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Cinnamoyl glucosides of catechin and dimeric procyanidins from young leaves of *Inga umbellifera* (Fabaceae)

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Abstract

The rapidly growing, nearly achlorophyllous, young leaves of *Inga umbellifera* express high concentrations of mono and dimeric 3-*O*-gluco-cinnamoyl catechin/epicatechin, rare forms of substituted flavan-3-ols. Here we present structures for five novel compounds in this class: three monomers [catechin-3-*O*-*β*-D-gluco(2-cinnamoyl)pyranoside, catechin-3-*O*-*β*-D-gluco(6-cinnamoyl) pyranoside, catechin-3-*O*-*β*-D-gluco(2,6-biscinnamoyl)pyranoside] and two dimeric procyanidins [catechin-3-*O*-*β*-D-glucopyrano-(4α→8)-catechin-3-*O*-*β*-D-gluco(2-cinnamoyl)pyranoside and catechin-3-*O*-*β*-D-glucopyrano-(4α→8)-epicatechin-3-*O*-*β*-D-gluco(6-cinnamoyl)pyranoside]. The young leaves of *Inga umbellifera* express high concentrations of 3-*O*-(cinnamoyl)glucosides of catechin and epicatechin.

Keywords: *Inga umbellifera*; Fabaceae; Young leaf secondary chemistry; Acetylated flavan-3-ol glycoside; Acetylated procyanidin glycoside; Cinnamoyl glycoside

1. Introduction

As part of a study of leaf developmental patterns in woody tropical forest plants, we are characterizing the secondary chemistry of expanding young leaves in *Inga umbellifera* (Mimosoideae, Fabaceae). This Neotropical species ranges from Nicaragua to central Brazil where it is frequently encountered in non-flooding moist forests (Pennington, 1997). The pattern of young leaf development in *I. umbellifera* differs from that of many other *Inga* species in that leaves are produced in rapidly expanding synchronous flushes and chlorophyll deployment is delayed until relatively late in leaf maturation (Kursar and Coley, 2003). We are investigating the form and function of defense metabolites that are associated with this pattern of leaf development. The leaves of *I. umbellifera*, like many *Inga* species, contain non-protein amino acids (Kite, 1997) and, in some populations, flavonoid metabolites (Harborne, 1997). We have found that young *I. umbellifera* leaves from populations growing in Panama synthesize high concentrations of an unusual class of flavans, catechin/epicatechin-3-*O*-*β*-D-gluco(cinnamoyl)pyranosides, as the major phenolic metabolite. Although flavan-3-ols such as catechin typically occur in nature as unsubstituted monomers or as proanthocyanidins (Bohm, 1998), they are occasionally encountered as gallates (Lewis et al., 1998; Malan, 1991; Nonaka et al. 1982), benzoates (Hwang et al., 2001; De Mello et al., 1996) and glycosides (Bae et al., 1994; Kolodziej et al., 1991; Zhang et al., 1988; Tschesche et al., 1980; Dokotsch et al., 1973). Considerably less common are acylated glycosides. The very few reports of compounds in this class include a coumaroyl/feruloyl di-substituted glucoside of catechin from *Cinnamomum cassia* (Morimoto et al., 1986) and two epicatechin-3-*O*-allo(cinnamoyl)pyranosides from the fern *Davallia divaricata* (Murakami et al., 1985). Here we present structures for five novel 3-*O*-gluco(cinnamoyl)pyranosides of flavan-3-ols: three catechin monomers, a catechin dimer and a mixed catechin/epicatechin dimer.

