Proanthocyanidins from the Leaves of *Machilus philippinensis*

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Seven proanthocyanidins (**2**−**8**) together with epicatechin (**1**) were isolated from the EtOH extract of the leaves of *Machilus philippinensis*. Of these, machiphilitannins A (**7**) and B (**8**) are new natural products, with respective IC_{50} values of 31.3 (**7**) and 18.4 μM (**8**) against α-glucosidase type IV from *Bacillus stearothermophilus*. Their structures were elucidated mainly on the basis of CD and 2D NMR analyses. In addition, aesculitannin B (**2**) showed inhibitory activity against α-glucosidase with an IC_{50} value of 3.5 μM. This work demonstrates for the first time that the purified proanthocyanidins possess inhibitory activity against α-glucosidase type IV.

Machilus philippinensis Merr. (Lauraceae) is a medium-sized evergreen tree distributed at an attitude of 500 to 1600 m in Taiwan and the Philippines. Our recent study indicated that acylated flavonol monorhamnosides from the CH_2Cl_2-soluble fraction of the leaf extract exhibited potent activities against α-glucosidase (*Bacillus stearothermophilus*, type IV),[1] which plays a key role in the digestion of dietary carbohydrates in humans. α-Glucosidase inhibitors such as acarbose could improve postprandial glucose control and have been used in the treatment of diabetes.[2] On the basis of the bioassay-guided approach, A-type proanthocyanidins from the *n*-BuOH-soluble fraction of *M. philippinensis* were isolated, characterized, and described here.

Results and Discussion

A continuing study on the CH_2Cl_2-soluble fraction of the EtOH extract of the leaves of *M. philippinense*[1] led to the isolation of a trimeric proanthocyanidin, **2**. The *n*-BuOH-soluble fraction, showing 55.9% inhibition at 10 μg/mL against α-glucosidase type IV from *Bacillus stearothermophilus*, was further subjected to column chromatography over Sephadex LH-20, Lobar RP-18, and semi-preparative RP-18 HPLC with the delivery systems described in the Experimental Section to give compounds **1** and **3**−**8** (Figure 1).

Compound **1** was identified as epicatechin by comparisons of its physical data with authentic references from our collection.[3]

The ESIMS spectra of compounds **2**−**4** showed the same $[M - H]^-$ ion at *m/z* 863.2. Their ^1H and ^{13}C NMR data were also very similar. Thus, they were structural isomers. The ^1H NMR spectrum of **2** revealed signals for three 3′,4′-disubstituted flavan-3-ol moieties, i.e., three ABX systems (δ 6.79−7.19) for the protons of rings B, E, and H, one AX system for two *meta*-coupled protons [H-6 (δ 5.84) and H-8 (δ 5.99) ($J = 2.3$ Hz)], and two singlets (δ 5.79 and 6.08) in the aromatic region, and one AX system for H-3 (δ 3.33) and H-4 (δ 3.94) ($J = 3.5$ Hz) of the C-2 and C-4 doubly linked epicatechin residue, two sets of signals characteristic for the H-2, H-3, and H-4 of a catechin residue (δ 4.61, d, $J = 9.3$ Hz, H-2; δ 4.56, t, $J = 9.3$ Hz, H-3; δ 4.51, d, $J = 9.3$ Hz, H-4; ring F), and an epicatechin residue (δ 4.36, H-2; δ 4.06, H-3; δ 2.87 and 2.77, H-4; ring I) (Supporting Information: Table 1S). The ^1H and ^{13}C NMR data (Table 1S) together with the ESIMS and CD data elucidated **2** as the known proanthocyanidin aesculitannin B[4] (Figure 1), containing an *ent*-catechin as unit II. The ^1H NMR spectrum of **3** differed from that of **2** only in the signals for the three aliphatic protons in ring F, appearing at δ 5.70 (brs, H-2), 4.12 (brs, H-3), and 4.56 (brs, H-4), verified by the COSY spectral analysis. Hence **3** contained three epicatechin moieties. By comparing the ^1H and ^{13}C NMR data (Table 1S) to those reported,[5] compound **3** was elucidated as cinnamtannin B-1 (Figure 1). The ^1H NMR spectrum of **4** (Table 1S) was similar to that of **3** except for the signals for the four aliphatic protons in ring I, appearing at δ 3.94 (d, $J = 9.0$ Hz, H-2), 3.66 (m, H-3), 3.04 (dd, $J = 16.2$, 6.0 Hz), and 2.41 (dd, $J = 16.2$, 10.2 Hz) (H-4s), verified by the COSY analysis, suggesting the unit III in **4** to be a catechin residue. The ^{13}C NMR spectrum showed similar C-2 signals in the flavan-3-ol units I and II of **3** and **4** but differed from the C-2 signal in the terminal unit (δ 83.3, **4**; δ 80.3, **3**), supporting the unit III in **4** to be the catechin moiety (Table 1S). The CD spectrum of **4** showed a strong positive Cotton effect (CE) at 229 nm, indicating the β-orientation of 4-flavanyl substituents.[6] These data established **4** as cinnamtannin D-1 (Figure 1).[7]

