Phytochemistry, Vol. 30, No. 12, pp. 4129–4135, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

DIMERIC AND TRIMERIC PROANTHOCYANIDINS POSSESSING A DOUBLY LINKED STRUCTURE FROM *PAVETTA OWARIENSIS**

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(*Received* 15 *April* 1991)

Key Word Index—*Pavetta owariensis*; Rubiaceae; stem bark; proanthocyanidins; doubly linked structures; dimer; trimers; thiolytic degradation.

Abstract—Pavetannin A-2, a new A-type proanthocyanidin, along with the trimers cinnamtannin B-1, pavetannin B-1, B-2, B-3, B-5 and B-6 have been isolated in their free phenolic form from the stem bark of *Pavetta owariensis*. Spectral data and partial acid-catalysed degradation established their structures as *ent*-epicatechin-(4α→8,2α→*O*→7)-catechin, epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4α→8)-epicatechin, epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4β→8)-*ent*-epicatechin, epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4β→8)-epicatechin, epicatechin-(4β→6,2β→*O*→7)-epicatechin-(4β→8)-epicatechin, epicatechin-(4β→6,2β→*O*→7)-catechin-(4β→8)-epicatechin, epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4α→8)-catechin, respectively.

INTRODUCTION

We previously reported the isolation of new antimicrobially active dimeric, trimeric and tetrameric proanthocyanidins with doubly linked structures from the bark of *Pavetta owariensis* [2]. In addition, the occurrence of catechin, epicatechin, *ent*-epicatechin, proanthocyanidin A-2, proanthocyanidin A-4 and pavetannin A-1 has been demonstrated [1]. Continued chemical investigations on the biologically active components of the title plant has resulted in the isolation of another A-type proanthocyanidin and six trimeric proanthocyanidins, all possessing a doubly linked structure.

RESULTS AND DISCUSSION

The proanthocyanidin fractions obtained by droplet counter current chromatography (DCCC) were subjected to chromatography on Sephadex LH-20 with ethanol [1]. Compounds **1**–**7** were obtained.

Compound **1** was characterized from a mixture (**1** and **8**) which was obtained in a yield of *ca* 0.002% as a brown amorphous powder. On TLC (EtOAc–HCO_2H–HOAc–H_2O; 140:2:1:59; upper phase; solvent 1), the components **1** and **8** were visualized at R_f 0.56 and 0.50 respectively, but efforts to separate these distinct bands by repeated chromatography on Sephadex LH-20 have hitherto failed. The FAB mass spectrum of the mixture indicated a $[M+H]^+$ ion at *m/z* 577, consistent with a biflavanoid moiety [3]. The ^{1}H NMR spectrum revealed the presence of two structurally related components in a ratio of 1:1.5. Due to the unequal intensities of the ^{1}H NMR signals, the resonances characteristic of each molecule were readily assigned. Compound **8**, R_f 0.56, was identified as proanthocyanidin A-2 by chromatographic and spectroscopic (^{1}H NMR) comparisons with authentic data [1]. The ^{1}H NMR spectrum obtained in acetone-d_6 of the second compound (**1**) was similar to those of both pavetannin A-1 (**9**) and proanthocyanidin A-4 (**10**) [1]. However, **1** differed from **9** and **10** with respect to the heterocyclic C and F ring signals. The appearance of a doublet ($J=8$ Hz) at δ4.57 due to H-2(F) was reminiscent of the presence of a 'lower' 2,3 *trans* flavanyl unit. The striking resemblance of the chemical shift of H-4(C) in the ^{1}H NMR spectra of **1** and **9**, δ4.38 and 4.34 respectively, which may be taken as indication of a quasiequatorial orientation of the C-4 substituent, when compared with the analogous proton (δ4.27) of the 4β-configuration **10** is noteworthy. As the chemical shifts of the A and D ring protons were close to those of pavetannin A-1 (**9**) and proanthocyanidin A-4 (**10**), a (4→8)-interflavanyl linkage is suggested.

In the ^{13}C NMR spectrum, the doubly linked structure of **1** was evident from the characteristic ketal carbon resonance at δ105.0. Other features of diagnostic value included the five aliphatic carbon signals in the high field region, of which three are oxygen bearing methines: C-3(C) at δ67.1, C-2(F) at δ83.1 and C-3(F) at δ67.7. The simultaneous presence of ^{13}C NMR signals at δ83.1 and δ67.1, attributable to C-2(F) and C-3(F) respectively, indicated a catechin 'terminal' unit. The C-3(C) signal at δ67.7 was close to that of both **9** and **10**, giving support for epicatechin as the 'upper' unit. The unsubstituted carbon signals at δ95.7 for C-6(D) and C-8(A), and δ98.4 for C-6(A) were in agreement with the C-4/C-8 nature of the interflavanoid linkage. From these considerations, **1** is *ent*-epicatechin-(4α→8,2α→*O*→7)-catechin and designated as pavetannin A-2.

*Part 2 in the series 'Proanthocyanidins from stem bark of *Pavetta owariensis*'. For Part 1, see ref. [2].

