JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Bioactive A-Type Proanthocyanidins from *Cinnamomum cassia*

K. Brian Killday,[†] Matthew H. Davey,[‡] Jan A. Glinski,[‡] Penggao Duan,[§] Ravikanth Veluri,[§] Gloria Proni,[⊥] F. Joseph Daugherty,[∥] and Michael S. Tempesta*[,∥]

[†]Bruker Biospin Corporation, Billerica, Massachusetts 01821, United States

[‡]PlantaAnalytica, Danbury, Connecticut 06810, United States

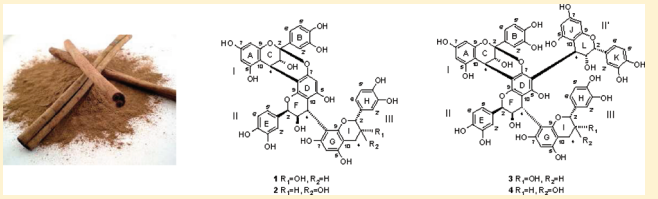
[§]Vertex Pharmaceuticals, Massachusetts 02139-4252, United States

[⊥]John Jay College of Criminal Justice, New York, New York 10019, United States

[∥]Phenolics, LLC, El Granada, California 94018-2439, United States

Supporting Information

ABSTRACT: Two trimeric proanthocyanidins, cinnamtannin B-1 (**1**) and cinnamtannin D-1 (**2**), have been isolated from the bark of *Cinnamomum cassia* along with the known tetramer parameritannin A-1 (**3**) and a previously unreported tetramer, named cassiatannin A (**4**). The structures of **1**−**4** were elucidated on the basis of 1D and 2D NMR, MS, and CD analyses and compared to the reported data. Proanthocyanidins (**1**−**4**) possess significant in vitro inhibitory activity against cyclooxygenase-2 (COX-2) at micromolar concentrations.



Cinnamon (*Cinnamomum cassia*) has been historically used as a spice for millennia. However, only recently has the bark been the subject of detailed chemical and biological evaluations. In initial testing of 50 well-known plant extracts, cinnamon showed remarkable potency to increase glucose metabolism, as measured by the epididymal fat cell assay.[1] Cinnamon extracts enriched in proanthocyanidins have also been shown to possess insulin potentiating activity.[2] Cinnamon has also been the subject of a placebo-controlled clinical study of diabetic patients for six weeks to evaluate effects on glucose and lipid metabolism.[3] Improvements in fasting glucose levels (18−29%), triglycerides (23−30%), LDL cholesterol (7−27%), and total cholesterol (12−26%) were noted over the course of the study in all three dose levels (1, 3, and 6 g/day), suggesting lower doses may also show beneficial effects. A-Type proanthocyanidins, prominently found in cinnamon, have been reported to have bioactivity in in vitro antidiabetic assays.[4] Proanthocyanidins with A-type linkages are known to be also present in cranberries, blueberries, plums, peanuts, curry, among others,[3] and are less common in nature than B-type linked proanthocyanidins occurring in grapes and grape seed extract, pine bark, and other woody biomass sources.[5]

Considerable recent research has explored the therapeutic applications of proanthocyanidins, which are primarily known for their antioxidant activity. These compounds have also been reported to demonstrate significant antibacterial, antiviral,[6−9] anticarcinogenic, anti-inflammatory, antiallergic, and vasodilatory effects. In addition, proanthocyanidins have been found to inhibit lipid peroxidation, platelet aggregation, and capillary permeability and fragility and to affect enzyme systems including phospholipase A2, cyclooxygenase (COX), and lipoxygenase. For example, proanthocyanidin monomers (i.e., catechins) and dimers have been used in the treatment of diseases associated with increased capillary fragility and have also been shown to have anti-inflammatory effects in animals.[10] On the basis of the anti-inflammatory reports, oligomeric proanthocyanidins may be useful components in the treatment of a number of conditions.[11] These findings stimulated our work to elucidate the COX-2 bioactive polyphenols from cinnamon and led to characterization of a new bioactive tetrameric A-type proanthocyanidin.[12,13]

RESULTS AND DISCUSSION

Commercial cinnamon consisting of the ground bark powder of *C. cassia* was extracted with acetone and fractionated by countercurrent chromatography followed by preparative HPLC and resulted in the isolation of two trimeric and two tetrameric proanthocyanidins (**1**−**4**). The HR-ESIMS spectra of compounds **1** and **2** both indicated molecular formulas of $C_{45}H_{36}O_{18}$. The ^{1}H and ^{13}C NMR spectra of **1** (600 MHz, methanol-d_4) were consistent with those of the trimeric proanthocyanidin cinnamtannin B-1, previously isolated from *C. zeylanicum*[14,15] (Supporting Information: Table 1S). The NMR data of **2** were similar to those of **1** with the exception of the resonances in the GHI moiety appearing at δ_H 3.94 (d, J = 9.2 Hz), 3.66 (ddd J = 10.1, 9.2, 6.0 Hz), 3.04 (dd, J = 16.2, 6.0), and 2.41 (dd, J = 16.2, 10.1) and δ_C 83.3, 70.1, and 30.7 consistent with the terminal unit being a catechin moiety. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Supporting Information: Table 1S) with the literature[4,16] established compound **2** as cinnamtannin

Received: November 8, 2010
Published: August 29, 2011

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 3 and 4 with HMBC Correlations[a]