The ESIMS data of **5** showed the $[M - H]^-$ ion at *m/z* 1151.2, indicating a tetrameric structure. The ^1H NMR spectrum exhibited exceptionally complex signals while measured at 298 K in methanol-d_4, caused by the dynamic isomerism due to the rotational hindrance around the interflavanyl bond.[4] The signals became sharper and analyzable while measured at lower temperature (280 K). The ^{13}C NMR data displayed signals for four 3′,4′-disubstituted flavan-3-ol units, where the C-3 methines appeared at δ 72.9, 67.2, 73.1, and 67.5, the ketal carbon (C-2) at δ 99.9, and the remaining C-2 methines at δ 77.0, 79.6, and 80.3 (Table 3S). The carbon signal at δ 109.9 assignable to C-6 (ring A) suggested the interflavanyl linkage of the upper two units between C-4 (ring L) and C-6 (ring A).[7,8] These NMR data together with the CD spectrum showing positive CEs around 232 nm, indicating the β-orientation for the C-4/C-6 bond,[6] elucidated **5** to be the known pavetannin C-1 (Figure 1).[8]

The ESIMS data of **6** showed the $[M - H]^-$ ion at *m/z* 1151.2, suggesting **6** to be a tetrameric proanthocyanidin. The ^1H NMR spectrum (methanol-d_4, 600 MHz) (Table 1) showed an AX system for H-3 (δ 3.30) and H-4 (δ 4.24) ($J = 3.5$ Hz) in ring C, and the ^{13}C NMR spectrum showed a characteristic signal for a C-2 ketal carbon (δ 100.1, s) in ring C, indicating **6** to be an A-type proanthocyanidin.[5] The ^{13}C NMR spectrum also showed characteristic signals for four C-3 methines (δ 66.8, 72.4, 67.4, and 71.3) and three C-2 methines (δ 76.6, 78.7, and 80.1) (Table 1), supporting **6** to be composed of four 3′,4′-disubstituted flavan-3-ol units. In addition, an oxygenated aryl carbon resonating at δ 148.5 (s, C-7, unit II) suggested the presence of a flavanyl substituent at C-6 of unit II.[5] Thus, compound **6** is a branched A-type proanthocyanidin. The ^1H NMR spectrum showed two sets of AX systems for H-6 and H-8 at δ 5.99 and 6.06 ($J = 2.1$ Hz) and δ 5.88 and 5.93 ($J = 2.1$ Hz), and one singlet at δ 6.08 (H-6, ring G) for three flavan-3-ol units, suggesting C-6 and C-8 of the DEF unit to be the linkage positions to C-4 of two other units. The ^1H and ^{13}C NMR assignments of **6** were made by 2D NMR analyses (HMBC,

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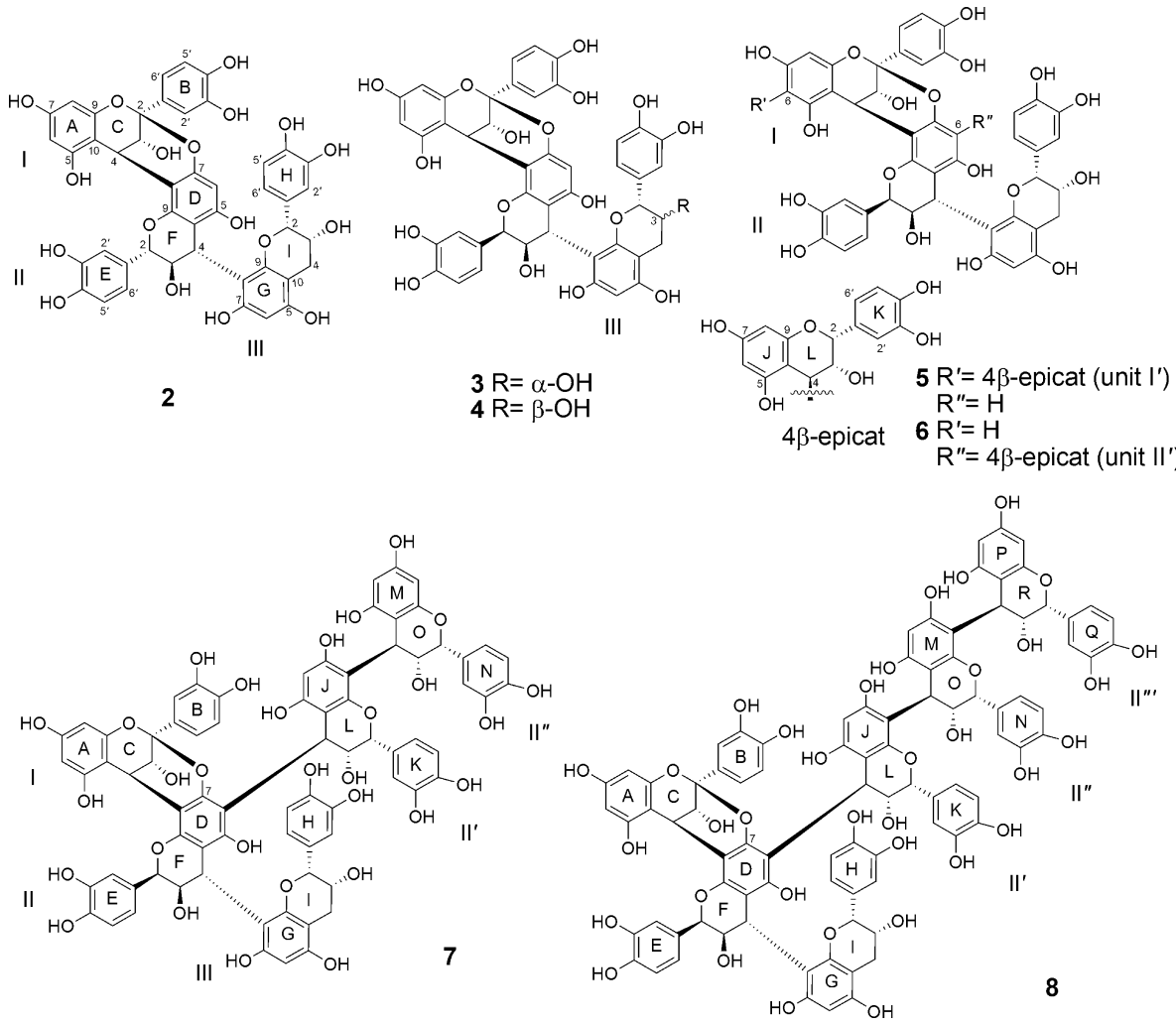

