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A-TYPE PROANTHOCYANIDINS FROM THE BARK OF *PARAMERIA LAEVIGATA*

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Abstract – Parameritannin A-3, a new tetrameric A-type proanthocyanidin, along with the cinnamtannin B-2, pavetannin C-1 and cinnamtannin D-1 have been isolated from the bark of *Parameria laevigata*. Complete assignments by spectroscopic analysis established their structures as epicatechin - (2□□ O□ 7, 4□□ 6) - epicatechin - (2□□ O□ 7, 4□□ 8) - epicatechin - (4□□ 8) - epicatechin, epicatechin-(4□□ 8)-epicatechin-(2□□ O□ 7, 4□□ 8)-epicatechin-(4□□ 8)-epicatechin, epicatechin-(4□□ 6)-epicatechin-(2□□ O□ 7, 4□□ 8)-epicatechin-(4□□ 8)-epicatechin, epicatechin-(2□□ O□ 7, 4□□ 8)-epicatechin-(4□□ 8)-catechin, respectively.

INTRODUCTION

During our investigation of proanthocyanidins from *Parameria laevigata* Moldenke (Apocynaceae), we have reported the isolation and structural elucidation of dimeric, trimeric and tetrameric proanthocyanidins possessing one or two double interflavonoid (A-type) linkages.[1] Continued chemical investigation of the proanthocyanidins of this plant has resulted in the isolation of another four A-type proanthocyanidins (**1**-**4**) together with epicatechin (**5**) (Figure 1). Among them, **1** was identified as novel A-type proanthocyanidin on the basis of spectroscopic evidence.