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2. Results and discussion

Fresh young leaves of *I. umbellifera* were homogenized and extracted with 80% aq. EtOH. After removal of the alcohol, the remaining aqueous phase was de-fatted with ligroin and subjected to a polarity fractionation. Compound **3** (Fig. 1) was extracted into Et_2O and compounds **1** and **2** (Fig. 1) were extracted into EtOAc. Compounds **4** and **5** (Fig. 2) were separated along with the majority of the flavanoid fraction by passing the aqueous suspension onto an octadecylsilane (ODS) solid-phase extraction column, washing with water and eluting with MeOH. Compounds **1**–**3** were purified using silica gel flash chromatography and were isolated as yellowish powders (**1** and **3**) or as a pink powder (**2**). Compounds **4** and **5** required separation by semi-preparative HPLC using an ODS column. They were isolated as white powders.

HRFAB mass spectra of compounds **1**–**3** gave an $[M+H]^+$ ion of *m*/*z* 583.1792 for **1,** a $[M+Na]^+$ ion of *m*/*z* 605.1631 for **2** and an $[M+H]^+$ ion of *m*/*z* 713.2259 for **3**. These indicated molecular formulas of $C_{30}H_{30}O_{12}$ for **1** and **2** and $C_{39}H_{36}O_{13}$ for **3**.

The 1D ^1H and ^{13}C NMR and DEPT spectra of **1**–**3** (Table 1) provided several clues as to their component structures. The presence of three isolated and two vicinal oxygen-bearing aromatic carbons as well as a single upfield methylene in each compound suggested a flavan-3-ol backbone. Compounds **1** and **2** possessed two additional, and compound **3** four additional, double-intensity aromatic peaks in the ^{13}C NMR spectra, indicating the presence of one and two symmetrical benzene rings respectively. The COSY/DEPT/HMQC spectra of each compound showed an isolated spin system of five oxymethine carbons and a single terminal methylene carbon, thus establishing a pyranose ring system. HMBC outlined the gross structural features of each compound: all three contained a 5,7-dihydroxychromane moiety with a 3,4-dihydroxyphenyl substituent at the C-2 position. This showed that the backbone was flavanoid of the catechin/epicatechin type. The *O*-glycosidic linkage was established as occurring at C-3 in each molecule. HMBC also showed that the mono-substituted aromatic systems were bonded through a two-carbon olefinic system to a carboxyl center. The 16 Hz couplings of the olefinic protons established this fragment to be a *trans*-cinnamoyl group. In each of compounds **1–3**, the carboxyl carbon correlated with a pyranose carbon but the patterns of esterification differed: in **1,** linkage occurred at the methylene carbon C′-6; in **2**, it occurred at C′-2, and in **3**, it occurred at both C′-2 and C′-6.

Relative stereochemistries for **1**–**3** were determined from ^1H–^1H couplings and from ROE correlations obtained from a 2D TROESY sequence. Compounds **1**–**3** all showed 6.5–8 Hz splittings between the C-2 and C-3 protons, indicating 1,2-diaxial relationships. This evidence coupled with the ROE observed in each case between H-C-2 and H_β-C-4 established the catechin-type relative stereochemistry for all three compounds. For compound **1**, the pyranose stereochemistry was inferred from the 1D proton/COSY spectra which showed first-order couplings of 8–9.5 Hz between each of the adjoining ring protons, including the anomeric

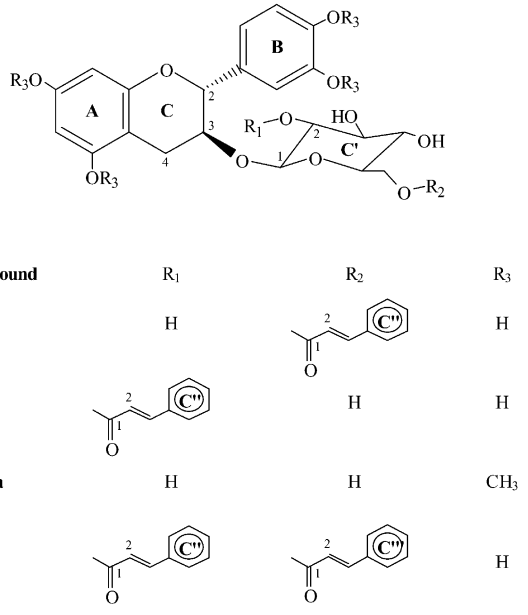

Fig. 1. Major monomeric flavanoids from *I. umbellifera*.