Figure 1. Structures of compounds **1**−**8**.

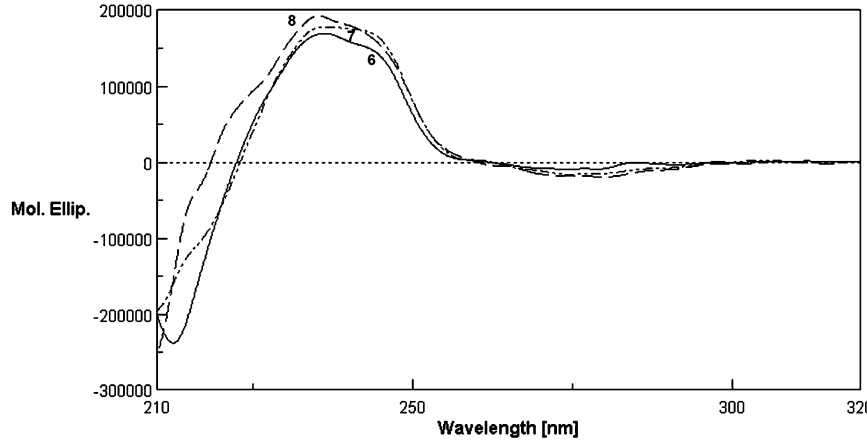

Figure 2. CD spectra of **6** (− − −), **7** (---), and **8** (—). (MeOH).

HMQC, COSY, and TOCSY) and are listed in Table 1. These data and the CD spectrum (Figure 2), showing a diagnostic positive CE at 236 nm for the *β*-orientation of the interflavonoid bonds, established **6** as the known parameritannin A-1 (Figure 1).[5]

Compound **7** had the molecular formula $C_{75}H_{60}O_{30}$, as deduced from HR-ESIMS, showing the quasi-molecular ion $[M - H]^-$ at *m/z* 1439.3092, indicating **7** to be a pentameric proanthocyanidin. The ^{1}H and ^{13}C NMR spectra were similar to those of **6**, except for the presence of additional signals for an epicatechin unit (Table 1), indicating **7** to possess a doubly linked flavan-3-ol structure. This comparison, together with the CD spectrum (Figure 2), showing a strong (+)-CE at 236 nm for the *β*-oriented C-4 flavan-3-ol substituents, indicated **7** to be the condensation product of **6** with an epicatechin unit linked to one of the following positions: unit I C-6 and C-8, unit II′ C-8, and unit III C-4. The ^{1}H NMR spectrum of **7** showed two AX systems for the *meta*-coupled H-6 and H-8 at *δ* 5.97 and 6.04 (J = 2.3 Hz), and *δ* 5.89 and 5.97 (J = 2.3 Hz), being resolvable while measured at 280 K (methanol-d_4) and verified by the COSY spectrum. These data also suggested a branched structure for **7** since only one such AX system present

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **6**, **7**, and **8** (methanol-d_4, AV-600, 298 K)