		3		4		
ring	no.	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)
			Unit I			
C	2	100.1, C		100.3, C		
	3	66.7, CH	3.27, d (3.6)	66.9, CH	3.51, d (3.7)	I: 10
	4	28.8, CH	4.23, d (3.6)	28.9, CH	4.03, d (3.7)	I: 2,3,5,9,10 II: 7,8,9
A	5	156.7, C		156.5, C		
	6	98.2, CH	5.97, d (2.3)	98.4, CH	5.94, d (2.4)	I: 8,10
	7	157.9, C		157.9, C		
	8	96.5, CH	6.04, d (2.3)	96.6, CH	6.02, d (2.4)	I: 6,7,10
	9	154.2, C		154.2, C		
	10	104.9, C		105.1, C		
B	1′	132.3, C		132.2, C		
	2′	115.8, CH	7.14, d (1.7)	115.7, CH	7.15, d (2.1)	I: 2,4′,6′
	3′	145.5, C		145.5, C		
	4′	146.7, C		146.7, C		
	5′	116.1, CH	6.88, d (8.2)	116.0, CH	6.86, d (8.4)	I: 1′,3′
	6′	119.9, CH	6.90, dd (8.2, 1.7)	120.2, CH	6.98, dd (8.4, 2.1)	I: 2,4′
			Unit II			
F	2	78.8, CH	5.64, brs	78.5, CH	5.54, brs	II: 3,4,1′,2′,6′
	3	72.4, CH	4.04, m	72.2, CH	4.00, m	
	4	38.4, CH	4.42, brs	38.3, CH	4.41, brs	II: 2,3,5,9,10 III: 7,8,9
D	5	154.2, C		154.2, C		
	6	107.6, C		108.1, C		
	7	148.4, C		148.4, C		
	8	106.9, C		106.7, C		
	9	150.3, C		150.4, C		
	10	107.2, C		107.1, C		
E	1′	131.7, C		131.2, C		
	2′	116.7, CH	7.30, d (1.5)	116.6, CH	7.24, d (2.1)	II: 2,4′,6′
	3′	145.9, C		145.9, C		
	4′	146.3, C		146.4, C		
	5′	116.1, CH	6.82, d (8.3)	116.0, CH	67.11, d (8.1)	II: 1′,3′
	6′	121.4, CH	7.21, dd (8.3, 1.5)	121.3, CH	7.11, dd (8.1,2.1)	II: 2,4′
			Unit III			
I	2	80.1, CH	4.09, d (9.0)	83.4, CH	3.95, d (9.4)	III: 3,4,1′,2′,6′
	3	67.4, CH	3.60, m	70.1, CH	3.65, ddd (10.8, 9.4, i	5.5)
	4	29.8, CH2	2.88, dd (16.2, 5.7)	30.7, CH2	3.05, dd (16.5, 6.5)	III: 2,3,5,9,10
	4		2.77, dd (16.2, 10.1)		2.43, dd (16.5, 10.8)	III: 2,3,5,9,10
G	5	156.1, C		155.6, C		
	6	96.4, CH	6.07, s	96.6, CH	6.06, s	III: 4*,5,7,8,10, II:4*
	7	155.5, C		155.2, C		
	8	108.8, C		108.7, C		
	9	155.6, C		155.3, C		
	10	99.7, C		101.7, C		
H	1′	132.8, C		131.2, C		
	2′	115.4, CH	6.63, d (1.7)	115.7, CH	6.69, d (1.9)	III: 2,4′,6′
	3′	145.7, C		144.4, C		
	4′	145.4, C		145.9, C		
	5′	115.8, CH	6.70, d (8.1)	116.0, CH	6.76, d (8.2)	III: 1′,3′
	6′	119.2, CH	6.24, dd (8.1, 1.7)	1120.1, CH	6.48, dd (8.2, 1.9)	III: 2,4′

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 1. Continued

		3		4		
ring	no.	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)
				Unit II′		
L	2	76.5, CH	4.73, brs	77.2, CH	4.80, brs	II′: 3,4,1′,2′,6′
	3	71.2, CH	4.09, brs	72.5, CH	4.06, brs	II′: 10
	4	37.6, CH	4.36, brs	37.2, CH	4.45, brs	II′: 2,3,5,9,10 II: 5,6,7
J	5	157.9, C		15779,C		
	6	96.0, CH	5.91, d (2.2)	95.9, CH	5.92, d (2.4)	II′: 5,8,10
	7	159.5, C		159.4, C		
	8	96.6 CH	5.87, d (2.2)	96.7 CH	5.86, d (2.4)	II′: 6,10
	9	159.4, C		159.4, C		
	10	99.1, C		99.1, C		
K	1′	131.6, C		131.9, C		
	2′	116.8, CH	7.10, d (1.6)	116.2, CH	7.13, d (2.0)	II′: 2,4′,6′
	3′	146.2, C		146.2, C		
	4′	146.3, C		146.1, C		
	5′	115.9, CH	6.90, d (8.2)	116.2, CH	6.90, d (8.3)	II′: 1′,3′
	6′	120.8, CH	6.91, dd (8.2, 1.6)	120.4, CH	6.87, dd (8.3, 2.0)	II′: 2,4′

[a] Methanol-J_4, AVIII-600, 280 K; *four-bond correlation.

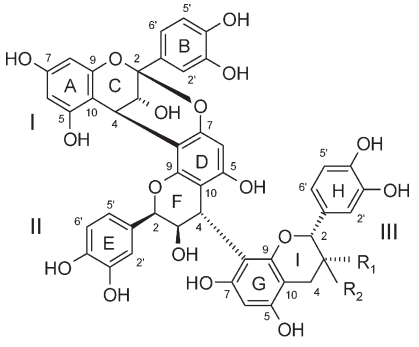


Figure 1. Structures of compounds **1** and **2**.