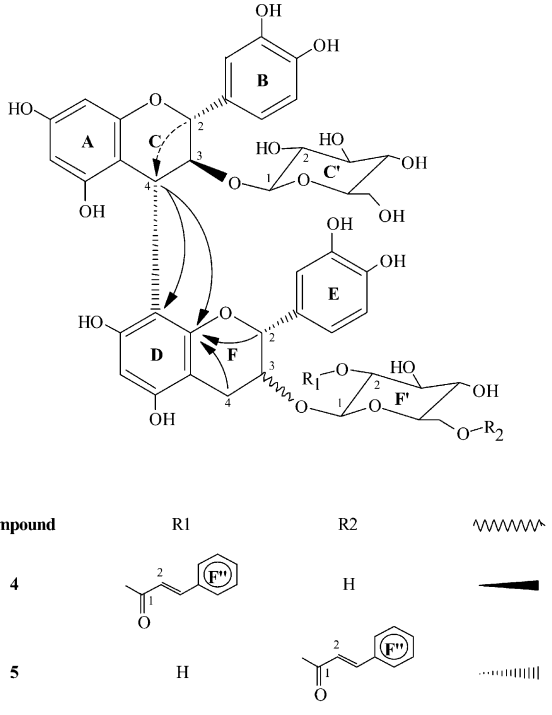

Fig. 2. Major dimeric flavanoids of *I. umbellifera*. Pertinent HMBC (⟶) and ROE (⋯⟶) correlations are indicated.

proton. The all-axial hydrogen configuration thus labeled the sugar moiety as β-D-glucose. In compounds **2** and **3**, complete glycoside stereochemistries could not be extracted from the 1D proton spectrum because of overlap of the D-3 and D-4 protons. In **2**, splittings between the protons at C′-1/2 (8 Hz), C′-2/3 (8 Hz) and C′-4/5 (9.5 Hz) established 1,2-diaxial relationships between each adjoining hydrogen. This evidence, coupled with the strong ROE that was observed between the C′-1 and C′-5 protons, again confirmed the β-D-glucose stereochemistry. In compound **3**, the 1D proton spectrum showed an 8 Hz coupling between the C′-1 and C′-2 protons, establishing a 1,2-diaxial relationship. The other pyranose couplings were obscured by overlaps or were non-first order. Analysis of the crosspeak between the C′-2 and C′-3 protons in the phase-sensitive COSY spectrum showed an active coupling of 9.5 Hz, indicating a 1,2-diaxial relationship. Strong ROE's were observed between the C′-1 and C′-5 protons as well as between the C′-2 and C′-4 protons. This latter interaction can only

Table 1
13C and 1H NMR chemical shifts in acetone-d_6 (1, 2, 3) and methanol-d_4 (**2a**); 1H multiplicities and coupling constants for **1**–**3**