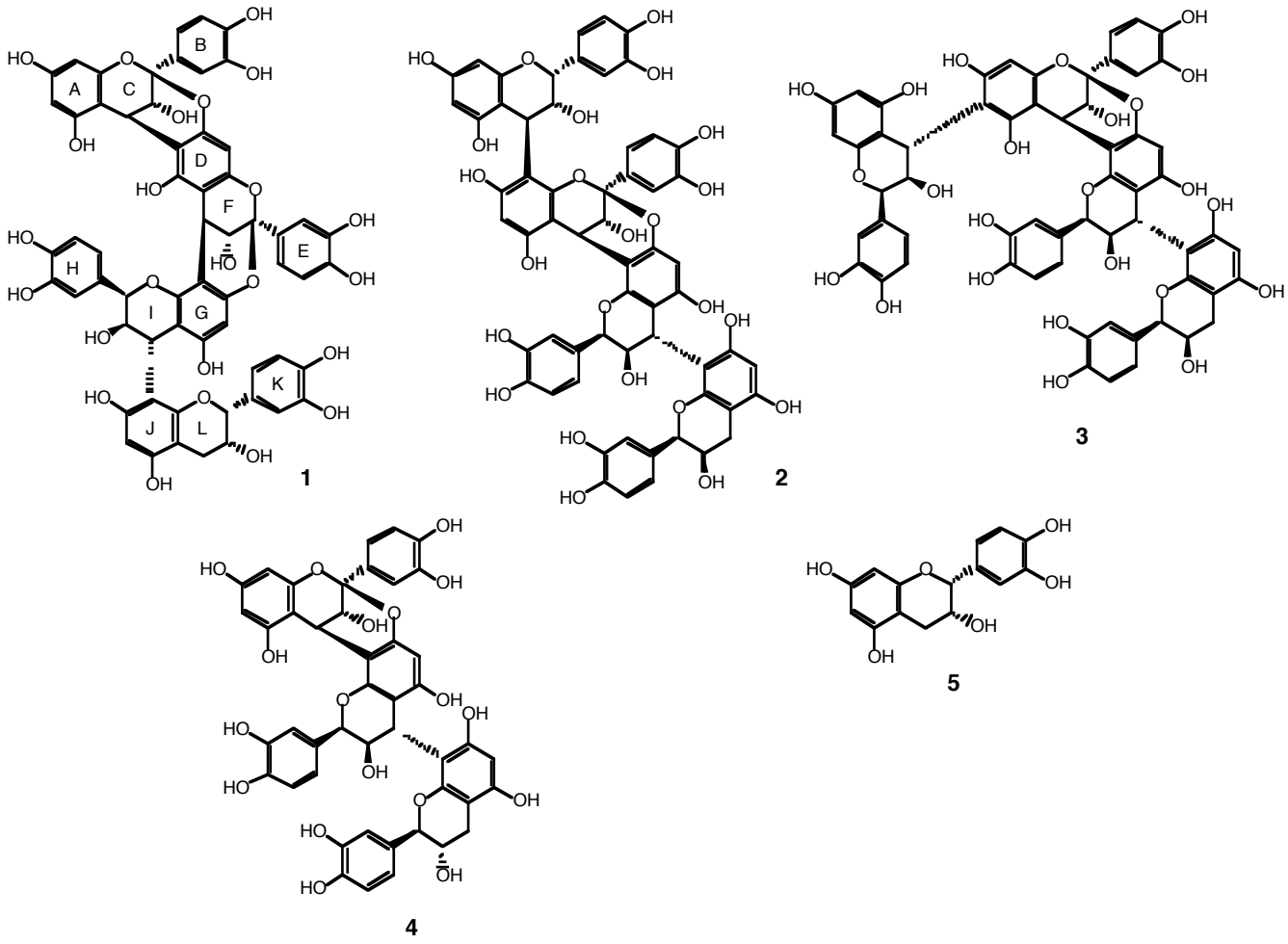

Figure 1. Chemical constituents from the bark of *P. laevigata*

RESULTS AND DISCUSSION

Compound (**1**) was obtained as a pale yellow amorphous powder and was positive to vanillin-sulfuric acid and anisaldehyde-sulfuric acid reagents, indicating it to be a proanthcyanidin. The MALDI-TOF-MS spectrum of **1** showed a principal ion peak at m/z 1173 which was consistent with the sodium ion adduct of a tetrameric proanthcyanidin. The ^{1}H-NMR spectrum indicated the presence of two A-type units from two AB coupling systems attributable to H-3, H-4 [one at 3.95 and 4.18 (each *d*, *J*=3.4 Hz), and the other at 3.25 and 4.21 (each, *d*, *J*=3.6 Hz)]. The presence of these doubly linked structures was supported by the ketal carbon signals at 100.37 and 99.95 in the ^{13}C-NMR spectrum. The presence of four flavanyl units was indicated by the ^{13}C-NMR spectral resonances at 66.98, 67.50, 67.61 and 72.35, each attributable to the C-3 of the flavan framework. In addition, the ^{13}C-NMR spectrum showed two carbon signals at 78.33 and 80.24 due to the C-2 of the I and L heterocyclic rings, respectively. From analysis of aromatic proton resonances, the presence of four 1, 3, 4-trisubstituted aromatic rings was suggested from four ABX coupling systems: [6.92 (*d*, *J*=2.1 Hz), 6.72 (*d*, *J*=8.2 Hz) and 6.75 (*dd*, *J*=8.2,

2.1 Hz)], [δ 7.30 (*d*, *J*=2.0 Hz), 6.84 (*d*, *J*=8.8 Hz) and 7.30 (*dd*, *J*=8.8, 2.0 Hz)], [δ 7.03 (*d*, *J*=2.2 Hz), 6.70 (*d*, *J*=8.3 Hz) and 6.91 (*dd*, *J*=8.3, 2.2 Hz)] and [δ 6.75 (*d*, *J*=1.9 Hz), 6.88 (*d*, *J*=8.2 Hz) and 6.62 (*dd*, *J*=8.2, 1.9 Hz)]. In addition, one set of *meta*-coupled protons at δ 5.91 and 5.93 (each, *d*, *J*=2.3 Hz) and three aromatic singlet protons at δ 5.66, 6.01 and 6.07 were observed. These observations show that **1** is a tetrameric proanthocyanidin consisting only of epicatechin-like units possessing two doubly linked structures. The sequence of each epicatechin-like unit was determined based on COLOC experiments (Figure 2).

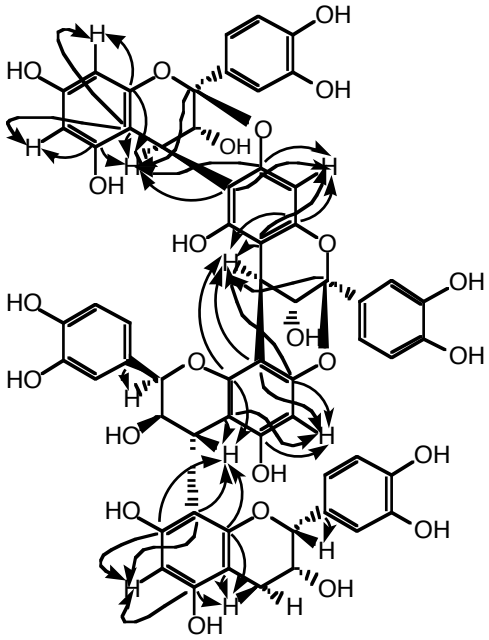

Figure 2 . Long-range correlations from the COLOC spectrum of **1**.