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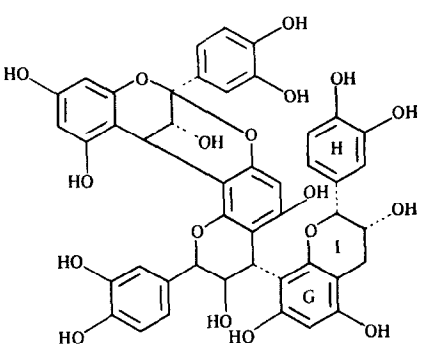

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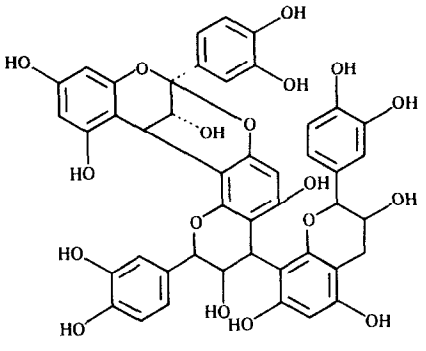

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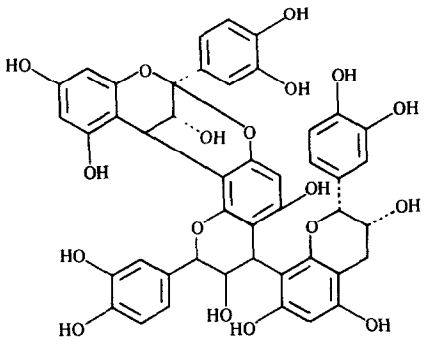

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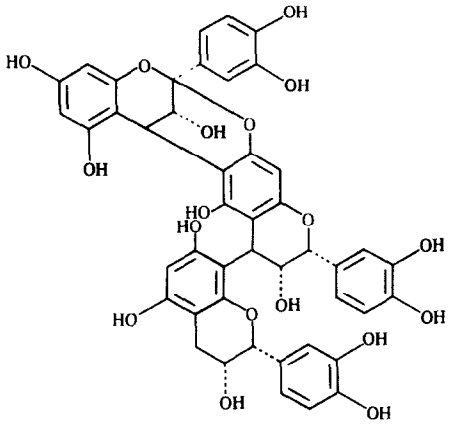

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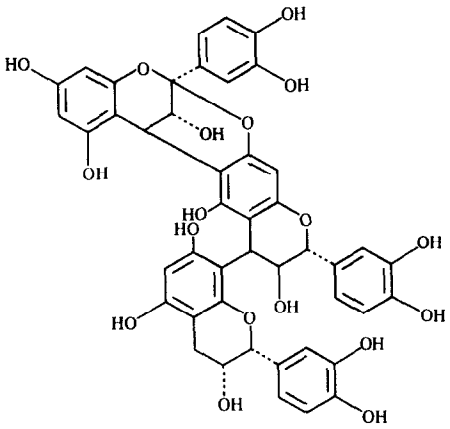

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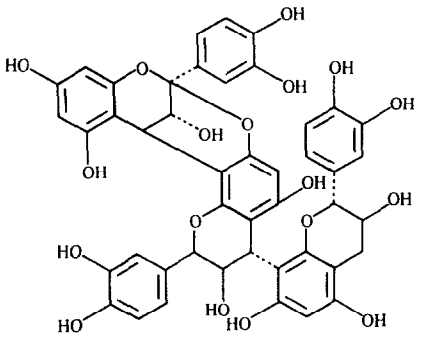

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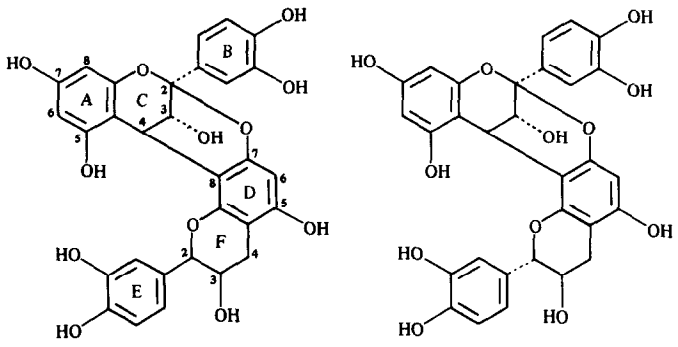
Compound **2**, responding positively to the vanillin–sulphuric and anisaldehyde–sulphuric reagents, was obtained in a yield of 0.080%. On TLC (solvent 1), **2** showed a R_f value at 0.29. In the FAB mass spectrum, a $[M+H]^+$ ion was detected at *m/z* 865, which suggested a triflavanoid moiety [4]. The ^1H NMR spectrum of **2** [in CD_3OD or $(CD_3)_2CO$] revealed the familiar presence of the effects of dynamic rotational isomerism at ambient temperatures [5, 6] and indicated two predominant rotameric forms. The ^1H and ^{13}C NMR (Table 1) spectra of **2** were indistinguishable from those of authentic cinnamtannin B-1, hence confirming the identity of the two compounds. Supporting evidence was available from the ^1H and ^{13}C NMR spectra of the acetylated derivative **2a** and the corresponding derivative of a reference sample which were virtually superimposable. Accordingly, the structure of compound **2** was unequivocally established as epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4α→8)-epicatechin, previously isolated from the bark of *Cinnamomum zeylanicum* (Lauraceae) by Nonaka and coworkers [7].

Compound **3**, R_f 0.28 (solvent 1), was isolated as a light brown amorphous powder in a yield of 0.135% and displayed similar colour response to the above spray reagents. The FAB mass spectrum of **3** indicated a $[M+H]^+$ ion at *m/z* 865 corresponding to a triflavanoid structure. Again, at room temperature, the ^1H NMR spectrum of **3** [in CD_3OD or $(CD_3)_2CO$], plagued by the adverse effects of dynamic rotational isomerism, indicated the presence of two predominant conformational isomers, the relative populations of the two states being 2:1 (in $(CD_3)_2CO$) and 7:1 (in CD_3OD) as judged from



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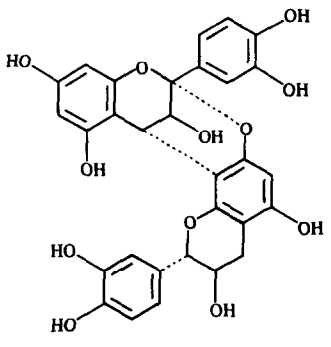