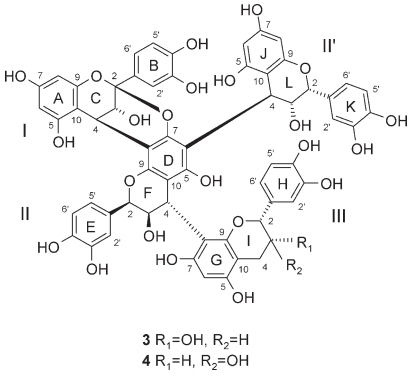


Figure 2. Structures of compounds **3** and **4**.

D-1. All ^{1}H and ^{13}C NMR resonances of **1** and **2** were assigned by analysis of the 2D NMR (HSQC, HMBC, COSY, TOCSY) data (Supporting Information).

The HR-ESIMS spectra of compounds **3** and **4** both indicated molecular formulas of $C_{60}H_{48}O_{24}$, suggesting A-type tetrameric proanthocyanidins. ^{1}H and ^{13}C NMR data of **3** and **4** at 298 K (700 MHz, methanol-d_4) indicated the presence of major and minor conformational isomers for each of these with broad resonances in the ^{1}H NMR spectrum. Lowering the temperature to 280 K significantly sharpened these resonances as previously reported for oligomeric proanthocyanidins.[4] Consequently, all spectroscopic data for these were obtained at 280 K. The ^{1}H NMR spectrum of **3** showed an AX system at δ_H 3.27, 4.23, (d 3.6 Hz) along with an acetal carbon at δ_C 100.1 consistent with a C-2/C-4 doubly linked epicatechin linkage between units I and II (ABC and DEF moiety). The ^{1}H and ^{13}C spectroscopic data (Table 1) suggested units II and III both were epicatechins consistent with the NMR data of compound **1**. The spectroscopic data indicated a branched chain proanthocyanidin with an epicatechin substituent at C-6 of unit II, which was confirmed by long-range HMBC correlations between H-4 (C-ring) and C-7, C-8, and C-9 (D-ring) and between H-4 (L-ring) and C-5, C-6, and C-7 (D-ring). Comparison of the ^{1}H and ^{13}C NMR spectra in methanol-d_4 (Table 1) with literature values[4] established this compound as parameritannin A-1, first reported from *Parameria laevigata*.[15] The ^{1}H and ^{13}C NMR spectra of **4** (Table 1) were similar to those of **3** with the exception of the resonances for the I-ring at δ_H 3.95 (d, J = 9.4 Hz), 3.65 (ddd J = 10.8, 9.4, 6.5 Hz), 3.05 (dd, J = 16.5, 6.5), and 2.43 (dd, J = 16.5, 10.8) and δ_C 83.4, 70.1, and 30.7, consistent with the terminal GHI moiety being catechin as in cinnamtannin D-1 (**2**). Long range four-bond ^{1}H−^{13}C couplings were observed in the HMBC between H-6 of unit III and C-4 of units II and III for both **3** and **4**, confirming the C-4 (unit II)/C-8(unit III) interflavanyl bond. All ^{1}H and ^{13}C NMR resonances of **3** and **4** were assigned (Table 1) by analysis of the 2D NMR (HSQC, HMBC, COSY, TOCSY) data (Supporting Information). Compound **4** has not been previously described.

The ^{1}H and ^{13}C NMR spectra of **1**−**4** in DMSO-d_6 at 298 K exhibit much more complex signals than in methanol-d_4. This can be attributed to the presence of conformational isomers due to rotational hindrance around the interflavanyl bonds,[17] as well

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 2. ^{1}H and ^{13}C NMR Data of the Conformational Isomers of Compounds 1 and 2 with HMBC Correlations[a]