In the COLOC spectrum, the methine proton signal at δ 4.18 (H-4, C-ring) due to the AB coupling system showed a long-range coupling to the quaternary carbon signals at δ 109.39 (C-6, D-ring), and δ 103.94 (C-10, A-ring) which correlated with the *meta*-coupled proton signals at δ 5.91 and 5.93 (H-6 and 8, A-ring). Another AB-coupling proton signal at δ 4.21 (H-4, F-ring) was long-range correlated to the quaternary carbon signals at δ 105.84 (C-8, G-ring), and δ 106.80 (C-10, D-ring) which caused a cross peak with an aromatic singlet proton signal at δ 6.07 (H-8, D-ring). Further correlations were observed between the methine proton signal at δ 4.46 (H-4, I-ring) and the quaternary carbon signals at δ 106.83 (C-10, G-ring) and 108.61 (C-8, J-ring). The interflavonoid linkage between each A-type unit was deduced to be C-4 (C-ring) / C-6 (D-ring) and C-4 (F-ring) / C-8 (G-ring) from reported ^{13}C-NMR chemical shifts.[2] The C-8 chemical shift of the extending unit of A-type proanthocyanidin, the [2→*O*→7, 4→8]-interflavonoid linkage (*ca.* δ 107.0), is distinguished from that of the C-6 [2→*O*→7, 4→6]-interflavonoid linkage (*ca.* δ 108.8).[2] The interflavonoid linkage between the third unit and the lower

epicatechin unit was assumed to be C-4 (I-ring) / C-8 (J-ring), as comparison of the ^{1}H-NMR spectrum of **1** and epicatechin showed the H-2 (L-ring) proton of **1** was shifted upfield by 0.54 ppm with respect to the H-2 proton of epicatechin. This upfield shift resulted from the magnetic anisotropic effect of the G-ring caused when the third unit was linked to the lower unit at the C-8 position (Figure 3).[3]

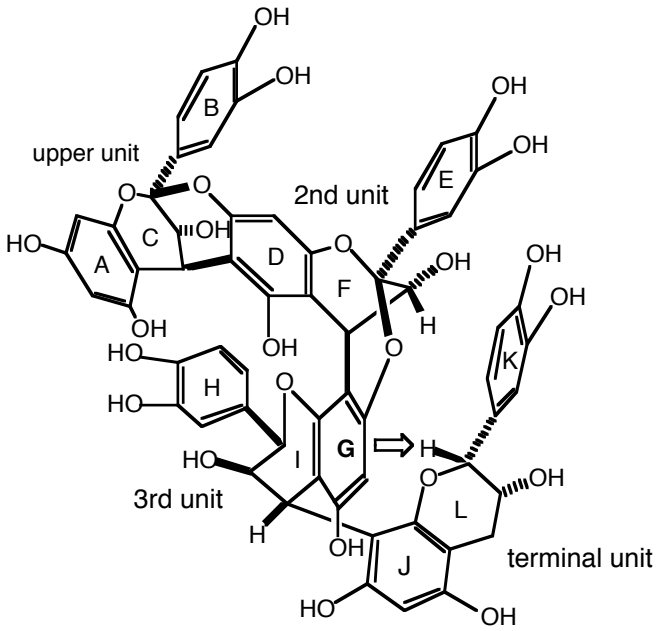

Figure 3. Anisotropic effect of **1**.

The stereochemistry of each C-4 configuration was determined from the CD spectrum. The positive Cotton effect at 234 nm in the CD spectrum suggests a □-orientation for each C-4 position.[4] Based on the above evidence, the structure of **1** was established as epicatechin - (2□□ *O*□ 7, 4□□ 6) - epicatechin - (2□□ *O*□ 7, 4□□ 8) - epicatechin - (4□□ 8) - epicatechin, which we have named parameritannin A-3. In this unique series of proanthocyanidins possessing two doubly linked structural elements only nine compounds have hitherto been reported.[1,5,6,7]

The MALDI-TOF-MS spectrum of compound (**2**) indicated an $[M+Na]^{+}$ ion at m/z 1175, consistent with a tetraflavonoid moiety. Also, the ^{1}H-NMR spectral resonance of the AB coupling systems were observed at □ 3.41 and 4.24 (each *d*, *J* =3.3 Hz), and ^{13}C-NMR spectral resonance of ketal carbon was observed at □ 100.25. These features suggest a tetrameric proanthocyanidin possessing an A-type structural element. In the ^{13}C-NMR spectrum, eight carbon signals were observed in the range □ 115-117, consistent with C-2' and C-5', and four carbon signals were observed in the range □ 119-122, consistent with the C-6' of B, E, H and K-rings. The chemical shifts of the C-3 signals for heterocyclic rings at □ 73.12, 72.54, 67.51 and 66.58 confirmed **2** to be a tetramer consisting only of epicatechin-like units. To determine the position of the interflavonoid linkage of each epicatechin-like unit, a COLOC study was carried out (Figure 4).