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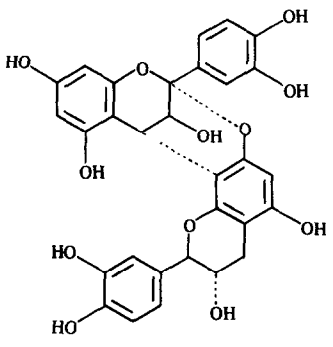

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Table 1. ^{13}C NMR spectral data for procyanidins **1–7** from *P. owariensis* (50.10 MHz; CD_3OD; δ-values)

Ring no.	C	(in Me_2CO-d_6) **1**	**2**	**3**	**4**	**5**	**6**	**7**
C	2	105.0	104.9	104.8	104.8	103.9	104.1	104.2
	3	67.1	67.5	67.5	67.4	67.3	67.3	66.9
	4	—	28.9	28.9	28.8	28.7	28.7	28.7
F	2	83.1	78.9	78.7	78.7	78.7	84.3	79.5
	3	67.7	72.6	72.5	72.5	72.3	73.0	73.0
	4	—	38.3	38.1	38.1	38.1	38.1	38.1
I	2		80.3	80.0	80.2	80.1	79.5	84.3
	3		67.3	67.2	67.2	67.3	66.9	68.1
	4		29.9	29.8	29.8	29.7	29.7	29.7
A	6	98.4	98.3	98.2	98.4	98.2	98.2	98.2
	8	95.7	96.6	96.6	96.6	96.4	96.4	97.2
D	6	95.7	96.6	96.6	96.6	106.93	107.45	97.2
	8	106.3	106.7	106.5	106.7	96.0	96.0	107.4
G	6		96.1	96.2	96.2	96.4	96.4	96.4
	8		106.5	106.2	106.4	106.5	106.2	106.9

—Overlapped with the solvent signal.

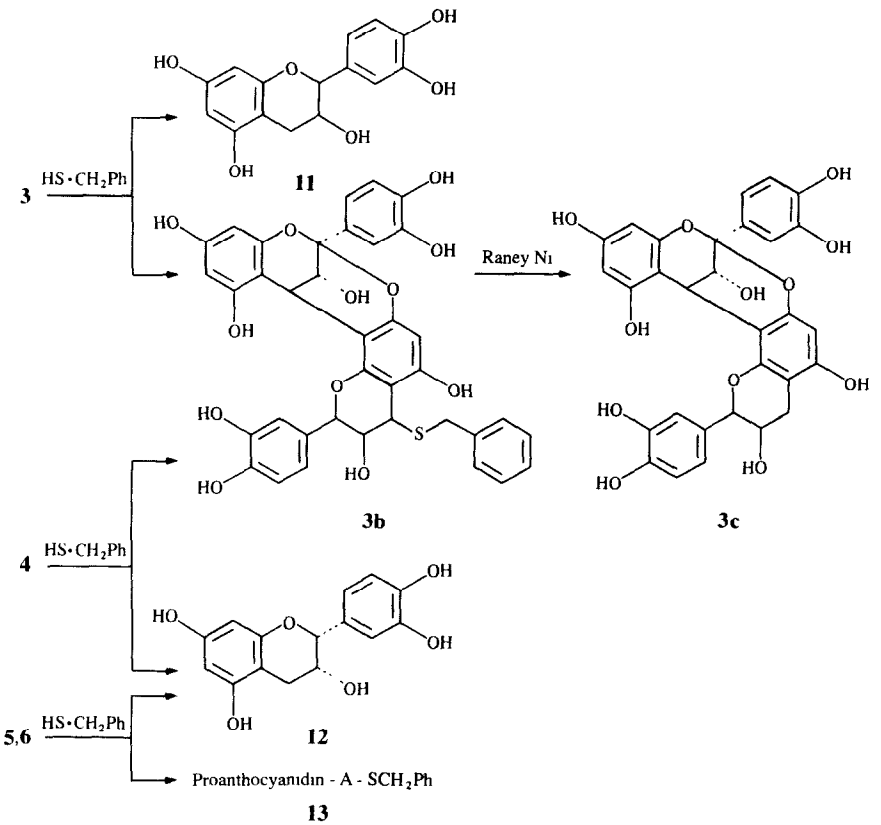
signal intensities. Structural similarity of **3** and cinnamtannin B-1 (**2**) became evident from comparison of their ^1H and ^{13}C NMR data. Notable differences, however, included slight shielding of C-4(F) and C-2(F) ($\Delta\delta -0.2$ in each instance) and of C-2(I) ($\Delta\delta -0.3$) in the ^{13}C NMR spectrum of **3** relative to that of **2**. The ^{13}C NMR spectrum (CD_3OD; ambient temperature; Table 1) exhibited three carbon signals at δ67.2, 67.4 and 72.5 corresponding to C-3 of the C, I and F heterocyclic rings [7, 8]. The presence of two epicatechin units clearly followed from the flavan C-2 signals at δ78.7 and 80.2 [9, 10]. Proof for the presence of an A-type unit was obtained from the Spin-Echo Fourier Transform (SEFT) ^{13}C NMR spectrum which displayed the characteristic