		6		**7**		**8**				**6**		**7**		**8**	
ring	no.	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)	ring	no.	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)
unit I								unit II′							
C	2	100.1, C		100.3, C		100.3, C		L	2	76.6, CH	4.75, brs	76.8, CH	4.98, brs	76.7, CH	5.02, brs
	3	66.8, CH	3.30, d (3.5)	67.0, CH	3.30, d (3.5)	67.3, CH	3.29, d (3.2)		3	71.3, CH	4.07, brs	71.1, CH	4.12, brs	70.6, CH	4.18, brs
	4	28.9, CH	4.24, d (3.5)	29.0, CH	4.24, d (3.5)	29.0, CH	4.26, d (3.2)		4	37.6, CH	4.38, brs	37.8, CH	4.52, brs	37.9, CH	4.53, brs
A	5	156.8, C		156.7, C		156.7, C		J	5	159.5, C		157.7, C		157.7, C	
	6	98.3, CH	5.99, d (2.1)	98.3, CH	5.97, d (2.2)	98.3, CH	5.98, d (1.8)		6	96.8, CH	5.88, d (2.1)	97.7, CH	6.00, s	97.9, CH	5.87, s
	7	157.9, C		157.9, C		157.9, C			7	159.4, C		158.1, C		158.2, C	
	8	96.5, CH	6.06, d (2.1)	96.5, CH	6.05, d (2.2)	96.5, CH	6.06, d (1.8)		8	96.1, CH	5.93, d (2.1)	107.4, C		107.8, C	
	9	154.3, C		154.5, C		154.4, C			9	158.0, C		155.0, C		155.0, C	
	10	104.9, C		105.0, C		105.0, C			10	99.3, C		100.3, C		100.4, C	
B	1′	132.3, C		132.5, C		132.4, C		K	1′	131.6, C		131.7, C		131.6, C	
	2′	115.9, CH	7.16, brs	115.9, CH	7.15, brs	115.9, CH	7.17, d (1.2)		2′	116.7, CH	7.11, brs	116.6, CH	7.10, brs	116.8, CH	7.11, brs
	3′	145.5, C		145.5, C		145.5, C			3′	146.3, C		146.2, C		146.3, C	
	4′	146.7, C		146.8, C		146.8, C			4′	145.9, C		145.9, C		145.8, C	
	5′	116.0, CH	6.91, d (8.2)	116.1, CH	6.91, d (8.1)	116.1, CH	6.93, d (8.4)		5′	116.0, CH	6.90, d (8.3)	116.1, CH	6.91, brd (8.1)	115.9, CH	6.94, d (8.4)
	6′	120.7, CH	6.94, brd (8.2)	120.2, CH	6.96, brd (8.1)	120.2, CH	6.99, dd (8.4, 1.2)		6′	119.9, CH	6.94, brd (8.3)	119.9, CH	6.98, brd (8.1)	119.9, CH	7.06, brd (8.4)
unit II								unit II″							
F	2	78.7, CH	5.66, brs	78.7, CH	5.66, brs	78.7, CH	5.63, brs	O	2			76.8, CH	5.01, brs	76.8, CH	5.19, brs
	3	72.4, CH	4.09, brs	72.5, CH	4.10, brs	72.4, CH	4.10, brs		3			73.8, CH	3.85, brs	73.4, CH	3.93, brs
	4	38.4, CH	4.43, brs	38.2, CH	4.49, brs	38.3, CH	4.48, brs		4			36.8, CH	4.54, brs	37.2, CH	4.58, brs
D	5	154.3, C		154.1, C		154.2, C		M	5			157.9, C		156.6, C	
	6	107.7, C		108.2, C		108.1, C			6			96.5, CH	5.91, d (1.9)	97.4, CH	5.85, s
	7	148.5, C		148.4, C		148.4, C			7			158.3, C		158.1, C	
	8	106.9, C		107.1, C		107.2, C			8			96.1, CH	5.99, d (1.9)	107.2, C	
	9	150.3, C		150.3, C		150.3, C			9			157.8, C		158.0, C	
	10	107.2, C		107.5, C		107.5, C			10			101.4, C		102.1, C	
E	1′	131.7, C		131.6, C		131.6, C		N	1′			132.9, C		132.9, C	
	2′	116.7, CH	7.31, d (1.5)	116.8, CH	7.31, d (1.8)	116.8, CH	7.31, d (1.2)		2′			115.0, CH	6.87, brs	114.8, CH	7.07, br, s
	3′	145.6, C		145.4, C		145.4, C			3′			146.4, C		146.3, C	
	4′	146.3, C		146.4, C		146.5, C			4′			145.2, C		145.0, C	
	5′	116.1, CH	6.84, d (8.2)	116.1, CH	6.83, d (8.2)	116.1, CH	6.83, d (8.4)		5′			116.1, CH	6.91, brd (8.1)	115.9, CH	6.91, d (7.8)
	6′	121.4, CH	7.21, dd (8.2, 1.5)	121.5, CH	7.19, dd (8.2, 1.8)	121.4, CH	7.21, dd (8.4, 1.2)		6′			119.4, CH	6.63, brd (8.1)	118.9, CH	6.65, brd (7.8)
unit III								unit II‴							
I	2	80.1, CH	4.11, brs	80.2, CH	4.01, brs	80.1, CH	3.96, brs	R	2					77.1, CH	5.07, brs
	3	67.4, CH	3.63, brs	67.4, CH	3.55, brs	67.0, CH	3.51, brs		3					73.4, CH	3.98, brs
	4	29.7, CH_2	2.78, m	29.7, CH_2	2.68, brd (16.9)	29.6, CH_2	2.69, brd (16.8)		4					37.2, CH	4.73, brs
					2.54, brd (16.9)		2.53, brd (16.8)								
G	5	155.5, C		155.4, C		155.4, C		P	5					157.1, C	
	6	96.6, CH	6.08, s	96.5, CH	6.06, s	96.5, CH	6.11, s		6					96.5, CH	6.01, brs
	7	156.1, C		156.0, C		156.1, C			7					158.5, C	
	8	108.8, C		108.6, C		108.6, C			8					96.3, CH	6.03, brs
	9	155.6, C		155.5, C		155.5, C			9					154.8, C	
	10	99.9, C		99.9, C		99.8, C			10					101.6, C	
H	1′	132.8, C		132.9, C		132.9, C		Q	1′					132.4, C	
	2′	115.4, CH	6.65, brs	115.4, CH	6.71, brs	115.5, CH	6.70, d (1.8)		2′					115.2, CH	6.90, brs
	3′	145.8, C		145.9, C		145.8, C			3′					145.8, C	
	4′	145.4, C		145.4, C		145.5, C			4′					145.9, C	
	5′	116.1, CH	6.72, d (8.0)	116.1, CH	6.74, d (8.0)	116.0, CH	6.74, d (7.8)		5′					116.1, CH	6.71, d (8.4)
	6′	119.2, CH	6.28, brd (8.0)	119.3, CH	6.41, brd (8.0)	119.3, CH	6.37, brd (7.8)		6′					119.3, CH	6.71, brd (8.4)