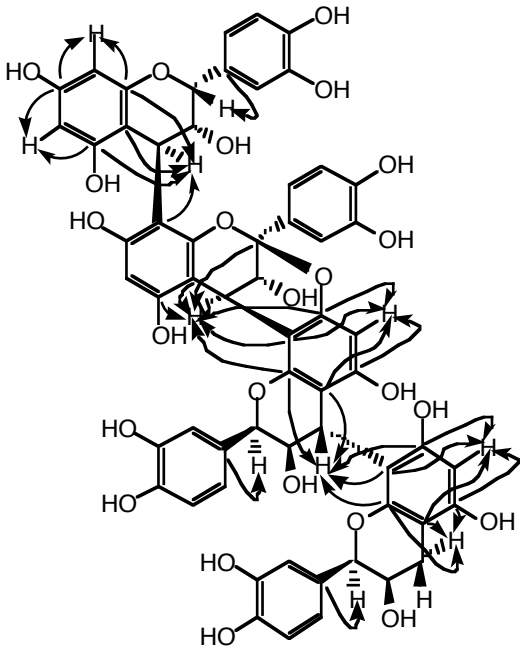

Figure 4 . Long-range correlations from the COLOC spectrum of **2**.

The methylene proton signals at δ 2.83 (H-4, L-ring) of the terminal unit showed long-range correlation to the carbon signal at δ 100.07 (C-10, J-ring) which coupled with the proton signal at δ 6.10 (H-6, J-ring). The proton signal at δ 4.57 (H-4, I-ring) showed long-range correlation to carbon signals at δ 108.77 (C-8, J-ring) and δ 106.75 (C-10, G-ring) which coupled with proton signals at δ 6.10 (H-6, J-ring) and δ 5.77 (H-6, G-ring), respectively. Further correlation was observed between the methine proton signal at δ 4.24 (H-4, F-ring) due to the AB coupling system and the carbon signal at δ 106.41 (C-8, G-ring) which correlated to the proton signal at δ 5.77 (H-6, G-ring). From these observations it was assumed that the structure of the 2nd, 3rd and terminal units was the same structure as cinnamtannin B-1. This was supported by comparison with the ^{13}C-NMR spectral data of cinnamtannin B-1.[1] Finally, the upper unit was linked to the C-8 of the 2nd unit, deduced from the extending carbon signal at δ 108.25 which showed a long-range correlation to the proton signal at δ 4.72 (H-4, C-ring) to be C-4 / C-8.[8] Considering the stereochemistry at C-4, the positive Cotton effect in the diagnostic wavelength region of the CD spectrum indicated a β-orientation for each C-4 flavanyl substituent.[4] The structure of **2** was therefore determined as epicatechin-(4β→8)-epicatechin-(2*O*→7, 4β→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-2).[6, 9]

In the MALDI-TOF-MS spectrum of compound (**3**) , an $[M+Na]^+$ ion peak at m/z 1175 was detected, corresponding to a tetraflavonoid structure. The ^{1}H-NMR spectrum proved to be exceedingly complex, presumably due to the effects of dynamic rotational isomerism at ambient temperature and rendering its interpretation impossible. In the ^{13}C-NMR spectrum, the ketal carbon signal at δ 99.79 indicated the presence of a doubly linked subunit. The ^{13}C-NMR spectrum displayed three carbon signals at δ 76.89,

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds (**1**) and (**2**) in CD_3OD and (**3a**) in $CDCl_3$