ketal carbon at δ104.8. The ^{1}H and ^{13}C NMR spectra of the acetate **3a**, recorded at ambient temperatures, were exceedingly complex, thus rendering their interpretation extremely difficult. The sequence of the flavanoid units in the trimeric chain was established by partial thiolytic degradation (Scheme 1) [11]. Thus, treatment of **3** with benzylthiol/acetic acid in ethanol yielded an *ent*-epicatechin (**11**) 'liberated' from the 'lower' terminal unit and identified by co-chromatography with an authentic reference (R_f 0.24; TLC analysis on cellulose with water), and 4-benzylthioproanthocyanidin **3b** derived from the 'upper' part. The FAB mass spectrum of **3b** showed a $[M+H]^+$ at *m/z* 699 and a fragment ion peak at *m/z* 575, corresponding to the loss of $C_6H_5CH_2SH$. Structural assesment of **3b** was effected by ^{1}H and ^{13}C NMR analysis and its conversion into **3c** by treatment with Raneynickel. On TLC (solvent 1), **3c** showed the same R_f value (0.57) as proanthocyanidin A-2 (**8**). Confirmation of the structural identity of **3c** and **8** was provided by comparison of their ^{1}H NMR data. Conjecture regarding the (4→8)-interflavanyl bonds of **3** followed tentatively from the general resemblance of the ^{1}H and ^{13}C NMR data of **3** and **2**. Supporting evidence, however, was available from the diagnostic chemical shifts of H-2(F) and H-2(I) located to relatively low magnetic field strength (δ5.05 and 5.27, respectively) [12]. Notable in the ^{13}C NMR spectrum of **3** was the conspicuous shielding of C-2(I) (δ80.0) relative to the same carbon in **2** (δ80.3). Such a feature is apparently characteristic of a 'lower' terminal *ent*-epicatechin unit. The strong positive Cotton effect at 237 nm in the CD curve of **3** was consistent with a 4β-flavanyl unit as depicted in the structure [5]. Thus, **3** is epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4β→8)-*ent*-epicatechin, designated as pavetannin B-1.

Compound **4**, R_f 0.27 (solvent 1), was obtained in a yield of 0.053% as a light brown amorphous powder. The FAB mass spectrum of **4** indicated a $[M+H]^+$ ion at *m/z* 865 and a fragmentation pattern identical to that of **2** and **3**. The ^{1}H and ^{13}C NMR spectra of **4** and **2** were superimposable, revealing their close structural resemblance. In addition, the ^{1}H and ^{13}C NMR spectra of the acetylated derivative **4a** were almost indistinguishable from those of **2a** and **3a**. Treatment of **4** with benzylthiol–acetic acid yielded epicatechin (**12**) and again 4-benzylthioprocyanidin **3b**, which, on desulphurization with Raney nickel produced **3c** (Scheme 1). Comparison of the physical data of the thioethers obtained from the thiolysis of **4** and **3**, confirmed the identity of the sulphur-containin products.

The interflavanoid linkage between the A-type unit and the 'lower' terminal epicatechin moiety was assumed to be C-4/C-8 based on the ^{13}C NMR chemical shifts of C-4(F), C-6 and C-8(D), which were identical to those of **3** (Table 1). The positive sign of the Cotton effect in the diagnostic wavelength region (220–240 nm) of the CD spectrum of the trimer **4** unequivocally indicated a β position of C-4 flavanyl substituents. Accordingly, the trimer **4**, designated as pavetannin B-2, was identified as epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4β→8)-epicatechin.

Compounds **5** and **6** were obtained as an inseparable



Scheme 1. Partial thiolytic degradation of compound **3**.

mixture in a yield of 0.006%. On TLC (solvent 1), both phenols migrated as a single band with R_f 0.25. The trimeric structure of **5** and **6** was indicated by the FAB mass spectrum data, showing a $[M+H]^+$ ion at *m/z* 865. Whereas the ^{1}H NMR spectrum of the mixture (in Me_2CO-d_6 or CD_3OD) was too complex to permit spectral interpretation, the presence of two structural isomers was evident from the SEFT-^{13}C NMR spectrum (in CD_3OD). Owing to inequal intensities (relative ratio, 1:3) the signals arising from each compound could be allocated (Table 1). The ^{13}C NMR data of the minor compound (**5**) exhibited a signal at δ103.9 due to a ketal carbon, along with C-2 signals at δ80.1 and 78.7 and C-3 signals at δ67.3 and 72.3, correlating with the presence of epicatechin units. Comparison of the ^{13}C NMR data of **5** and pavetannin B-1 (**3**) (Table 1) revealed their close structural resemblance. However, notable differences between their spectra included conspicuous deshielding of C-6(D) ($\Delta\delta -10.3$) and shielding of C-2(C) ($\Delta\delta -0.9$) and C-8(D) ($\Delta\delta -10.5$) in **5** relative to **3**, suggesting a (4→6)-interflavanyl linkage. A diagnostic feature in the ^{13}C NMR data of the major compound (**6**) was the presence of signals at δ84.3 and 73.0, attributable to C-2 and C-3 of ring F, respectively, characteristic of a catechin unit. These chemical shifts were similar to those of pavetannin A-2 (**1**). Apart from this, the C-6 and C-8(D) carbon signals of **6** were closer to those of **5** than to those of **2**–**4**, indicating the same mode of bonding. The positive sign of the Cotton effect in the diagnostic wavelength region (220–240 nm) of the CD spectrum of the mixture (**5** and **6**) suggested a 4β-flavanyl substituent for both compounds. Additional structural information was sought via chemical degradation. Partial thiolytic cleavage of the free phenolic mixture yielded an A-type benzylthioproanthocyanidin (**13**), which showed a $[M+H]^+$ ion at *m/z* 699, in addition to epicatechin (**12**) (Scheme 1). Owing to the low quantity of sample, the structure of the thioether (**13**) could not be established unambiguously. On acetylation the mixture gave two products, **5a** and **6a**, with R_f values at 0.35 and 0.37, respectively (toluene–Me_2CO; 2:1; solvent 2). The ^{13}C NMR spectrum displayed duplicated ketal carbons at δ97.32, 97.62, 97.89 and 98.10, corresponding to rotameric forms of two A-type proanthocyanidins. Notable were the shifts of H-4(F) at δ4.19, 4.38, 4.45 and 4.53 (J = 4.0 Hz) reminiscent of those of the acetates of epicatechin-(4β→6)-epicatechin (δ4.15 and 4.32) and epicatechin-(4β→6)-catechin (δ4.35 and 4.45) [13]. The terminal unit of **5a** and **6a** was assumed to be (−)-epicatechin, because the C-4(I) carbon resonances of the acetylated mixture (δ26.2, 29.69 and 26.3, 29.71, respectively) were closer to those of **2a** (δ26.1 and 29.6) and **4a** (δ26.5 and 29.7) than to those of **3a** (δ26.2 and 26.3) characterized by an *ent*-epicatechin 'terminal' moiety. This was consistent with thiolytic studies (see above). Upon consideration of the above results, compounds **5** and **6**, designated as pavetannin B-3 and B-5, were tentatively characterized as epicatechin-(4β→6,2β→*O*→7)-epicatechin-(4β→8)-epicatechin, and epicatechin-(4β→6,2β→*O*→7)-catechin-(4β→8)-epicatechin, respectively.