		1a		1b			2a		2b		
ring	no.	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)
						unit I					
C	2	98.5, C		98.4, C			98.4, C		98.5, C		
	3	66.4, CH	3.85, m	65.0, CH	3.43, m	I: 10	66.4, CH	3.84,m	64.8, CH	3.51, m	I: 10
	3		5.31 d (4.0) OH		4.34 br OH	I: 2,3,4		5.32 d (3.4) OH			I: 2,3,4
	4	28.0, CH	4.29, d (3.5)	27.5, CH	4.06, d (3.4)	I: 2,3,5,9,10 II: 7,8,9	28.0, CH	4.28, d (3.1)	27.3, CH	3.84, brs	I: 2,3,5,9,10 II: 7,8,9
A	5	156.0, C	7.90 s OH	155.5, C	7.39 s OH	I: 5,6,10	156.3, C		155.4, C		
	6	96.6, CH	5.90, d (2.2)	96.8, CH	5.79, d (2.2)	I: 5,7,10	96.6, CH	5.90, d (2.2)	96.8, CH	5.76, d (2.2)	I: 5,7,10
	7	156.5, C		156.3, C			156.5, C		156.3, C		
	8	94.8, CH	5.89, d (2.2)	94.8, CH	5.84, d (2.2)	I: 6,7,10	94.7, CH	5.89, d (2.2)	94.8, CH	5.84, d (2.2)	I: 6,7,10
	9	152.7, C		152.6, C			152.8, C		152.7, C		
	10	103.1, C		103.9, C			103.0, C		104.0, C		
B	1′	131.0, C		130.9, C			131.0, C		130.8, C		
	2′	114.7, CH	7.03, d (2.1)	115.7, CH	6.97, d (1.8)	I: 2,4′,6′	114.7, CH	7.03, d (1.8)	114.9, CH	7.00, d (1.7)	I: 2,4′,6′
	3′	144.4, C		144.2, C			144.3, C		144.2, C		
	4′	145.4, C		144.2, C			145.5, C		144.2, C		
	5′	114.7, CH	6.77, d (8.3)	114.9, CH	6.76, d (8.2)	I: 1′,3′	114.7, CH	6.77, d (8.3)	114.9, CH	6.80, d (8.2)	I: 1′,3′
	6′	117.9, CH	6.88, dd (8.3, 2.1)	117.9, CH	6.70, dd (8.2, 1.8)	I: 2,4′	117.9, CH	6.88, dd (8.2, 1.8)	118.1, CH	6.79, dd (8.2,1.7)	I: 2,4′
						Unit II					
F	2	76.6, CH	5.13, brs	76.3, CH	5.41, brs	II: 3,4,1′,2′,6′	76.7, CH	5.21, brs	76.3, CH	5.26, brs	II: 3,4,1′,2′,6′
	3	71.0, CH	3.78, m	69.6, CH	3.92, m		70.5, CH	3.80, m	69.5, CH	3.90, m	
	4	36.7, CH	4.48, brs	37.1, CH	4.30, brs	II: 2,3,5,9,10 III: 7,8,9	36.4, CH	4.46, brs	37.1, CH	4.26, brs	II: 2,3,5,9,10 III: 7,8,9
D	5	155.0, C	8.86, s OH	154.0, C	8.56, s OH	II: 5,6,10	154.9, C		154.0, C		
	6	94.1, CH	5.95, s	94.0, C	5.67 s	II: 4*,5,7,8,10 I: 4*	94.1, CH	5.95, s	94.0, C	5.68 s	II: 4*,5,7,8,10 I: 4*
	7	150.0, C		148.9, C			149.9, C		148.8, C		
	8	104.5, C		104.2, C			104.4, C		104.1, C		
	9	151.0, C		150.4, C			152.7, C		150.4, C		
	10	105.2, C		105.4, C			105.4, C		105.1, C		
E	1′	130.1, C		130.1, C			130.2, C		129.8, C		
	2′	114.9, CH	6.94, d (1.8)	115.9, CH	7.19, d (1.8)	II: 2,4′,6′	115.09, CH	6.97, d (2.1)	115.7, CH	7.14, d (1.4)	II: 2,4′,6′
	3′	144.7, C		144.8, C			144.4, C		144.5, C		
	4′	144.7, C		144.7, C			144.7, C		145.3, C		
	5′	115.2, CH	6.72, d (8.4)	114.9, CH	6.73, d (8.2)	II: 1′,3′	115.15, CH	6.73, d (8.3)	114.8, CH	6.72, d (8.2)	II: 1′,3′
	6′	118.4, CH	6.93, dd (8.4, 1.8)	119.7, CH	7.00, dd (8.2, 1.8)	II: 2,4′	119.0, CH	6.94, dd (8.3, 2.1)	119.6,CH	7.00,dd (8.2,1.4)	II: 2,4′

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 2. Continued

		1a		1b			2a		2b		
ring	no.	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)	δ_C m	δ_H m (J/HZ)	δ_C m	δ_H m (J/HZ)	HMBC (unit: C#)
						Unit III					
I	2	77.5, CH	4.95, brs	78.5, CH	4.25, brs	III: 3,4,1′,2′,6′	80.1, CH	4.48, d (5.1)	81.6, CH	3.81, d (8.7)	III: 3,4,1′,2′,6′
	3	64.7, CH	4.19, m	64.8, CH	3.70, m		66.1, CH	3.94,m	67.5, CH	3.50, m	
	4	27.5, CH2	2.70, dd (16.4, 4.4)	28.8, CH2	2.64, dd (16.4, obs)	III: 2,3,5,9,10	25.8, CH2	2.44, dd (16.2, 4.6)	29.9, CH2	2.82, dd (15.8,5.4)	III: 2,3,5,9,10
	4		2.37, dd (16.4, 4.6)		2.50, dd (16.4, 4.4)	III: 2,3,5,9,10		2.39, dd (16.2, 5.4)		2.27, dd (15.8,9.8)	III: 2,3,5,9,10
G	5	154.0, C		154.2, C			153.8, C		154.0, C		
	6	95.7, CH	5.81, s	94.9, CH	6.07, s	III: 4*,5,7,8,10, II: 4*	95.7, CH	5.80, s	95.2, CH	6.06, s	III: 4*,5,7,8,10, II: 4*
	7	152.6, C	8.36 s OH	153.8, C		III: 6,7,8	154.3, C	8.36 s OH	153.8, C		III: 6,7,8
	8	106.8, C		107.2, C			106.7, C		107.3, C		
	9	154.0, C		154.0, C			152.5, C		153.6, C		
	10	98.8, C		98.4, C			98.5, C		99.7 C		
H	1′	130.1, C		131.2, C			131.2, C		130.9, C		
	2′	114.9, CH	6.94,d (1.8)	114.7, CH	6.70, d (1.8)	III: 2,4′,6′	113.5, CH	6.76,d (1.8)	114.6, CH	6.61, d (1.4)	III: 2,4′,6′
	3′	144.7, C		144.2, C			144.7, C		144.5, C		
	4′	144.7, C		143.9, C			144.4, C		144.2, C		
	5′	115.2, CH	6.72, d (8.4)	114.4, CH	6.68, d (8.1)	III: 1′,3′	115.2, CH	6.65, d (8.1)	114.8, CH	6.70, d (8.2)	III: 1′,3′
	6′	118.9, CH	6.93, dd (8.4,1.8)	117.6, CH	6.53, dd (8.1, 1.8)	III: 2,4′	117.5, CH	6.81, dd (8.1,1.8)	118.0, CH	6.47, dd (8.2,1.4)	III: 2,4′

[a] DMSO-d_6, AVIII-700, 298 K; *four-bond correlation.