		1		**2**		**3a**	
Ring	No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Upper unit							
C	C-2	100.37		77.03	4.96 (br s)	73.87	5.47 (br s)
	C-3	67.61	3.95 (d, 3.4)	73.12	3.90 (br s)	70.59	5.19 (br t, 1.5)
	C-4	29.63	4.18 (d, 3.4)	37.33	4.72 (br s)	34.48	4.61 (br s)
A	C-5	156.26		158.26 [1]		149.81 [2]	
	C-6	98.27	5.91 (d, 2.3)	96.62	5.97 (d, 2.2)	109.94	6.65 (d, 2.3)
	C-7	158.07		157.84 [1]		146.69 [2]	
	C-8	96.76	5.93 (d, 2.3)	96.10	6.00 (d, 2.2)	107.65	6.70 (d, 2.3)
	C-9	153.94		155.48 [1]		154.66	
	C-10	103.94		101.69		110.95	
B	C-1'	132.23		132.39		135.72	
	C-2'	115.66	6.92 (d, 2.1)	115.29	7.11 (d, 1.8)	122.94	7.46 (d, 2.1)
	C-3'	146.09		145.55		141.81	
	C-4'	146.75		145.87		142.99	
	C-5'	115.77	6.72 (d, 8.2)	115.85	6.65 (d, 8.2)	123.22	7.07 (d, 8.1)
	C-6'	119.84	6.75 (dd, 8.2, 2.1)	119.28	6.56 (dd, 8.2, 1.8)	124.30	7.17 (dd, 8.1, 2.1)
2nd unit							
F	C-2	99.95		100.25		97.14	
	C-3	66.98	3.25 (d, 3.6)	66.58	3.41 (d, 3.3)	67.13	4.94 (d, 3.9)
	C-4	29.21	4.21 (d, 3.6)	29.11	4.24 (d, 3.3)	27.77	4.48 (d, 3.9)
D	C-5	151.10		155.31		147.82	
	C-6	109.39		99.54	5.94 (s)	117.27	
	C-7	152.24		155.31		148.47	
	C-8	97.13	6.07 (s)	108.25		109.28	6.94 (s)
	C-9	152.70		156.75		151.97	
	C-10	106.80		105.04		113.20	
E	C-1'	132.24		132.42		135.03	
	C-2'	116.45	7.30 (d, 2.0)	116.72	7.33 (d, 2.0)	125.28	7.57 (d, 2.1)
	C-3'	145.61		145.87		141.74	
	C-4'	146.28		146.26		142.65	
	C-5'	116.52	6.84 (d, 8.8)	116.14	6.85 (d, 8.3)	123.00	7.20 (d, 8.4)
	C-6'	121.04	7.30 (dd, 8.8, 2.0)	121.33	7.20 (dd, 8.3, 2.0)	125.03	7.51 (dd, 8.4, 2.1)

Table 1. *Continued*

		1		**2**		**3a**	
Ring	No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
3rd unit							
I	C-2	78.33	5.67 (br s)	78.85	5.71 (br s)	76.10	5.41 (br d, 1.9)
	C-3	72.35	4.16 (br d, 2.0)	72.54	4.14 (br d, 1.4)	70.04	5.14 (br d, 2.8)
	C-4	37.95	4.46 (t-like)	38.37	4.57 (t-like)	33.49	4.52 (br s)
G	C-5	155.88		155.82		149.73	
	C-6	96.08	5.66 (s)	96.02	5.77 (s)	104.70	6.46 (s)
	C-7	151.03		151.05		150.16	
	C-8	105.84		106.41		108.58	
	C-9	151.54		151.70		151.16	
	C-10	106.83		106.75		107.89	
H	C-1'	131.41		131.71		134.35	
	C-2'	115.66	7.03 (d, 2.2)	116.67	7.24 (d, 2.0)	121.72	7.32 (d, 2.0)
	C-3'	145.71		145.77		141.67	
	C-4'	146.62		146.54		142.12	
	C-5'	115.66	6.70 (d, 8.3)	115.90	6.88 (d, 8.3)	122.84	7.04 (d, 8.4)
	C-6'	119.78	6.91 (dd, 8.3, 2.2)	120.03	6.99 (dd, 8.3, 2.0)	125.52	7.13 (dd, 8.4, 2.0)
Terminal unit							
L	C-2	80.24	4.27 (br s)	80.25	4.39 (br s)	76.63	5.09 (br s)
	C-3	67.50	3.74 (t-like)	67.51	3.86 (br s)	66.32	5.46 (m)
	C-4	29.83	2.72 (m)	29.80	2.83 (m)	26.29	2.98 (dd, 18.0, 4.6)
							2.89 (br d, 18.0)
J	C-5	156.03		155.97		148.50	
	C-6	96.51	6.01 (s)	96.49	6.10 (s)	110.77	6.51 (s)
	C-7	155.76		155.48		147.26	
	C-8	108.61		108.77		118.32	
	C-9	155.56		155.73		151.67	
	C-10	100.02		100.07		110.03	
K	C-1'	133.18		133.07		135.38	
	C-2'	115.53	6.75 (d, 1.9)	115.35	6.82 (d, 1.8)	121.57	7.17 (d, 1.9)
	C-3'	145.30		145.37		141.59	
	C-4'	145.46		145.78		141.97	
	C-5'	115.95	6.68 (d, 8.2)	116.03	6.80 (d, 8.3)	123.22	7.09 (d, 8.0)
	C-6'	119.42	6.62 (dd, 8.2, 1.9)	119.37	6.74 (dd, 8.3, 1.8)	123.54	7.06 (dd, 8.0, 1.9)

All assignments are based on the ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and COLOC spectral data.

Coupling patterns and coupling constants (J) in Hz are given in parentheses.

Symbol[1, 2] in each column may be interchanged.