The structure of pavetannin B-4, a minor trimer, was tentatively established from the NMR data of an acetylated mixture. Its characterization as well as the assignment of both proton and carbon resonances of the acetylated derivatives **2a**–**6a** by means of 2D-NMR will be the subject of a separate paper.

Compound **7**, present in low concentrations was identified on the basis of NMR data of a chromatographically homogeneous mixture, comprising the compounds **3** and **7** in a yield of 0.013%. The ^{13}C NMR spectrum suggested the presence of two trimeric proanthocyanidins (relative ratio, 1:7), each containing an A-type structure. The signals arising from the major compound were superimposable with those of **3**, which as such was identified as pavetannin B-1. The ^{13}C NMR data of the minor compound (**7**) included a signal at δ104.1 due to a ketal carbon, along with signals at δ79.5 and 84.3, corresponding to epicatechin and catechin C-2 signals, respectively. Deshielding of C-3 (I) (δ68.1) relative to the same carbons in **2**–**6** (Table 1), when taken in conjunction with the C-2 signal at δ84.3, indicated a 'terminal' catechin unit. The 'upper' unit was characterized as proanthocyanidin A-2 by comparison of the heterocyclic carbon resonances of **7** with those of **2**–**5**. The relative configuration of the interflavanoid linkage between the A-2 and catechin unit could not be determined unequivocally on the basis of circular dichroism, as the positive Cotton effect in the CD spectrum does not necessarily reflect a 4β-flavanyl moiety in the presence of the dominating compound **3**. However, the 4α arrangement was established on the basis of the C-2(F) signal of **7** [14]. Thus, **7**, designated as pavetannin B-6, was identified as epicatechin-(4β→8,2β→*O*→7)-epicatechin-(4α→8)-catechin.

The present study reports for the first time on the occurrence in relatively high yields of a series of trimeric proanthocyanidins possessing doubly linked flavanyl units within the Rubiaceae. The isolation of these compounds extends the range of natural proanthocyanidins possessing an A-type unit. On the other hand, it is interesting from a biosynthetic point of view that the bark of *P. owariensis* essentially contains dimeric and trimeric proanthocyanidins with a doubly linked structure, assumed to be derived from singly linked precursors [15]. The conspicuous absence of the B-type proanthocyanidins in this plant source contrasts with the co-occurrence of both types in most previous reports from higher plant sources. The reason for an immediate oxidative transformation of B- to A-type proanthocyanidins remains obscure.

EXPERIMENTAL

General and isolation [1]. *Thiolytic degradation.* A mixt. of proanthocyanidin (150 mg), benzylmercaptan (1.5 ml) and HOAc (1.5 ml) in EtOH (10 ml) was refluxed for 12 hr with stirring. The reaction mixt. was concd under red. pres., and the oily residue chromatographed over Sephadex LH-20 (EtOH), affording a thioether, a flavan-3-ol or proanthocyanidin, and other unknown coloured compounds.

Desulphurization of the thioether derivative. The thioether (10 mg) in 1 ml EtOH–HOAc (9:1) was shaken at 50° with Raney nickel (W-4) (10 drops) for 60 min. After removal of the catalyst by filtration, the filtrate was concd under red. pres. The residue was purified by Sephadex LH-20 chromatography (60% aq. MeOH) to give the free proanthocyanidin.

Pavetannin A-2 (**1**). Amorphous solid; R_f 0.50 (solvent 1), λ_{max}^{EtOH} 279 nm; FAB-MS: *m/z* 599 $[M+Na]^+$, 577 $[M+H]^+$, 425, 407 and 287; ^{1}H NMR (199.50 MHz; Me_2CO-d_6): δ4.00 [*m*, 1H, H-3(F)], 4.20 [*d*, J = 4 Hz, H-3(C)], 4.38 [*m*, H-4(C)], 4.57 [*d*, J = 8 Hz, H-2], 6.00 [*d*, J = 2 Hz, H-6(A)], 6.09 [*d*, J = 2 Hz, H-8 (A)], 6.13 [1H, *s*, H-6(D)], 6.77–7.13 (6H, B and E-ring protons); ^{13}C NMR (50.10 MHz; Me_2CO-d_6): C-4(F) overlapped with the

solvent signal; δ67.1 [C-3(C)], 67.7 [C-3(F)], 83.1 [C-2(F)], 95.7 [C-8(A)], 106.3 [C-8(D)], 98.4 [C-6(A)], 95.7 [C-6(D)], 105.0 [C-2(C)], 102.3 [C-4a(A)], 100.8 [C-4a(D)], 151.4, 153.5, 155.1, 155.6, 156.8 and 157.3 [C-5, C-7 and C-8a (A and D)], 115.0, 115.4, 119.7 and 122.3 [C-2, C-5 and C-6 (B and E)], 130.5 and 131.8 [C-1 (B and E)], 144.4 and 144.7 [C-3 (B and E)], 145.4 and 145.6 [C-4 (B and E)].