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 3. ^{1}H and ^{13}C NMR Data of the Conformational Isomers of Compounds 3 and 4 with HMBC Correlations[a]

		3a		3b			4a		4b		
ring	no.	δ_C m	δ_H m (J/Hz)	δ_C m	δ_H m (J/Hz)	HMBC (unit: C#)	δ_C m	δ_H m (J/Hz)	δ_C m	δ_H m (J/Hz)	HMBC (unit: C#)
						unit I					
C	2	98.6, C		98.8, C			98.8, C		98.8, C		
	3	64.7, CH	3.58, m	65.8, CH	3.89, m	I: 10	65.0, CH	3.49, m	65.9, CH	3.88, m	I: 10
	3									5.33, d (4.0) OH	I: 2,3,4
	4	27.4, CH	4.10, bs	28.0, CH	4.37, bs	I: 2,3,5,9,10 II: 7,8,9	27.4, CH	3.83, d (3.5)	28.1, CH	4.35, d (3.4)	I: 2,3,5,9,10 I: 7,8,9
A	5	155.4, C		155.7, C			155.3, C	7.16, s OH	155.7, C	7.72, s OH	I: 5,6,10
	6	96.8, CH	5.78, d (2.4)	96.7, CH	5.89, d (2.2)	I: 5,7,10	96.9, CH	5.75, d (2.3)	96.8, CH	5.89, d (2.2)	I: 5,7,10
	7	156.3, C		156.3, C			156.3, C		156.5, C		
	8	94.7, CH	5.87, d (2.4)	94.6, CH	5.90, d (2.2)	I: 6,7,10	94.7, CH	5.87, d (2.3)	94.6, CH	5.91, d (2.2)	I: 6,7,10
	9	153.0, C		152.9, C			153.1, C		153.1, C		
	10	103.8, C		103.4, C			103.9, C		103.3, C		
B	1′	130.7, C		130.7, C			130.7, C		130.6, C		
	2′	115.6, CH	7.04, d (1.8)	115.6, CH	7.07, d (1.9)	I: 2,4′,6′	115.5, CH	7.07, d (1.7)	115.7, CH	7.08, d (1.9)	I: 2,4′,6′
	3′	144.8, C		144.7, C			144.9, C		144.8, C		
	4′	145.1, C		144.9, C			145.1, C		145.4, C		
	5′	114.9, CH	6.69, d (8.1)	115.1, CH	6.70, d (8.0)	I: 1′,3′	115.0, CH	6.76, d (8.2)	115.2, CH	6.71, d (8.3)	I: 1′,3′
	6′	117.9, CH	6.73, dd (8.1, 1.8)	118.0, CH	6.93, dd (8.0, 1.9)	I: 2,4′	118.2, CH	6.84, dd (8.2, 1.7)	118.0, CH	6.92, dd (8.3, 1.9)	I: 2,4′
						Unit II					
F	2	76.2, CH	5.40, brs	76.6, CH	6.00, brs	II: 3,4,1′,2′,6′	76.1, CH	5.30, brs	76.8, CH	5.06, brs	II: 3,4,1′,2′,6′
	3	69.3, CH	3.91, m	70.8, CH	3.81, m		69.2, CH	3.87, m	70.4, CH	3.88, m	
	3							4.96, d (4.2) OH		4.50, d (5.2) OH	II: 2,3,4
	4	37.1, CH	4.14, brs	37.1, CH	4.31, brs	II: 2,3,5,9,10 HI: 7,8,9	37.0, CH	4.14, brs	36.8, CH	4.23, brs	II: 2,3,5,9,10 III 7,8,9
D	5	152.1, C	5.54, s OH	153.1, C	5.87, s OH	II: 5,6,10	152.0, C	5.47, s OH	153.1, C	5.88, s OH	II: 5,6,10
	6	106.3, C		107.5, C			106.7, C		106.9, C		
	7	146.2, C		147.0, C			146.3, C		146.8, C		
	8	105.1, C		105.2, C			105.0, C		105.2, C		
	9	148.6, C		148.9, C			148.7, C		149.2, C		
	10	106.0, C		105.5, C			105.8, C		106.2, C		
E	1′	129.7, C		129.6, C			129.5, C		129.8, C		
	2′	115.9, CH	7.20, d (1.7)	115.2, CH	6.95, d (1.8)	II: 2,4′,6′	115.9, CH	7.15, d (l.6)	115.3, CH	6.97, d (1.8)	II: 2,4′,6′
	3′	144.7, C		144.8, C			144.9, C		144.9, C		
	4′	144.9, C		144.6, C			145.0, C		145.0, C		
	5′	115.2, CH	6.73, d (8.1)	115.2, CH	6.71, d (8.2)	II: 1′,3′	115.9, CH	6.76, d (8.1)	115.1, CH	6.73, d (8.2)	II: 1′,3′
	6′	119.7, CH	7.03, dd (8.1, 1.7)	119.1, CH	6.91, dd (8.2, 1.8)	II: 2,4′	119.8, CH	7.01, dd (8.1, 1.6)	119.3, CH	6.93, dd (8.2, 1.8)	II: 2,4′