C#	**1**		**2**		**2a**		**3**	
	δ ^{13}C	δ ^{1}H, mult., Hz	δ ^{13}C	δ ^{1}H, mult., Hz	δ ^{13}C	δ ^{1}H, mult., Hz	δ ^{13}C	δ ^{1}H, mult., Hz
C-2	79.6	4.90 *d* 6.5	80.4	4.65 *d* 8.0	80.5	4.98 *d* 6.5	80.2	4.68 *d* 7.8
C-3	76.3	4.19 *m*	74.8	4.21 *m*	75.4	4.31 *m*	75.6	4.17 *m*
C-4	26.7	2.91 *dd* 16.5, 5.5 (α)	27.7	2.90 *dd* 16.5, 5.5 (α)	26.9	2.80 *dd* 16.5, 5.5 (α)	24.7	3.04 *dd* 16.5, 5.5 (α)
		2.76 *dd* 16.5, 7.0 (β)		2.63 *dd* 16.5, 8.0 (β)		2.72 *dd* 16.5, 6.5 (β)		2.67 *dd* 16.5, 8.0 (β)
A-5	152.7		157.1		160.2		157.0	
A-6	96.3	6.04 *d* 2.5	96.3	6.03 *d* 2.5	92.5	6.13 *d* 2.5	96.3	6.02 *d* 2.5
A-7	157.7		157.8		161.3		157.7	
A-8	95.5	5.91 *d* 2.5	95.5	5.85 *d* 2.5	94.3	6.11 *d* 2.5	95.4	5.83 *d* 2.5
A-9	156.5		156.7		156.5		156.6	
A-10	100.3		100.5		102.9		100.4	
B-1	132.1		131.9		133.5		131.7	
B-2	114.2	6.90 *d* 2.0	115.4	6.90 *d* 2.0	112.8	7.01 *d* 1.6	115.3	6.90 *d* 2.0
B-3	145.8		145.8		150.4		145.7[a]	
B-4	145.7		145.8		150.5		145.6[a]	
B-5	115.9	6.81 *d* 8.5	115.8	6.81 *d* 8.0	112.0	6.93 *d* 8.5	115.8	6.78 *d* 8.0
B-6	119.4	6.78 *dd* 8.5, 2.0	120.1	6.71 *dd* 8.0, 2.0	120.7	6.96 *dd* 8.5, 1.5	120.1	6.73 *dd* 8.0, 2.0
C′-1	104.1	4.32 *d* 8.0	101.1	4.27 *d* 8.0	103.6	4.05 *d* 7.5	101.5	4.39 *d* 8.0
C′-2	74.9	3.13 *dd* 9.0, 8.0	74.7	4.78 *dd* 8.0, 8.0	75.3	3.1 *dd* 9.0, 7.5	74.4	4.84 *m*
C′-3	77.6	3.32 *dd* 9.0, 9.0	76.2	3.43 *o*	77.9	3.18 *dd* 9.0, 9.0	76.0	3.50 *o*
C′-4	71.4	3.37 *dd* 9.5, 9.0	72.0	3.43 *o*	71.7	3.23 *dd* 9.0, 9.0	71.7	3.50 *o*
C′-5	75.0	3.54 *ddd* 9.5, 6.5, 2.0	77.9	3.29 *ddd* 9.5, 6.0, 2.0	78.3	3.14 *m*	75.0	3.55 *o*
C′-6	64.6	4.59 *dd* 12.0, 2.0	63.0	3.70 *dd* 12.0, 6.0	63.0	3.85 *dd* 12.0, 2.3	64.3	4.64 *dd* 12.0, 2.0
		4.29 *dd* 12.0, 6.5		3.29 *dd* 12.0, 2.0		3.64 *dd* 12.0, 6.0		4.34 *dd* 12.0, 6.0
C″-1	167.2		166.3				166.1	
C″-2	118.9	6.58 *d* 16.0	119.6	6.56 *d* 16.5			119.5	6.50 *d* 16.0
C″-3	145.5	7.69 *d* 16.0	145.1	7.66 *d* 16.5			145.1	7.64 *d* 16.0
C″-4	135.3		135.7				135.6	
C″-5	129.1	7.61 *m*	129.2	7.75 *ddd* 8.0, 1.5, 0.5			129.2	7.75 *o*
C″-6	129.8	7.40 *o*	129.9	7.45 *o*			129.8	7.45 *o*
C″-7	131.1	7.40 *o*	131.0	7.45 *o*			131.0	
C″-8	129.8	7.40 *o*	129.9	7.45 *o*			129.8	7.45 *o*
C″-9	129.1	7.61 *m*	129.2	7.75 *ddd* 8.0, 1.5, 0.5			129.2	7.75 *o*
C‴-1							167.1	
C‴-2							118.9	6.60 *d* 16.0
C‴-3							145.6	7.64 *d* 16.0
C‴-4							135.3	
C‴-5							129.2	7.60 *m*
C‴-6							129.8	7.41 *o*
C‴-7							131.1	7.42 *o*
C‴-8							129.8	7.41 *o*
C‴-9							129.2	7.60 *m*
Ome					56.6	3.83 *s*		
Ome					56.6	3.82 *s*		
Ome					56.0	3.79 *s*		
Ome					55.9	3.73 *s*		