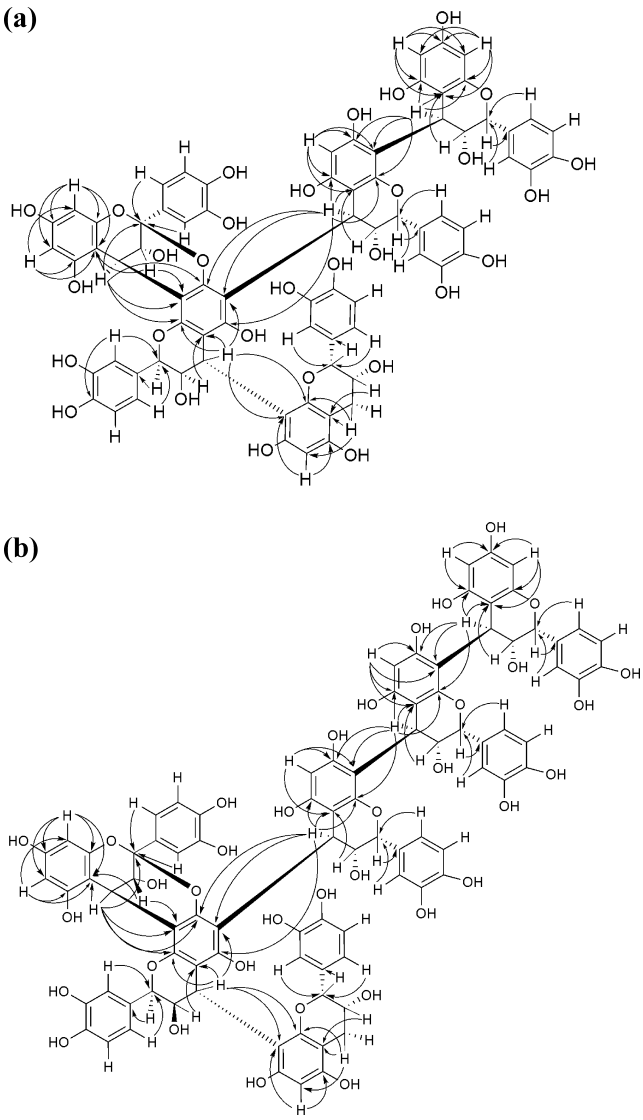

Figure 3. Key HMBC correlations of compounds **7** (a) and **8** (b) (methanol-d_4, 600 MHz).