Table 2. ^{1}H- and ^{13}C-NMR Spectral data for Compound (**3**) in CD_3OD

Ring	No.	^{13}C	^{1}H	Ring	No.	^{13}C	^{1}H
C	2	76.89	5.00	I	2	79.44	5.77
	3	72.77	4.00		3	72.95	4.03
	4	37.81	4.67		4	38.35	4.62
A	6	96.09	5.95	G	6	96.17	5.85
	8	96.42	6.04		8	106.17	
	10	101.54			10	106.45	
F	2	99.79		L	2	80.22	4.39
	3	67.12	3.37		3	67.33	3.86
	4	29.12	4.13		4	29.74	2.84
D	6	109.79		J	6	96.50	6.11
	8	97.39	5.99		8	108.80	
	10	105.04			10	100.01	

79.44 and 80.22 corresponding to the C-2, and four carbon signals at □ 72.77, 67.12, 72.95 and 67.33 attributable to the C-3 of the C, F, I, and L heterocyclic rings, indicating a tetramer consisting only of epicatechin-like units. The ^{13}C-NMR spectrum of **3** was quite close to that of **2**, except for the extending carbon signal of the upper unit [□108.25 for **2** and □ 109.79 for **3**] .[8] From this finding, the linkage between the upper and 2nd units was assumed to be C-4 / C-6. In order to clarify in detail the assignment of ^{1}H- and ^{13}C-NMR spectral signals, acetylation of **3** was attempted, yielding peracetate (**3a**). Full assignment of ^{1}H- and ^{13}C-NMR spectral signals of **3a** using ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and COLOC (Figure 5) has been carried out.

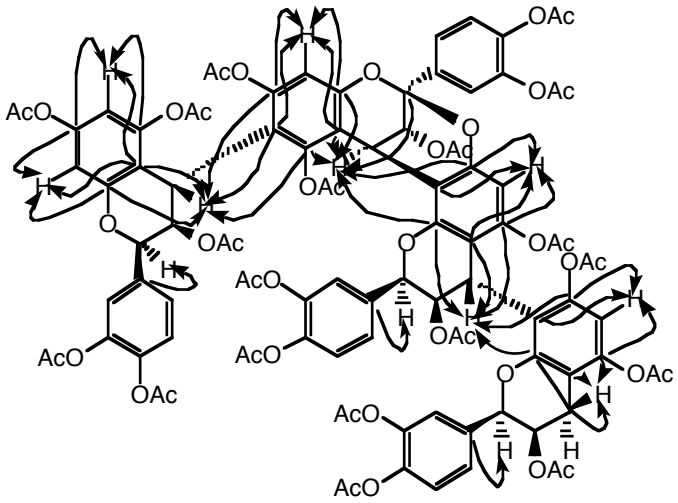

Figure 5. Long-range correlations from the COLOC spectrum of **3a**.

Finally, the absolute configuration at C-4 of each flavan unit was established by CD measurement. The strong positive Cotton effect in the diagnostic wavelength region (220-240nm) indicated the □-configuration.[4] The structure of **3** was therefore characterized as epicatechin-(4□→6)-epicatechin-(2□→*O*

→7, 4→8)-epicatechin-(4→8)-epicatechin (pavetannin C-1).[6]

The HR-negative-FAB-MS spectrum of compound (**4**) showed an $[M-H]^-$ ion at m/z 863.1824, corresponding to trimeric proanthocyanidin with one A-type unit. The ^{1}H- and ^{13}C-NMR spectra of **4** resembled those of cinnamtannin B-1, except for the signals of the terminal unit, the significant difference being that the $^3J_{H\text{-}2,\ H\text{-}3}$ coupling constant of 9.1Hz corresponding to the terminal unit, indicated that terminal unit was a catechin-like moiety. The positive Cotton effect in the diagnostic wavelength region of the CD spectrum suggested that the absolute configuration at C-4 was a -orientation.[4] In addition, the proton signal for H-2 (I-ring) appeared at a higher magnetic field than that for the H-2 of catechin. This upfield shift was attributable to the magnetic anisotropic effect of the D-ring (Figure 6).[3]