s = singlet, *d* = doublet, *dd* = double doublet, *ddd* = doublet of double doublets, *o* = overlap, *m* = multiplet.
[a] Interchangeable assignment.

occur if H-C′-4 is axial, again confirming the pyranose to have all-axial proton stereochemistry. As in compounds **1** and **2** therefore, the sugar moiety in **3** is β-D-glucose.

Absolute stereochemistries of the flavan moiety in compounds **1–3** were determined by their circular dichroic (CD) properties. Cotton effects (CE's) associated with the 280 nm (^1L_b) transition of flavan-3-ols are reliable indicators of stereochemistry at C-2, with the *R* and *S* configurations consistently showing negative and positive CE's respectively (van Rensburg et al., 1999). CD spectra of **1–3** recorded in this region gave results which were not readily interpretable however. Compound **1** showed a negative CE ($[\theta] = -3{,}020$, 282 nm) while **2** gave a positive CE ($[\theta] = 14{,}850$, 289 nm) and **3** showed no CE at 280 nm. To resolve these discrepancies, compounds **1–3** were treated with an excess of diazomethane at 22 °C. These reaction conditions led to the simultaneous methylation of phenolic hydroxyls and deacylation of the sugar moieties of these compounds. The resulting derivatives had identical ^1H NMR spectra. The compound formed by methylation/deacylation of **2**, compound **2a** (Fig. 1 and Table 1), was fully characterized by 1 and 2D NMR spectroscopic analysis and was shown to be 5,7,3′,4′-tetra-*O*-methylcatechin-3-*O*-β-D-glucopyranoside, with a predicted molecular formula of $C_{25}H_{32}O_{11}$. This was confirmed by ESI-MS which gave a molecular ion, $[M+Na]^+$, of *m*/*z* 531.2 amu. The CD spectra of all three deacylation products gave negative CE's at 280 nm, indicating that they share the same absolute stereochemistry at C-2. The deacyl derivative of **1** was then converted to 5,7,3′,4′-tetra-*O*-methylcatechin (TMC) by acid hydrolysis of the glucoside. The ^1H NMR spectrum of this compound was identical to standard TMC that had been prepared from (+)-catechin. In addition, ESI-MS gave a molecular ion, $[M+H]^+$, of *m*/*z* 347.4 amu for the derivative of compound **1**, indicating the molecular formula of TMC, $C_{19}H_{22}O_6$. Finally, the CD spectra of standard TMC and the TMC of plant origin were measured. Both gave negative CE's in the ^1L_b transition, $[\theta] = -1757$ (280 nm) and −2639 (279 nm), respectively. From these results, we conclude that compounds **1–3** have the same 2*R*, 3*S* absolute stereochemistry, the flavan-3-ol enantiomer most commonly found in plants. Spectral studies of these three cinnamoyl glucosides demonstrate that, while CD analysis is a useful tool for determination of the absolute stereochemistry of flavans, CE's can vary widely depending on the nature of substituents near chiral centers. Chemical conversion of the unknown to a form that permits unequivocal compar-

Table 2
^{13}C and ^1H NMR chemical shifts for **4** and **5** in methanol-d_4, ^1H multiplicities and coupling constants for **4–5**