in unit I could be observed if no branch existed. The HMBC spectrum of **7** showed the correlations of H-6 (δ 5.97), H-8 (δ 6.05), and H-4 (δ 4.24, d) to C-10 (δ 105.0), and H-4 and H-3 to the ketal C-2 (δ 100.3) in unit I (Figure 3a), thus eliminating **7** as the product of **6** linked to the additional epicatechin unit at either C-6 or C-8 of unit I and also confirming the location of the doubly linked interflavonoid linkage between unit I and unit II. The HMBC spectrum showed the correlations of unit I H-4 (δ 4.24) and unit II′ H-4 (δ 4.52, brs) to unit II C-7 (δ 148.4), indicating the linkage between unit II C-6 and unit II′ C-4, which confirmed the branched structure. The upfield-shifted C-7 signal in unit II of **7** relative to the corresponding signals in **2**−**4** (δ 148.4 vs δ 151.1 in **3** and **4**), caused by the γ-effect from the unit II′ structure,[9] also supported such a branched structure. The linkage between the branched units (units II′ and II″) is evidenced by comparison of the ^{13}C NMR data measured in methanol-d_3 and methanol-d_4. This H−D exchange experiment distinguished the C-9 signals from those of C-5 and C-7 in the region 148.4−158.2 ppm.[10,11] The isotopic shift was observed for those carbons bearing phenolic OH groups but having little effect on those non-phenolated but oxygenated aryl carbons such as C-9s and C-7 of unit II. The data shown in Tables 2 and 4S indicate that the signals at δ 148.4, 150.3, 154.5, 155.0, 155.5, and 157.8 are little influenced by the isotopic effect, thus assignable to those non-phenolated but oxygenated aryl carbons. Further analysis of the HMBC spectrum indicated the $^3J_{H-C}$ correlation of unit II′ H-4 and unit II″ H-4 (δ 4.54, brs) to unit II′ C-9 (δ 155.0) (Figure 3a), which did not show isotopic shifts as indicated above, suggesting the additional epicatechin (unit II″) to be C-4 linked to unit II′ C-8. The 2D spectrum also supported the linkage between unit II C-4β and unit III C-8 by the observation of correlations of unit II H-4 (δ 4.49) and unit III H-6 (δ 6.06) to unit III C-8 (δ 108.6). In addition, the correlation of H-6 and H-4s (δ 2.54 and 2.68) to C-10 (δ 99.9) confirmed unit III to be one of the terminal residues. These data composed together thus established **7** as epicatechin-(4β→8,2β→*O*→7)-[epicatechin-(4β→8)-epicatechin-(4β→6)]-epicatechin-(4β→8)-epicatechin, i.e., epicatechin-(4β→8)-parameritannin A-1 (Figure 1).

Compound **8** had the molecular formula $C_{90}H_{72}O_{36}$, as deduced from HR-ESIMS, showing the quasi-molecular ion $[M - H]^-$ at *m*/*z* 1727.3726, indicating **8** to be a hexameric proanthocyanidin. This additional flavan-3-ol residue was also C-4β linked to the C-8 of another epicatechin unit, as verified by the ^{1}H NMR spectrum, showing three broad singlets in the aliphatic regions, assignable to H-2−H-4 of this additional unit (Table 1). The similarity of the ^{1}H and ^{13}C NMR data between **7** and **8** in the units I−III (Table 1) thus suggested the additional epicatechin unit to be located in the branch part. By comparison of isotopic shifts in the H−D exchange experiment,[10,11] the C-9 signals [δ 150.3, 154.4, 154.8, 155.0 (2C), and 158.0] were identified (Table 2 and Supporting Information). The HMBC spectrum designated the chemical shift of unit II′ H-4 at δ 4.53 (brs) by showing the shift correlation of this proton and unit I H-4 (δ 4.26, d, J = 3.2 Hz) to unit II C-7 (δ 148.4, s), which also suggested a linkage between unit II C-6 and unit II′ C-4. This 2D spectrum showed further correlation of unit II′ H-4 and a proton at δ 4.58 (brs) to unit II′ C-9 (δ 155.0), designating unit II″ H-4 at δ 4.58. This unit II″ proton and a proton at δ 4.73 (brs) were observed to be ^3J-correlated to unit II″ C-9 (δ 158.0, s) (Figure 3b), designating unit II‴ H-4 at δ 4.73 and suggesting the additional epicatechin moiety (unit II‴) to be C-4 linked to C-8 of the unit II″. On the basis of these analyses and the CD spectrum (Figure 2), showing a strong (+)-CE at 235 nm for the β-oriented C-4 flavan-3-ol substituents, compound **8** was elucidated as epicatechin-(4β→8,2β→*O*→7)-[epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→6)]-epicatechin-(4β→8)-epicatechin, i.e., epicatechin-(4β→8)-epicatechin-(4β→8)-parameritannin A-1 (Figure 1).

The ^{1}H and ^{13}C NMR assignments of **7** and **8** were made unambiguously by analysis of 2D NMR data (HMBC, HMQC, COSY, and TOCSY) and are listed in Table 1. During analysis of both COSY and TOCSY spectra of **7** measured at 295 K, the long-range relayed correlation of H-6 (δ 5.91) and H-8 (δ 5.99) to H-4 (δ 4.54) in unit II″, and unit II′ H-6 (δ 6.00) to unit II″ H-4, were observed, the latter of which confirmed the linkage between unit II′ and unit II″ in **7**. Similar analysis of the 2D spectra of **8** measured at 285 K also revealed the long-range correlation (^5J) of H-6 and H-8/H-4 (unit II‴)/H-6/H-4 (unit II″)/H-6 (unit II′), confirming the linkage among the units II′−II‴.

The CD data of **6**−**8** (Figure 2) demonstrated the additive effect of the epicatechin chromophore. With the increment of this unit, the molar ellipticity for the positive Cotton effect around 235 nm increased accordingly as reported.[12]

Compounds **7** and **8** are new A-type oligomeric proanthocyanidins and are named machiphilitannins A and B, respectively.

