg) and 0.25 (22.1 mg). The R_f 0.36 band yielded starting material **10**. Acetylation of the R_f 0.31 band followed by prep. TLC in MeOH–benzene–Me_2CO (5:2:3) gave an R_f 0.43 band (36.9 mg) which was further purified by prep. TLC in benzene–EtOAc–Me_2CO (21:3:1, ×2) to give bands at R_f 0.65 (16.1 mg) and 0.51 (14.5 mg).

The latter band yielded a compound with ^1H NMR, CD and MS data identical to those of the natural product derivative **2**. The R_f 0.65 band gave 7,3′,4′-tri-*O*-methyl-*ent*-butiniflavan-(4β → 8)-5,7,3′,4′-tetra-*O*-methyl-3-*O*-acetylepicatechin **12** as a *light-brown solid* (Found: M^+, 686.2723. $C_{39}H_{42}O_{11}$ requires M^+, 686.2727); δ_H (Table 1); CD $[\theta]_{234.8}$ −17, $[\theta]_{237.3}$ −383, $[\theta]_{239.8}$ 8102, $[\theta]_{256.0}$ 36, $[\theta]_{271.8}$ −5765, $[\theta]_{280.1}$ 50 and $[\theta]_{286.1}$ 6729.

Acetylation of the R_f 0.25 band followed by prep. TLC in benzene–EtOAc (13:7, ×4) gave a fraction at R_f 0.63 (8.3 mg) with ^1H NMR, CD and MS data identical to those of the natural product derivative **4**.

3.5. 7,3′,4′-Tri-O-methylbutiniflavan-(4β → 8)-5,7,3′,4′,5′-penta-O-methyl-3-O-acetylepigallocatechin 6

Methylation of a portion (200 mg) of fraction C_5 followed by prep. TLC in benzene–Me_2CO (8:2) gave three bands at R_f 0.61 (26.5 mg), 0.50 (24.9 mg) and 0.36 (18.8 mg). Acetylation of the R_f 0.50 band followed by prep. TLC in toluene–2-butanone (9:1) gave compound **6** (R_f 0.21, 5.3 mg) as a *rustic-brown amorphous solid*. (Found: M^+, 716.2831. $C_{40}H_{44}O_{12}$

requires M^+, 716.2833); δ_H (Table 1); CD $[\theta]_{284.4}$ -10530, $[\theta]_{244.6}$ 47980 and $[\theta]_{230.8}$ 5902.

The diastereomeric mixture obtained from the $TiCl_4$ catalyzed coupling of **9** and **11** (Scheme 1) was separated by prep. TLC in benzene–Me_2CO (9:1, ×2) to give three bands at R_f 0.61 (129 mg), 0.54 (22 mg) and 0.41 (12.3 mg). The R_f 0.61 band yielded starting material **11**. Acetylation of the R_f 0.54 band followed by prep. TLC in benzene–Me_2CO (9:1, ×2) gave two bands at R_f 0.52 (6.6 mg) and 0.43 (4.5 mg). The R_f 0.52 band yielded 7,3′,4′-tri-*O*-methyl-*ent*-butiniflavan-(4β → 8)-5,7,3′,4′,5′-penta-*O*-methyl-3-*O*-acetylepigallo-catechin **14** as a *light-brown amorphous solid.* (Found: M^+, 716.2835. $C_{40}H_{44}O_{12}$ requires M^+, 716.2833); δ_H (Table 1); CD $[\theta]_{238.8}$ -1595, $[\theta]_{240.7}$ 7, $[\theta]_{246.5}$ 9881, $[\theta]_{258.4}$ 22, $[\theta]_{273.1}$ -3481, $[\theta]_{279.5}$ 98, $[\theta]_{285.9}$ 5553 and $[\theta]_{298.9}$ 201.

The R_f 0.43 band was identified as a diastereomer of **14** viz. 7,3′,4′-tri-*O*-methylbutiniflavan-(4α → 8)-5,7,3′,4′,5′-penta-*O*-methyl-3-*O*-acetylepigallocatechin **13**. (Found: M^+, 716.2830. $C_{40}H_{44}O_{12}$ requires M^+, 716.2833); δ_H (Table 1); CD $[\theta]_{232.0}$ 698, $[\theta]_{235.6}$ 1671, $[\theta]_{237.3}$ 103, $[\theta]_{243.7}$ -21880, $[\theta]_{255.1}$ 27, $[\theta]_{274.3}$ 5356 and $[\theta]_{282.9}$ 22.

Acetylation of the R_f 0.41 band followed by prep. TLC in benzene–Me_2CO (9:1, ×2) gave a band at R_f 0.40 with ^1H NMR, CD and MS data identical to those of the natural product derivative **6**.

3.6. 7,3′,4′-Tri-O-methylbutein ***7***

Physical data corresponded to those in the literature (Van der Westhuizen, et al., 1980).

3.7. 7,3′,4′-Trimethoxyflavanone ***8***

Physical data identical to those in the literature (Van der Westhuizen, et al., 1980).

3.8. 7,3′,4′-Trimethoxyflavan-4-ol ***9***

Compound **8** was treated with $NaBH_4$ in EtOH to give the diastereomeric mixture **9** (Hatano, et al., 1997; Malan, et al., 1997). δ_H ($CDCl_3$) δ 7.42 (d, *J* 8.5, H-5), 6.60 (dd, *J* 8.5 and 2.5, H-6), 6.46 (d, *J* 2.5, H-8), 6.90 (d, *J* 8.5, H-5′), 7.01 (dd, *J* 8.5 and 2.5, H-6′), 7.00 (d, *J* 2.5, H-2′), 5.12 (dd, *J* 2.0 and 12.0, H-2), 5.07 (br m, *J* 6.0 and 12.0, H-4), 2.50 (ddd, *J* 2.0, 6.0 and 13.0, H-3_{ax}), 2.16 (ddd, *J* 12.0, 12.0 and 13.0, H-3_{eq}), 3.93, 3.91 and 3.78 (3 × s, OMe).

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