Cinnamtannin B-1 (**2**). Amorphous solid (400 mg); R_f 0.29 (solvent 1); λ_{max}^{EtOH} 280 nm; FAB-MS: *m/z* 887 $[M+Na]^+$, 865 $[M+H]^+$, 713, 575, 533, 369, 337, 287. ^{1}H NMR (199.50 MHz; CD_3OD): δ2.83 [2H, *m*, H-4(I)], 3.96–4.55 (heterocyclic protons), 5.69–6.09 (A, E and G-ring protons), 6.73–7.31 (B, E and H-ring protons). ^{13}C NMR (see Table 1). Acetylation of **2** (27 mg) gave an amorphous compound **2a** (20 mg); R_f 0.51 (solvent 2); FAB-MS: *m/z* 1453 $[M+H]^+$; IR ν^{KBr} cm^{-1}: 1770, 1625, 1610, 1510, 1480, 1440, 1375, 1250, 1180, 1050, 1020, 900, 840, 800; ^{1}H NMR (199.50 MHz; $CDCl_3$) δ1.25–2.32 ($MeCO_2$), 2.90–3.09 [*m*, H-4(I)]; C, F, and I-ring protons: δ4.31 (2H, *m*), 4.79 (1H, *br s*), 5.00 (2H, *m*), 5.21 (3H, *m*), 5.43 (2H, *m*), 5.53 (1H, *m*), 5.71 (1H, *m*); A, D and G-ring protons: δ6.25 (1H, *s*), 6.43 (1H, *d*, *J* = 2 Hz), 6.52 (1H, *d*, *J* = 2 Hz), 6.58 (1H, *s*), 6.59 (1H, *s*), 6.61 (1H, *s*), 6.72 (1H, *d*, *J* = 2 Hz), 6.85 (1H, *d*, *J* = 2 Hz); B, E, and H-ring protons: δ7.07–7.55 (9H, *m*); ^{13}C NMR (50.10 MHz; $CDCl_3$): δ19.38–21.08 ($MeCO_2$–) 26.1 and 29.6 [C-4(I)], 27.3, 33.4 and 33.6 [C-4 (C and F)]; 66.3, 67.8, 69.7 and 70.4 [C-3 (C, F and I)]; 75.6 and 75.7 [C-2 (F and I); 97.2 and 98.2 [C-2(C)], 104.2, 104.7, 106.6, 107.1, 110.3 and 110.8 [C-6 and C-8 (A, D and G)]; 121.3, 121.6, 122.7, 122.9, 123.2, 123.6, 124.2, 125.2, 125.4 and 125.8 [C-2, C-5 and C-6 (B, E and H)]; 107.2, 108.1, 108.4, 108.7, 109.8, 110.9, 112.9, 114.1, 116.6, 117.3, 134.7, 135.3, 141.7, 142.0, 142.4, 142.7, 142.9, 147.6, 147.7, 148.0, 148.7, 149.5, 150.1, 151.8, 153.3, 153.7, and 153.9 [C-4a, C-5, C-7 and C-8a (A, D and G); C-8 (D and G); C-1, C-3 and C-4 (B, E and H)]; 167.8–170.3 ($MeCO_2$–).

Pavetannin B-1 (**3**). Brown amorphous solid (675 mg); R_f 0.28 (solvent 1); FAB-MS *m/z* 887 $[M+Na]^+$, 865 $[M+H]^+$, 713, 575, 533, 369, 337, 287; CD $[\theta]_{288}$O, $[\theta]_{288}$ −9030, $[\theta]_{262}$O, $[\theta]_{237}$ +41 270, $[\theta]_{215}$O; ^{1}H NMR (199.50 MHz; CD_3OD): δ2.83 [*m*, 2H, H-4(I)], 3.96–4.55 (heterocyclic protons), 5.71–6.12 (A, E and G-ring protons), 6.75–7.33 (B, E and H-ring protons). ^{13}C NMR (see Table 1); acetylation treatment of **3** (100 mg) with pyridine–Ac_2O (overnight) yielded 90 mg **3a**, an amorphous compound; R_f 0.49 (solvent 2); IR ν^{KBr} cm^{-1}: 1780, 1625, 1610, 1510, 1485, 1440, 1380, 1240, 1185, 1110, 1050, 1020, 900, 845, 800; CD $[\theta]_{234}$ +53 864, $[\theta]_{265}$ +27 323, $[\theta]_{283}$ −4293; FAB-MS *m/z* 1453 $[M+H]^+$; ^{1}H NMR (199.50 MHz; $CDCl_3$) δ1.25–2.29 ($MeCO_2$); δ2.90–2.98 [*m*, H-4(I)]; C, F and I-ring protons: δ4.31 (2H, *m*), 4.63 (1H, *d*, *J* = 4 Hz), 4.79 (1H, *br s*), 4.99 (1H, *d*, *J* = 4 Hz), 5.02 (1H, *d*, *J* = 4 Hz), 5.20 (2H, *m*), 5.37 (1H, *m*), 5.42 (1H, *br s*), 5.53 (1H, *m*), 5.71 (1H, *m*); A, D, and G-ring protons: δ6.25 (1H, *s*), 6.43 (1H, *d*, *J* = 2 Hz), 6.52 (1H, *d*, *J* = 2 Hz), 6.58 (1H, *s*), 6.59 (1H, *s*), 6.62 (1H, *s*), 6.72 (1H, *d*, *J* = 2 Hz), 6.85 (1H, *d*, *J* = 2 Hz); B, E and H-ring protons: δ7.07–7.55; ^{13}C NMR (50.10 MHz; $CDCl_3$): δ19.4–21.0 ($MeCO_2$–); δ26.1[C-4(I)]; δ27.4 and 33.5 [C-4 (C and F)]; δ66.3, 66.7, 67.8, 69.7 and 70.3 [C-3 (C, F and I)]; δ75.3, 75.5 [C-2 (F and I)]; δ97.8 and 98.3 [C-2(C)]; δ104.2, 104.7, 106.7, 107.1, 109.9, 110.3, 110.8 and 110.9 [C-6 and C-8 (A, D and G)]; C-2, C-5 and C-6 (B, E and F): (see 2a) δ107.2, 108.1, 108.4, 108.7, 109.8, 110.9, 112.9, 113.9, 116.6, 117.7, 134.7, 135.3, 141.3, 141.7, 142.0, 142.4, 142.8, 147.7, 148.0, 148.5, 149.5, 150.1, 151.8, 153.3 and 153.9 [C-4a, C-5, C-7 and C-8a (A, D and G); C-8 (D and G); C-1, C-3 and C-4 (B, E and H)]; δ167.8, 168.3, 168.7, 168.9, 169.2, 169.6, 169.9, 170.0 and 170.3 ($MeCO_2$–). Reaction of **3** (155 mg) with benzylthiol gave epicatechin (8 mg) and **3b** (17 mg).