The inhibitory effects of compounds **1**−**8** against α-glucosidase type IV from *Bacillus stearothermophilus* were assayed, and the results are summarized in Table 3. Aescultitannin B (**2**) is the most potent, with an IC_{50} value of 3.5 μM. The results indicate that the A-type trimeric proanthocyanidins with the same interflavanyl linkage show quite different activity, dependent on the composition. That is, the presence of *ent*-catechin as the unit II (**2**) showed the highest activity (IC_{50} 3.5 μM), and next is catechin as the unit III (**4**) (IC_{50} 92.9 μM); the worst is the epicatechin trimer **3**, which is

Table 2. Isotopic Shift[a] ($\Delta\delta$/ppm) of C-5, C-7, and C-9 in **6**, **7**, and **8** by Comparison of ^{13}C NMR Signals in Methanol-d_3 and Methanol-d_4 (see Supporting Information)

	unit I			unit II			unit III			unit II′			unit II″		unit II‴
no.	**6**	**7**	**8**	**6**	**7**	**8**	**6**	**7**	**8**	**6**	**7**	**8**	**7**	**8**	**8**
5	0.04	0.05	0.05	0.13	0.15	0.16	0.08	0.12	0.09	0.04	0.07	0.08	0.05	0.08	0.08
7	0.05	0.05	0.06	0.00	0.00	0.00	0.05	0.05	0.04	0.08	0.10	0.12	0.03	0.15	0.05
9	−0.01	−0.03	−0.01	0.03	0.03	0.05	−0.01	−0.04	0.00	0.00	0.02	0.01	0.00	−0.02	0.03

[a] The values of isotopic shift [$\Delta\delta = \delta$(methanol-d_3) − δ(methanol-d_4)] were calculated using the signal of a well-buried carbon (C-7 in unit II) as internal standard, i.e., $\Delta\delta = 0$, in each compound.

Table 3. Inhibitory Effect of Compounds **1**−**8** and Acarbose against α-Glucosidase

compound	IC_{50}[a] (μM)
1	>100.0
2	3.5 ± 0.0
3	>100.0
4	92.9 ± 2.6
5	10.5 ± 1.8
6	>100.0
7	31.3 ± 5.7
8	18.4 ± 0.2
acarbose	0.049 ± 0.003

[a] IC_{50} values were calculated from the dose−response curve of six concentrations of each sample in triplicate.

almost inactive (IC_{50} > 100 μM). For the A-type tetramers, compound **5**, with the branch at the unit I C-6, had better activity than **6**, with the branch at the unit II C-6 (IC_{50} 10.5 vs >100 μM). For the A-type oligomers with the same branch linkage at the unit II C-6, the more epicatechin units there are in the branch part, the better the inhibitory activity appears to be, i.e., **8** > **7** > **6** (IC_{50} 18.4 vs 31.3 vs >100 μM). Such structure−activity relationship for the A-type oligomeric proanthocyanidins will be useful as a reference for the development of α-glucosidase inhibitors for blood glucose control in diabetic patients.

Some bioactivities of proanthocyanidins, such as antioxidant activity, potentiating insulin action,[13] and effect on hyperlipidemia through lowering levels of triglyceride, total cholesterol, and nonesterified fatty acids,[14] have been reported. The oligomeric procyanidin mixtures from French maritime pine bark extract (pycnogenol), which exhibited antidiabetic effects in patients,[15] showed inhibitory activity against α-glucosidase.[16] Our work demonstrates for the first time that the purified proanthocyanidins do possess such inhibitory activity. These compounds could play an important role as biomarkers in the quality control of antidiabetic oligomeric procyanidins.

Experimental Section

General Experimental Procedures. The optical rotations were recorded on a JASCO DIP-370 polarimeter. UV spectra were measured in MeOH on a Hitachi 150-20 double-beam spectrophotometer. The CD spectra were recorded on a J-720 spectropolarimeter. ^{1}H, ^{13}C, and 2D NMR spectra were obtained on a Bruker AV400 NMR spectrometer for **1**−**4** and a Bruker Avance III 600 NMR spectrometer, equipped with a 5 mm cryoprobe, for **5**−**8** (methanol-d_4, δ_H 3.30 and δ_C 49.0) using standard pulse programs. The HR-ESIMS data were measured on a micrOTOF orthogonal ESI-TOF mass spectrometer (Bruker, Daltonik, Bremen, Germany). TLC analyses were carried out on silica gel plates (KG60-F_{254}, Merck). Semipreparative HPLC separations were performed on a Phenomenex RP-18 column (Prodigy ODS-3, 250 × 10 mm, 5 μm). The microplate spectrophotometer for bioassay was SPECTRAmax PLUS (Molecular Devices, USA).

Plant Material. The leaves of *M. philippinensis* were collected in September 2007 in Fu-shan Research Center, Taiwan Forestry Research Institute, Yilan County, Taiwan. A voucher specimen (NTUSP200709MP) was authenticated by Mr. Jer-Tone Lin, Associate Researcher, Taiwan Forestry Research Institute, and was deposited in the herbarium library of that institute.