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 3. Continued

		3a		3b			4a		4b		
ring	no.	δ_C m	δ_H m (J/Hz)	δ_C m	δ_H m (J/Hz)	HMBC (unit: C#)	δ_C m	δ_H m (J/Hz)	δ_C m	δ_H m (J/Hz)	HMBC (unit: C#)
						Unit III					
I	2	78.6, CH	4.10, brs	77.4, CH	4.79, brs	III: 3,4,1′,2′,6′	81.7, CH	3.85, d (9.1)	80.5, CH	4.63, d (8.3)	III: 3,4,1′,2′,6′
	3	64.7, CH	3.58, m	64.4, CH	4.13, m		67.2, CH	3.51, m	66.3, CH	3.80, m	
	3							4.73, d (3.7) OH		4.90, d (4.6) OH	III: 2,3,4
	4	29.0, CH2	2.54, m	28.4, CH2	2.67, dd (16.2, 5.1)	III: 9,10	30.1, CH2	2.87, dd (16.1, 5.8)	27.3, CH2	2.49, dd (16.1, 6.8)	III: 2,3,5,9,10
	4		2.54, m		2.44, dd (16.2, 7.1)	III: 9,10		2.22, dd (16.1, 10.1)	2.35, dd	(16.1, 5.0)	III: 2,3,5,9,10
G	5	154.4, C		154.9, C			153.8, C	9.11, s OH	153.9, C	8.98, s OH	III: 5,10
	6	95.2, CH	6.03, s	95.5, CH	5.99, s	III: 4*,5,7,8,10, II: 4*	95.4, CH	6.04, s	95.5, CH	5.82, s	III: 4*,5,7,8,10, II: 4*
	7	153.6, C		154.3, C			153.8, C	9.08, s OH	154.4, C	8.37, s OH	III: 6,7,8
	8	106.7, C		105.7, C			106.8, C		106.1, C		
	9	153.8, C		152.9, C			153.7, C		152.7, C		
	10	98.2, C		99.6, C			99.7, C		99.1, C		
H	1′	130.4, C		130.9, C			130.6, C		130.8, C		
	2′	114.1, CH	6.99, d (1.7)	114.6, CH	6.61, brs	III: 2,4′,6′	114.5, CH	6.58, d (1.4)	113.7, CH	6.72, d (1.6)	III: 2,4′,6′
	3′	144.4, C		144.4, C			144.4, C		144.7, C		
	4′	143.9, C		144.0, C			145.0, C		144.3, C		
	5′	115.0, CH	6.56, d (8.0)	114.3, CH	6.61, d (8.2)	III: 1′,3′	115.5, CH	6.75, d (8.0)	115.0, CH	6.48, d (8.1)	III: 1′,3′
	6′	117.7, CH	6.69, dd (8.0,1.7)	117.1, CH	6.14, brd (8.2))	III: 2,4′	118.4, CH	6.29, dd (8.0,1.4)	117.9, CH	6.60, dd (8.1, 1.6)	) III: 2,4′
						Unit II′					
L	2	75.4, CH	4.55, brs	76.0, CH	4.80, d (7.0)	II′: 3,4,1′,2′,6′	76.0, CH	4.71, brs	76.0, CH	4.63, brs	II′: 3,4,1′,2′,6′
	3	70.1, CH	3.75, m	72.3, CH	3.67, m	II′: 10	70.9, CH	3.82, m	72.1, CH	3.69, m	II′: 10
	3							4.63, d (5.8) OH		II′: 2,3,4	
	4	35.5, CH	4.25, brs	35.3, CH	4.48, brs	II′: 2,3,5,9,10 II: 5,6,7	35.3, CH	4.32, brs	35.5, CH	4.47, brs	II′: 2,3,5,9,10 II: 5,6,7
J	5	157.7, C	9.23, s OH	157.5, C		II′: 5,6,10	157.6, C	9.22, s OH	157.9, C	9.37, s OH	II′: 5,6,10
	6	95.6, CH	5.82, d (2.2)	95.5, CH	5.83, d (2.4)	II′: 5,8,10	95.7, CH	5.80, d (2.2)	95.7, CH	5.90, d (2.2)	II′: 5,8,10
	7	156.4, C		156.3, C			156.4, C		156.1, C		
	8	94.5, CH	5.77, d (2.2)	94.6, CH	5.78, d (2.2)	II′: 6,10	94.5, CH	5.78, d (2.2)	94.7, CH	5.86, d (2.2)	II′: 6,10
	9	156.2, C		156.3, C			156.1, C		156.3, C		
	10	97.7, C		98.5, C			97.9, C		97.5, C		

dx.doi.org/10.1021/np1007944 |J. Nat. Prod. 2011, 74, 1833–1841

Table 3. Continued

		3a		3b			4a		4b		
ring	no.	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)	HMBC (unit: C#)	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)	HMBC (unit: C#)
K	1′	130.1, C		130.7, C			130.5, C		130.3, C		
	2′	115.5, CH	6.94, d (1.6)	114.9, CH	6.92, d (1.7)	II′: 2,4′,6′	115.0, CH	6.93, d (1.6)	115.6, CH	7.06, d (1.6)	II′: 2,4′,6′
	3′	145.1, C		145.0, C			145.2, C		145.1, C		
	4′	144.7, C		144.7, C			144.7, C		144.8, C		
	5′	114.8, CH	6.77, d (8.2)	115.1, CH	6.72, d (8.1)	II′: 1′,3′	115.0, CH	6.70, d (8.2)	115.1, CH	6.76, d (8.2)	II′: 1′,3′
	6′	118.1, CH	6.50, dd (8.2, 1.6)	118.4, CH	6.51, dd (8.1, 1.7)	II′: 2,4′	118.5, CH	6.50, dd (8.2, 1.6)	118.3, CH	6.57, dd (8.2, 1.6)	II′: 2,4′

[a] DMSO-d_6, AVIII-700, 298 K; *four-bond correlation.