4-Benzylthioproanthocyanidin-A2 (**3b**). FAB-MS *m/z* 721 $[M+Na]^+$, 699 $[M+H]^+$, 575 $[MH-124]^+$, 557, 547, 287. ^{1}H NMR (199.50 MHz, Me_2CO-d_6) δ4.04 (2H, *s*, CH_2S), 4.11–4.30 [4H, *m*, H-3 and H-4 (F and C)], 5.33 [1H, *s*, H-2(F)], 5.95 [*d*, *J* = 2 Hz, H-6(A)], 6.04 [*d*, *J* = 2 Hz, H-8(A)], 6.14 [1H, *s*, H-6(D)], 6.80–7.42 (11H, *m*, aromatic protons). ^{13}C NMR (50.10 MHz, Me_2CO-d_6) δ36.8 (CH_2S), 43.6 [C-4(F)], 66.9 [C-3(C)], 69.7 [C-3(F)], 76.1 [C-2(F)], 95.6, 96.5 and 97.5 [C-6 and C-8(A), C-6(D)], 99.3 and 101.7 [C-4a (A and D)], 103.1 [C-2(C)], 106.4 [C-8(D)], 114.7, 115.0, 115.1 and 115.9 [C-2 and C-5 (B and E)], 119.1 and 120.4 [C-6 (B and E)], 127.1, 128.7, 129.2 [C-2, C-3, C-4, C-5 (toluene-thiolyl ring)] 129.8 and 131.6 [C-1 (B and E)], 139.1 [C-1 (toluene-thiolyl ring)], 144.4, 145.1 and 145.6 [C-3, C-4 (B and E)], 150.9, 152.7 and 153.2 [C-5 and C-7 (A and D)], 156.2, 156.4 and 157.3 [C-7 and C-8a (A and D)]. Desulphurization of **3b** (13 mg) with Raney nickel afforded proanthocyanidin A-2 (7 mg) [1].

Pavetannin B-2 (**4**). Brown amorphous solid (265 mg); R_f 0.27 (solvent 1); FAB-MS *m/z* 887 $[M+Na]^+$, 865 $[M+H]^+$; ^{1}H NMR (199.50 MHz; CD_3OD): δ2.83 [2H, *m*, H-4(I)], 3.86–4.55 (heterocyclic protons), 5.71–6.12 (A, E and G-ring protons), 6.75–7.33 (B, E and H-ring protons); ^{13}C NMR (see Table 1); acetylation of **4** (50 mg) gave the acetate derivative **4a** (19 mg); R_f 0.47 (solvent 2); FAB-MS: *m/z* 1453 $[M+H]^+$; IR ν^{KBr} cm^{-1}: 1770, 1625, 1610, 1510, 1480, 1440, 1375, 1250, 1180, 1050, 1020, 900, 840, 800; ^{1}H NMR (199.50 MHz; $CDCl_3$) δ1.25–2.31 ($MeCO_2$), 2.89–2.91 [*m*, H-4(I)]; C, F and I-ring protons: δ4.32 (2H, *m*), 4.62 (*d*, *J* = 4 Hz), 4.79 (1H, *br s*), 5.00 (2H, *m*), 5.20 (3H, *m*), 5.41 (2H, *m*), 5.53 (1H, *m*,), 5.70 (1H, *m*); A, D and G-ring protons: δ6.25 (1H, *s*], 6.43 (1H, *d J* = 2 Hz,), 6.52 (1H, *d*, *J* = 2 Hz), 6.59 (2H, *s*), 6.62 (1H, *s*), 6.72 (1H, *d*, *J* = 2 Hz), 6.84 (1H, *d*, *J* = 2 Hz); B, E and H-ring protons: δ7.07–7.55 (9H, *m*); ^{13}C NMR 50.10 MHz; $CDCl_3$): δ19.5–21.1 ($MeCO_2$), 26.5 and 29.7[C-4(I)], 27.5, 33.5 and 33.7 [C-4 (C and F)]; 66.4, 66.7, 67.9, 69.8 and 70.5 [C-3 (C, F and I)]; 75.6 and 75.7 [C-2 (F and I); 97.8 and 98.3 [C-2(C)], 104.2, 104.8, 106.7, 107.1, 110.3 and 110.8 [C-6 and C-8 (A, D and G)]; 121.4, 121.7, 122.8, 123.0, 123.2, 123.6, 124.3, 125.3, 125.5 and 125.9 [C-2, C-5 and C-6 (B, E and H)]; 107.1, 108.1, 108.4, 108.7, 109.8, 110.9, 112.9, 114.1, 116.6, 117.3, 134.8, 135.4, 141.7, 142.0, 142.4, 142.7, 142.9, 147.6, 147.7, 148.0, 148.7, 149.5, 150.1, 151.8, 153.3, 153.7 and 153.9 [C-4a, C-5, C-7 and C-8a (A, D and G); C-8 (D and G); C-1, C-3 and C-4 (B, E and H)]; 167.8–170.3 ($MeCO_2$–); thiolytic degradation of **4** (50 mg) afforded epicatechin (4 mg) and **3b** (12 mg).