Extraction and Isolation. The EtOH extract (160 g) of the dried leaves of *M. philippinensis* (774 g) was divided into fractions soluble in CH_2Cl_2 (38.30 g), EtOAc (10.51 g), *n*-BuOH (31.02 g), and H_2O (74.12 g) by a liquid−liquid partitioning process.[1] Part of the CH_2Cl_2-soluble fraction (23.5 g) was fractionated on a Sephadex LH-20 column (2.6 L, MeOH) to give three fractions. Fraction 3 (890 mg) was separated by an RP-18 column (LiChroprep RP-18, size B, 310 × 25 mm; 40−63 μm, Merck), eluted by a stepwise gradient of MeOH−H_2O from 5:95 to 100:0, to give **2** (7.8 mg) and a fraction (21.5 mg) containing kaempferol-3-*O*-α-L-rhamnopyranoside 3″,4″-di-*E*-*p*-coumarate and 3″-*E*,4″-*Z*-di-*p*-coumarate.[1] The *n*-BuOH-soluble fraction (22.10 g out of 31.02 g), which showed 55.9% inhibition against α-glucosidase at 10 μg/mL, was fractionated on a Sephadex LH-20 column (2.6 L, MeOH) to give eight fractions. Fraction 4 (706.9 mg), showing 44.6% inhibition against α-glucosidase at 10 μg/mL, was treated with MeOH (5 mL) to give a precipitate (56.6 mg), which was identified as quercetin 3-*O*-β-D-galactopyranoside.[17] The residue (647.0 mg) obtained after evaporation of the filtrate was separated on the same Lobar RP-18 column as above, using a stepwise gradient of MeOH−H_2O from 25:75 to 65:35, to give six subfractions. Subfraction 2 (46.3 mg) was found to be pure **1**. Fraction 6 (976.3 mg), showing 33.4% inhibition against α-glucosidase at 10 μg/mL, was separated on the same Lobar RP-18 column, using a stepwise gradient of MeOH−H_2O from 5:95 to 40:60, to give eight subfractions. Subfractions 7 and 2 were found to be **3** (344.9 mg) and **6** (81.8 mg), respectively. Subfractions 4 (21.7 mg) and 5 (18.5 mg) were further purified on a semipreparative RP-18 HPLC column, using 12% MeCN in H_2O, flow rate 2.5 mL/min, to give **7** (3.4 mg, t_R = 29.0 min) and **8** (5.4 mg, t_R = 22.5 min), respectively. Fraction 7 (521.3 mg), showing 96.0% inhibition against α-glucosidase at 10 μg/mL, was separated on the same Lobar RP-18 column, delivered by a stepwise gradient of MeOH−H_2O from 10:50 to 50:50, to give five subfrations. Subfraction 4 was found to be **5** (3.6 mg). Subfraction 2 (31.4 mg) was further purified on the semipreparative RP-18 HPLC column, using 10% MeCN in H_2O, flow rate 2.5 mL/min, to give **4** (2.6 mg, t_R = 23.7 min).

Machiphilitannin A (7): off-white, amorphous powder; $[\alpha]^{27}_D$ +18.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 278.0 (4.61) nm; CD (MeOH) $\Delta\varepsilon_{236}$ +54.14, $\Delta\varepsilon_{274}$ −4.69, $\Delta\varepsilon_{279}$ −4.38 (Figure 2); ^{1}H and ^{13}C NMR data, see Table 1; HMBC, see Figure 3a; $^-$ESIMS *m*/*z* (rel int %) 1438.8 (100, [M − H]$^-$); $^-$HRESIMS *m*/*z* 1439.3092 [M − H]$^-$ (calcd for $C_{75}H_{59}O_{30}$, 1439.3091).

Machiphilitannin B (8): off-white, amorphous powder; $[\alpha]^{27}_D$ +4.3 (*c* 0.10, MeOH,); UV (MeOH) λ_{max} (log ε) 280.0 (4.34) nm; CD (MeOH) $\Delta\varepsilon_{235}$ +58.60, $\Delta\varepsilon_{274}$ −5.42, $\Delta\varepsilon_{279}$ −5.70 (Figure 2); ^{1}H and ^{13}C NMR data, see Table 1; HMBC, see Figure 3b; $^-$ESIMS *m*/*z* (rel int %) 1727.4 (100, [M − H]$^-$); $^-$HR-ESIMS *m*/*z* 1727.3726 [M − H]$^-$ (calcd for $C_{90}H_{71}O_{36}$, 1727.3725).

Additional Data for Parameritannin A-1 (6): CD, see Figure 2 and Table 5S; ^{1}H and ^{13}C NMR data, see Table 1.

Assay for α-Glucosidase Activity. The inhibitory activities against α-glucosidase type IV from *Bacillus stearothermophilus* were measured following the reported method.[1] Compounds **1**−**8** were dissolved in 10% MeOH. The positive control was acarbose (Bayer), whose IC_{50} value against the same enzyme was found to be 0.049 μM.

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Supporting Information Available: ^{1}H and ^{13}C NMR data of **1**−**5** (Tables 1S−3S); isotopic shift in ^{13}C NMR data of **6**, **7**, and **8** (Table 4S); UV, $[\alpha]^{27}_D$, and CD data of **5** and **6** (Table 5S); and 1D (^{1}H and

^{13}C NMR) and 2D spectra of **7** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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