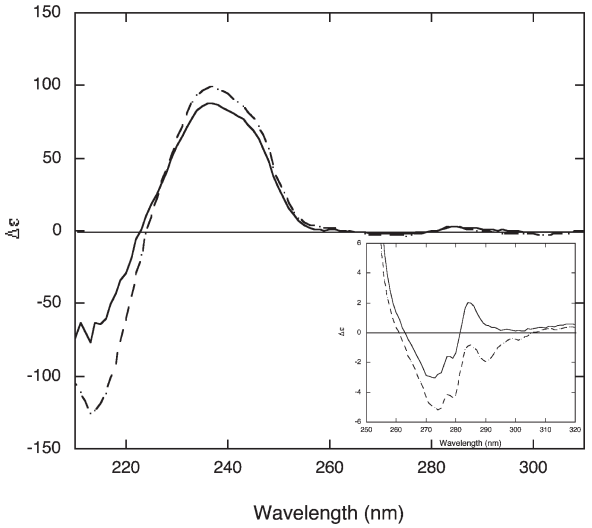


Figure 3. CD spectra of parameritannin A-1, **3** (- - -), and cassiatannin A, **4** (——), recorded in MeOH. In the inset is shown a magnified 320−250 nm region.

as the presence of the exchangeable protons in the spectra. Compounds **1** and **2** each exhibited two sets of resonances assignable to two conformational isomers present in a ratio of 1.4:1 and 2.5:1, respectively, and analysis of the 2D NMR data at high field (700 MHz, Supporting Information) permitted the complete ^{1}H and ^{13}C NMR assignments for all conformers (Table 2). The major conformers for these are designated **1a** and **2a** and the minor conformers **1b** and **2b**. The exchangeable proton resonances provided additional key structural information. The HMBC correlations between H-4 of the F-ring, between C-5 and C-10 of the D-ring, and between OH-5 and C-10, C-5, and C-6 allow unambiguous assignment of the D-ring proton to be H-6 rather than H-8, further establishing the C-4−C-8 linkage between units I and II. The ^{1}H and ^{13}C NMR spectra of **3** and **4** exhibited exceptionally complex signals. Compound **3** exhibited resonances for a major and a minor conformer with a ratio of 1.5:1, while **4** contained a 1:1 mixture of conformers. The 2D NMR data (Supporting Information) permitted the complete assignments of the conformational isomers of these (Table 3). The major and minor isomers of **3** are designated as **3a** and **3b**, respectively, whereas those of **4** are assigned as **4a** and **4b** by similarity of the chemical shifts of the I, II, and II′ subunits to those of **3a** and **3b**.

The CD spectra obtained for the tetramer parameritannin A-1 (**3**) and cassiatannin A (**4**) are very similar and characterized by a weak Cotton effect at 275 nm and a strong positive Cotton effect at 238 nm followed by a negative Cotton effect at 215 nm (Figure 3). These bands are assigned, respectively, to the ^1L_b, ^1L_a, and ^1B electronic transitions of the aromatic moieties in the flavan-3-ol rings. The structure differences between **3** and **4** are reflected in different intensities for the CD signals of the two compounds, with parameritannin A-1, **3**, providing higher intensities. The Cotton effect at 238 nm is consistent with the β-orientation of the C-4 flavan-3-ol groups.[18] On the basis of the relative configuration determination via NMR, together with correlation of the Cotton effects previously reported,[15] the absolute configurations of **3** and **4** as depicted are in accord with other related epicatechin-based natural products, which are designated 2*S*.

The inhibitory effects of proanthocyanidins **1**−**4** against the COX-2 enzyme isolated from human recombinant Sf9 cells were determined following published procedures.[19] Each compound was tested in triplicate at 3-log dilutions of 10, 100, and 1000 μg/mL concentrations, with all four compounds exhibiting significant inhibition at all levels. The tetramers **3** (% inhibition: 14, 86, 94) and **4** (% inhibition: 38, 52, 97) display higher activity in this assay than the trimers **1** (% inhibition: 19, 27, 86) and **2** (% inhibition: 2, 44, 95). These results are in accord with the number of proanthocyanin units−bioactivity relationship of B-type proanthocyanidins noted earlier when tested for antiviral activity,[8] but A-type proanthocyanins are not necessarily good models for prediction purposes. Indeed, more recent testing of related oligomeric proanthocyanidins for α-glucosidase bioactivity presents a more ambiguous picture.[4]

EXPERIMENTAL SECTION

General Experimental Procedures. The CD spectra were recorded on a Jasco J810 in the Chemistry Department of Columbia University using circular 1 cm path-length circular quartz cuvettes from Hellma. The concentrations for the solutions of **3** and **4** in MeOH were determined by UV spectroscopy (data not presented) on a Jasco V630 UV−vis instrument using rectangular Hellma quartz cuvette with path-length of 1 cm. The extinction coefficient used for both compounds is log e = 4.41 in MeOH at 280 nm, as described by other authors for compound **3**.[15] The concentrations for compounds **3** and **4** in Figure 3 are, respectively, 4.13×10^{-6} and 7.61×10^{-6} in Figure 3, while, in the inset, they are 2.98×10^{-5} and 3.78×10^{-5}. ^{1}H, ^{13}C, and 2D NMR spectra were obtained on a Bruker Avance III 600 NMR spectrometer equipped with a 5 mm DCH cryoprobe and on a Bruker Avance III 700 NMR spectrometer equipped with a 5 mm TCI cryoprobe using standard pulse programs. Spectra were recorded in methanol-d_4 and DMSO-d_6 and referenced to the solvent resonances (methanol-d_4, δ_H 3.30, δ_C 49.0; DMSO-d_6, δ_H 2.50, δ_C 39.5). The HR-ESIMS data were measured on a Bruker Daltonics micrOTOF-Q ESI-TOF mass spectrometer.