Table 3. ^{1}H-NMR Spectral Data for Compound (**4**) and catechin in CD_3OD

		4		catechin
Ring	No.	^{1}H	Ring	^{1}H
Upper unit				
C	H-2			
	H-3	3.47 (d, 3.5)		
	H-4	4.00 (d, 3.5)		
A	H-6	5.94 (d, 2.3)		
	H-8	6.01 (d, 2.3)		
B	H-2'	7.09 (d, 2.1)		
	H-5'	6.85 (d, 8.3)		
	H-6'	6.95 (dd, 8.3, 2.1)		
Middle unit				
F	H-2	5.51 (br s)		
	H-3	4.06 (br s)		
	H-4	4.53 (br s)		
D	H-6	5.84 (s)		
	H-8			
E	H-2'	7.23 (d, 2.0)		
	H-5'	6.84 (d, 8.1)		
	H-6'	7.08 (dd, 8.1, 2.0)		
Terminal unit				
I	H-2	3.95 (d, 9.1)	C	4.57 (d, 7.5)
	H-3	3.68 (ddd, 10.1, 9.1, 6.1)		3.98 (ddd, 8.1, 7.5, 5.4)
	H-4	3.05 (dd, 16.1, 6.1)		2.85 (dd, 16.1, 5.4)
		2.43 (dd, 16.1, 10.1)		2.51 (dd, 16.1, 8.1)
G	H-6	6.10 (s)	A	5.86 (d, 2.3)
	H-8			5.93 (d, 2.3)
H	H-2'	6.75 (d, 1.9)	B	6.84 (d, 2.0)
	H-5'	6.76 (d, 8.1)		6.76 (d, 8.1)
	H-6'	6.66 (dd, 8.1, 1.9)		6.72 (dd, 8.1, 2.0)

Coupling patterns and coupling constants (J) in Hz are given in parentheses.

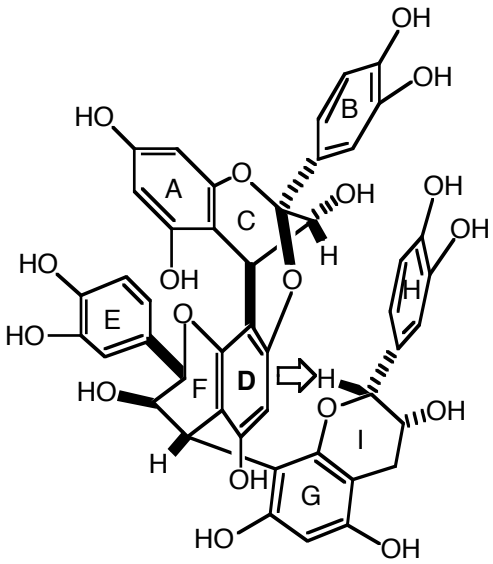

Figure 6. Anisotropic effect of **4**.

Therefore, the terminal unit of **4** was catechin. From all the preceding evidence, the structure of **4** was established as epicatechin-(2→*O*→7, 4→8)-epicatechin-(4→8)-catechin, and this compound has been named cinnamtannin D-1.[10]

EXPERIMENTAL

General procedures and plant material. General experimental procedures and plant material have been described in earlier publication.[1] MALDI-TOF-MS spectrum were performed with a Shimadzu Kratos Kompact MALDI 4.

Extraction and isolation. The isolation of proanthocyanidin fraction from *P. laevigata* have been published earlier.[1] Briefly, **1**-**5** were obtained from proanthocyanidin fraction by repetitious column chromatography on Sephadex LH-20 using MeOH-H_2O (2:1), Rp-18 using acetonitrile-H_2O and MeOH-H_2O systems and silica gel using AcOEt.

Parameritannin A-3 [epicatechin-(2□□ *O*□ 7, 4□□ 6)-epicatechin-(2□□ *O*□ 7, 4□□ 8)-epicatechin-(4□□ 8)-epicatechin] (1): Pale yellow amorphous powder; MALDI-TOF-MS: m/z 1173 $[M+Na]^+$; CD: $[\theta]_{274}$ –16300, $[\theta]_{234}$ +309400, $[\theta]_{209}$ –269800; IR ν_{max}^{KBr} cm^{-1}: 3391, 1611, 1522, 1448, 1286, 1063; UV λ_{max}^{MeOH} nm (log ε): 212 (4.97), 231sh (4.79), 280 (4.16); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see Table 1.

Cinnamtannin B-2 [epicatechin-(4□□ 8)-epicatechin-(2□□ *O*□ 7, 4□□ 8)-epicatechin-(4□□ 8)-epicatechin] (2): Pale yellow amorphous powder; MALDI-TOF-MS: m/z 1175 $[M+Na]^+$; CD: $[\theta]_{229}$ +213800, $[\theta]_{206}$ -133900; IR ν_{max}^{KBr} cm^{-1}: 3350, 1616, 1522, 1447, 1283, 1065; UV λ_{max}^{MeOH} nm (log ε): 214 (5.08), 280 (4.23); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see Table 1.

Pavetannin C-1 [epicatechin-(4□□ 6)-epicatechin-(2□□ *O*□ 7, 4□□ 8)-epicatechin-(4□□ 8)-epicatechin] (3): Pale yellow amorphous powder; MALDI-TOF-MS: m/z 1175 $[M+Na]^+$; CD: $[\theta]_{234}$ +216700, $[\theta]_{211}$ –174100, $[\theta]_{201}$ +121800; IR ν_{max}^{KBr} cm^{-1}: 3380, 1614, 1520, 1448, 1283, 1065; UV λ_{max}^{MeOH} nm (log ε): 211 (5.11), 281 (4.19); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see Table 2, carbon signals of downfield: 158.16-151.01 (C-5, 7, 9), 146.49-145.23 (C-3', 4'), 133.03, 132.54, 132.26, 131.37 (C-1'), 122.16, 119.85, 119.53, 119.26 (C-6'), 117.37, 116.14, 115.95, 115.84, 115.72, 115.67, 115.47, 115.30 (C-2', 5').