C#	**4**		**5**		C#	**4**		**5**	
	$\delta^{13}C$	δ^1H, mult., Hz	$\delta^{13}C$	δ^1H, mult., Hz		$\delta^{13}C$	δ^1H, mult., Hz	$\delta^{13}C$	δ^1H, mult., Hz
C-2	82.5	4.27 *d* 9.5	82.7	4.38 *d* 10.0	D-6	96.6	6.06 *s*	96.4	6.05 *s*
C-3	81.2	4.47 *o*	82.2	4.69 *dd* 10.0, 6.5	D-7	155.6		154.9	
C-4	37.5	4.45 *o*	37.5	4.56 *d* 6.5	D-8	109.3		109.5	
A-5	158.7		159.0		D-9	155.3		153.9	
A-6	97.0	5.76 *d* 2.0	97.0	5.75 *d* 2.0	D-10	103.0		101.7	
A-7	157.2		157.5		E-1	131.4		131.8	
A-8	97.5	5.87 *d* 2.0	97.7	5.93 *d* 2.0	E-2	116.8	6.62 *o*	114.1	6.47 *br s*
A-9	157.2		157.2		E-3	145.9		145.7	
A-10	107.2		107.3		E-4	146.4		145.9	
B-1	132.2		132.5		E-5	116.2	6.71 *d* 8.5	116.4	6.53 *br d* 8.0
B-2	116.9	6.68 *d* 1.5	116.6	6.96 *d* 1.5	E-6	121	6.39 *br d* 8.5	119	6.04 *m*
B-3	145.8		146.7		F′-1	101.9	4.02 *d* 8.5	102.3	4.47 *d* 7.5
B-4	146.5		146.1		F′-2	75.4	4.71 *dd* 9.0, 8.5	75.4	3.18 *dd* 9.0, 7.5
B-5	116.6	6.64 *d* 8.5	116.4	6.76 *d* 8.0	F′-3	76.4	3.29 *o*	77.6	3.41 *dd* 9.0, 9.0
B-6	120.9	6.33 *dd* 8.5, 1.5	121.2	6.70 *dd* 8.0, 1.5	F′-4	71.9	3.29 *o*	72	3.38 *m*
C′-1	103.5	3.40 *d* 8.0	103.9	3.63 *d* 8.5	F′-5	78.3	3.10 *m*	75.6	3.61 *m*
C′-2	75.6	2.82 *dd* 9.5, 8.0	75.9	2.90 *dd* 8.5, 8.5	F′-6	62.8	3.84 *br d* 12.0	65.1	4.61 *dd* 12.0, 1.5
C′-3	77.2	2.92 *dd* 9.5, 9.5	77.3	3.02 *dd* 9.0, 8.5			3.64 *dd* 12.0, 6.2		4.32 *dd* 12.0, 6.0
C′-4	71	3.10 *dd* 10.0, 9.5	71.4	3.10 *dd* 9.0, 9.0	F″-1	168.2		168.8	
C′-5	77	2.68 *m*	77.1	2.83 *m*	F″-2	119.5	6.60 *o*	118.7	6.56 *d* 16.0
C′-6	62.7	3.55 *dd* 11.5, 2.5	63	3.63 *o*	F″-3	146.6	7.70 *o*	146.9	7.71 *d* 16.0
		3.43 *dd* 11.5, 4.0		3.41 *o*	F″-4	136.1		135.8	
F-2	81.8	4.50 *d* 8.5	77.7	5.23 *d* 4.5	F″-5	129.7	7.73 *m*	129.5	7.52 *m*
F-3	76.3	3.98 *m*	74.2	4.25 *m*	F″-6	130.2	7.46 *o*	130.1	7.37 *o*
F-4	29.6	2.96 *dd* 16.5, 6.0 (α)	25.3	2.71 *dd* 17.0, 4.6 (β)	F″-7	131.6	7.44 *o*	131.6	7.37 *o*
		2.63 *dd* 16.5, 7.0 (β)		2.50 *dd* 17.0, 4.6 (α)	F″-8	130.2	7.46 *o*	130.1	7.37 *o*
D-5	155.2		155.8		F″-9	129.7	7.73 *m*	129.5	7.52 *m*

d = doublet, *dd* = double doublet, *ddd* = doublet of double doublets, *o* = overlap, *m* = multiplet, br = broad.

ison to an enantiomer of known stereochemistry will generally be necessary to arrive at a reliable determination of absolute configuration.