Pavetannin B-3 (**5**) *and B-5* (**6**). Brown amorphous solid (30 mg); R_f 0.25 (solvent 1); FAB-MS *m/z* 887 $[M+Na]^+$, 865 $[M+H]^+$; the ^{1}H NMR spectrum was complicated by conformational isomerism; ^{13}C NMR (see Table 1); CD $[\theta]_{234}$ +99 000, $[\theta]_{265}$ +36 771, $[\theta]_{284}$ −3771; acetylation of the mixt. (12 mg) gave the acetate derivative **5a** and **6a** (8 mg); R_f 0.37 and 0.35 (solvent 2); FAB-MS: *m/z* 1453 $[M+H]^+$; IR ν^{KBr} cm^{-1}: 1770, 1625, 1610, 1510, 1480, 1440, 1375, 1240, 1180, 1115, 1049, 1047, 1020, 900, 840, 800; ^{1}H NMR (600.1 MHz; $CDCl_3$) δ1.25–2.31 ($MeCO_2$–), 2.90–3.07 [*m*, H-4(I)]; C, F, and I-ring protons: δ4.19 (*d*, *J* = 4 Hz), 4.38 (*d*, *J* = 4 Hz), 4.45 (*d*, *J* = 4 Hz), 4.53 (*d*, *J* = 4 Hz), 4.57 (*d*, *J* = 4 Hz), 4.66 (*s*), 4.77 (*s*), 4.86 (*d*, *J* = 4 Hz), 4.89 (*d*, *J* = 4 Hz), 5.16 (*d*, *J* = 4 Hz), 5.20 (*br s*), 5.30 (*d*, *J* = 4 Hz), 5.33 (*m*), 5.41 (*s*), 5.51 (*m*), 5.69 (*m*); A, D and G-ring protons: δ6.28 (*s*), 6.33 (*s*), 6.40 (*s*), 6.48 (*d*, *J* = 2 Hz), 6.51 (*d*, *J* = 2 Hz), 6.57 (*s*), 6.64 (*s*), 6.73 (*s*), 6.85 (*d*, *J* = 2 Hz), 6.88 (*d*, *J* = 2 Hz); B, E and H-ring protons: δ6.98–7.53 (*m*); ^{13}C NMR (150 MHz; $CDCl_3$): δ19.5–21.1 ($MeCO_2$–), 26.22, 26.34, 29.69 and 29.71 [C-4(I)]; 27.19, 27.22, 27.33, 27.37, 30.94, 33.66 and 33.75 [C-4 (C and F)]; 66.29, 66.49, 66.93, 68.03, 69.99 and 70.86 [C-3 (C, F and I)]; 75.57, 75.68, 76.75 [C-2 (F and I); 97.32, 97.62,

97.89 and 98.13 [C-2(C)]; 103.92, 104.14, 104.37, 105.20, 107.24, 107.43, 108.19, 108.97, 109.41, 109.64, 109.89, 109.96, 110.33, 110.89, 110.99, 113.18, 113.27, 116.43, 116.59 and 118.04 [C-4a, C-8a, C-6 and C-8 (A, D and G)]; 121.33, 121.85, 122.19, 122.32, 122.87, 123.00, 123.09, 123.24, 123.39, 123.69, 123.77, 124.34, 124.53, 124.89, 124.99, 125.06, 125.51, 125.91 and 126.18 [C-2, C-5 and C-6 (B, E and H)]; 134.19, 134.38, 134.69, 134.74, 135.51, 135.59, 135.71, 141.17; 141.68, 141.73, 141.77, 141.85, 142.12, 142.19, 142.44, 142.93, 143.25, 147.60, 147.76, 147.84, 148.41, 148.58, 149.59, 149.84, 150.14, 150.66, 151.60, 151.82, 151.87, 151.91, 152.05, 153.76 and 153.84 [C-5 and C-7 (A, D and G); C-8 (D and G); C-1, C-3 and C-4 (B, E and H)]; 167.72–170.52 ($MeCO_2$–). Reaction of the mixt. **5** and **6** (15 mg) with benzylthiol gave epicatechin and an A-type proanthocyanidin (FAB-MS *m/z* 721 $[M+Na]^+$, 699 $[M+H]^+$, 575, 557, 287).

Pavetannin B-5 (**7**). Brown amorphous solid; R_f 0.28 (solvent 1); FAB-MS *m/z* 887 $[M+Na]^+$, 865 $[M+H]^+$; ^{13}C NMR (see Table 1).

Acknowledgements—We are grateful to Dr G. Nonaka (Faculty of Pharmaceutical Sciences, Kyushu University, Japan) for the generous supply of authentic samples of proanthocyanidin A-2 and cinnamtannin B-1. The authors also acknowledge the Belgian National Fund for Scientific Research (N.F.W.O.) for financial support (grants no. 30089.87 and 30019.89) A. M. Baldé is a recipient of a grant from the Belgian General Agency for Development and Co-operation (ABOS). L. A. Pieters is a senior research assistant of the NFWO.

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