Acetylation of 3. Compound (**3**) (200 mg) was acetylated with Ac_2O (2 mL)-pyridine (2 mL) and the

product was purified by column chromatography on silica gel using hexane-AcOEt-acetone (3: 2: 1) to yield peracetate (**3a**) (200 mg).

Peracetate (3a): Colorless amorphous powder; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1, methyl protons of -$COCH_3$: 2.23(x2), 2.22, 2.21, 2.20(x2), 2.19, 2.16, 2.07, 1.98, 1.84, 1.83. 1.80, 1.77, 1.64, 1.55, 1.44, 1.33, 1.32 (each 3H, s), carbonyl carbons of -$COCH_3$: 170.63, 170.61, 169.89, 168.84, 168.80, 168.57, 168.31, 168.12, 168.09, 167.98(x2), 167.95, 167.92, 167.87, 167.85, 167.79, 167.56, 167.43, 166.93, methyl carbons of $COCH_3$: 20.87, 20.69, 20.59(x3), 20.57, 20.53, 20.49, 20.48, 20.47, 20.44, 20.38, 20.16, 20.11, 19.87, 19.73, 19.59, 19.49, 18.88.

Cinnamtannin D-1 [epicatechin-(2□□ *O*□ 7, 4□□ 8)-epicatechin-(4□□ 8)-catechin] (4): Pale yellow amorphous powder; HR-negative-FAB-MS: m/z 863.1826 $[M-H]^-$; CD: $[\theta]_{241}$ +131900, $[\theta]_{208}$ –249800; IR ν_{max}^{KBr} cm^{-1}: 3244, 1616, 1522, 1437, 1286, 1062; UV λ_{max}^{MeOH} nm (log ε): 231 (4.88), 281 (4.09); ^{13}C-NMR (100 MHz, CD_3OD): upper unit: 100.07 (C-2), 67.22 (C-3), 30.65 (C-4), 156.66 (C-5), 98.38 (C-6), 157.82 (C-7), 96.56 (C-8), 154.22 (C-9), 104.99 (C-10), 132.45 (C-1'), 115.86 (C-2'), 145.88 (C-3'), 146.66 (C-4'), 116.25 (C-5'), 120.06 (C-6'), middle unit: 78.68 (C-2), 72.51 (C-3), 38.31 (C-4), 155.86 (C-5), 96.12 (C-6), 151.07 (C-7), 106.31 (C-8), 151.74 (C-9), 106.58 (C-10), 131.54 (C-1'), 116.55 (C-2'), 145.50 (C-3'), 146.32 (C-4'), 115.75 (C-5'), 121.07 (C-6'), terminal unit: 83.33 (C-2), 70.10 (C-3), 28.86 (C-4), 155.39 (C-5), 96.59 (C-6), 155.59 (C-7), 108.83 (C-8), 155.39 (C-9), 101.84 (C-10), 132.71 (C-1'), 116.25 (C-2'), 145.77 (C-3'), 145.99 (C-4'), 115.80 (C-5'), 120.02 (C-6'); ^{1}H-NMR (400 MHz, CD_3OD): see Table 3.

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REFERENCES

1. K. Kamiya, C. Watanabe, H. Endang, M. Umar, and T. Satake, *Chem. Pharm. Bull.*, 2001, **49**, 551.
2. H. Lou, Y. Yamazaki, T. Sasaki, M. Uchida, H. Tanaka, and S. Oka, *Phytochemistry*, 1999, **51**, 297.
3. N. Tanaka, R. Orii, K. Ogasa, H. Wada, T. Murakami, Y. Saiki, and C. M. Chen, *Chem. Pharm. Bull.*, 1991, **39**, 55.
4. J. J. Botha, D. A. Young, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1213.

5. S. Morimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, 1987, **35**, 4717.
6. A. M. Balde, T. D. Bruyne, L. Pieters, H. Kolodziej, D. V. Berghe, M. Claeys, and A. Vlietinck, *Phytochemistry*, 1995, **38**, 719.
7. F. Calzada, R. Cedillo-Rivera, R. Bye, and R. Mata, *Planta Medica*, 2001, **67**, 677.
8. L. J. Porter, R. H. Newman, L. Y. Foo, H. Wong, and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1217.
9. G. Nonaka, S. Morimoto, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2139.
10. S. Morimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, 1988, **36**, 33.