Plant Material. Powdered cinnamon lot # 7A11-1172 was purchased from Monterey Bay Spice Company.

Extraction and Isolation. The powdered cinnamon (0.45 kg) was extracted with 2.5 L of 70% aqueous acetone for 20 h. The resulting extract was evaporated to dryness on a Rotovap to produce a red solid foam (ca. 45 g). This extract was fractionated by countercurrent chromatography, using a Kromaton FCPC, with a 1 L rotor (Kromaton Technologies, Sainte Gemmes sur Loire, France). The rotor was filled with the lower phase of a solvent system containing EtOAc−MeOH−H_2O (6:1:5 v/v). Eight grams of the extract was dissolved in a mixture of both phases each of 50 mL and pumped into the rotor using Waters HPLC pump model 590. The rotational speed was set at 800 rpm, and the upper phase of the solvent system was pumped at 8 mL/min. Eluent was collected into test tubes at 24 mL/tube using a Gilson race track fraction collector model 220. The content of the test tubes was analyzed by HPLC, and the eluent was combined into two fractions. The first fraction (tubes 52 to 68) contained the proanthocyanidin trimers **1** and **2** (1.42 g), and tubes 72 to 96 as the main HPLC peaks contained proanthocyanidin tetramers **3** and **4** (0.87 g). Isolation of pure compounds **1**−**4** was done by preparative HPLC, using a Waters system consisting of a controller and a pump model 600, Rheodyne 7125 sample loop, and a UV detector model 600. A chromatographic column YMC ODS 150 × 30 mm, 120 nm, 5 μm was used in a gradient of MeCN from 8% to 17% over 30 min.

Cassiatannin A (4): off-white, amorphous powder; CD (MeOH) $\Delta\varepsilon_{235}$ +87.87, $\Delta\varepsilon_{274}$ −2.29 (Figure 3); ^{1}H and ^{13}C NMR data and HMBC correlations in methanol-d_4, see Table 1; ^{1}H and ^{13}C NMR data and HMBC correlations in DMSO-d_6, see Table 3; HR-ESIMS m/z 1153.2605 $[M - H]^-$ (calcd for $C_{60}H_{49}O_{24}$, 1153.2608).

ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR data of **1**−**4** (Tables 1S−4S), 1D and 2D NMR spectra (S1−S15), and HR-ESIMS spectra (S16−S21). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel/fax: 650-726-5725. E-mail: natprod@aol.com.

ACKNOWLEDGMENT

We thank Dr. T. Jolas (Cerep, Paris, France) for performing the cyclooxygenase studies.

REFERENCES

(1) Broadhurst, C. L.; Polansky, M. M.; Anderson, R. A. *J. Agric. Food Chem.* **2000**, *48*, 849–852.
(2) Anderson, R. A.; Broadhurst, C. L.; Polansky, M. M.; Schmidt, W. F.; Khan, A.; Flanagan, V. P. *J. Agric. Food Chem.* **2004**, *52*, 65–70.
(3) Khan, A.; Safdar, M.; Khan, M. M. A.; Khattak, K. N.; Anderson, R. A. *Diabetes Care* **2003**, *26*, 3215–3218.
(4) Lin, H.-S.; Lee, S.-S. *J. Nat. Prod.* **2010**, *73*, 1375–1380.
(5) Tempesta, M. S.; Barrett, M. In *Botanical Medicine from Bench to Bedside*; Cooper, R., Kronenberg, F., Eds.; Mary Ann Liebert: New Rochelle, NY, 2009; p 209.
(6) Erdelmeier, C. A.; Cinatl., J., Jr.; Rabenau, H.; Doerr, H. W.; Biber, A.; Koch, E. *Planta Med.* **1996**, *62*, 241–245.
(7) DeBruyne, T.; Pieters, L. *J. Nat. Prod.* **1999**, *62*, 954–958.
(8) Takechi, M.; Tanaka., T.; Takehara, M.; Nonaka, G. I.; Nishioka, I. *Phytochemistry* **1985**, *24*, 2245–2250.
(9) Fukuchi, K.; Sakagami, H.; Okuda, T.; Hatano, T.; Tanuma, S. *Antiviral Res.* **1989**, *11*, 285–298.
(10) Beladi, I.; Pusztai, R.; Mucsl, I.; Makay, M.; Gabor, M. *Ann. N.Y. Acad. Sci.* **1977**, *284*, 358.
(11) Fine, A. M. *Altern. Med. Rev.* **2000**, *5*, 144–151.
(12) Tempesta, M. S., Glinski, J. A., Killday, K. B. U.S. Patent Application 20100029756, 2010.
(13) Tempesta, M. S.; Glinski, J. A.; Davey, M.; Kane, R.; Killday, K. B. 7th Joint Meeting of GA, AFERP, ASP, PSI and SIF, Athens, Greece, August 5, 2008; SL122.
(14) Nonaka, G.; Morimoto, S.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2139–2145.
(15) Kamiya, K.; Watanabe, C.; Endang, H.; Umar, M.; Satake, T. *Chem. Pharm. Bull.* **2001**, *49*, 551–557.
(16) Kamiya, K.; Ohno, A.; Horii, Y.; Endang, H.; Umar, M.; Satake, T. *Heterocycles* **2003**, *60*, 1697–1708.
(17) Morimoto, S.; Nonaka, G. I.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 4717–4729.
(18) Botha, J. J.; Young, D. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1213–1219.
(19) Glaser, K.; Sung, M. L.; O'Neill, K.; Belfast, M.; Hartman, D.; Carlson, R.; Kreft, A.; Kubrak, D.; Hsiao, C. L.; Weichman, B. *Eur. J. Pharmacol.* **1995**, *281*, 107–111.