Having unambiguous structures for compounds **1**–**3** greatly expedited structural solutions of **4** and **5** (Fig. 2). Relative to **1**–**3**, ^{13}C NMR spectra of **4** and **5** (Table 2) showed double the number of flavanoid and sugar resonances. These included the readily identifiable vicinal and isolated phenolic carbons of catechin/epicatechin and the anomeric glycoside carbons. These data suggested that the molecules were dimers of flavanoid glycosides. Analysis of DEPT, HSQC and COSY spectra gave two pyranose ring systems. For each compound, HMBC, with supporting evidence from 1D proton and COSY spectra, yielded a single cinnamate moiety and two flavan-3-ols of the catechin/epicatechin type. This predicted an empirical formula of $C_{51}H_{52}O_{23}$. HRMALDI mass spectra of each compound provided confirmation, giving an $[M+Na]^+$ ion of m/z 1055.2844 for compound **4** and an $[M+Na]^+$ ion of m/z of 1055.2796 for compound **5**.

HMBC correlations permitted unequivocal determination of the interflavanoid bond position. In both **4** and **5**, the lower unit D-8 and D-9 carbons shared correlations with upper unit H-C-4. In addition, D-9 had correlations with H-F-2 and H-F-4, thus establishing the linkage as C4→C8 in each case (Fig. 2) and placing **4** and **5** in the 'B' series of procyanidins. Unlike other dimers in this class, **4** and **5** did not show duplications of ^{13}C and ^{1}H signals due to atropisomerism, suggesting that the glycosides prevented subunit rotation around the interflavanyl bond. HMBC showed that the glycosidic linkages in both **4** and **5** occur at C-3 and F-3 carbons, respectively. The single cinnamate substitution, for both molecules, appeared on the sugar moiety of the lower flavanoid subunit though at different sites: in **4**, it was at the F′-2 position, in **5**, it was at the F′-6 position. Fortuitous dispersion of the proton resonances in both of the upper-unit sugar moieties permitted straightforward stereochemical assignments: coupling constants of 8-9.5 Hz between vicinal protons indicated all-axial proton configurations and, as in **1**–**3**, β-D-glucose. Overlaps made the lower-unit sugars more difficult to decipher. In **4**, splittings at the anomeric proton (8.5 Hz) and H-F′-2 (8.5 and 9 Hz) indicated axial interactions between the protons at F′-1 and F′-3. In addition, H-F′-1 and H-F′-2 showed strong ROE correlations to H-F′-5 and H-F′-4 respectively, establishing all-axial proton orientations. Similarly in **5**, a 7.5 Hz splitting of the anomeric proton was observable in the 1D proton NMR spectrum. Examination of a 1D trace through H-F′-3 in the F2 dimension of an HMQC spectrum revealed an 8 Hz/8 Hz double doublet indicating axial orientations of the protons at F′-1 through F′-4. ROE correlations between H-F′-1, H-F′-3 and H-F′-5 established all-axial proton configurations. β-D-Glucose therefore is the only pyranose structure in **4** and **5**.

In compound **4**, ^{13}C NMR shifts of 82.5 and 81.8 ppm, respectively at the position 2 carbon of the upper and lower flavanoid subunit, as well as 8.5–9.5 Hz doublets for the attached protons, indicated 2,3-*trans* stereochemistry for both subunits. These observations were confirmed using ROESY sequences. In the lower subunit, a 2D TROESY experiment showed a strong ROE correlation between H-F-2 and H_β-F-4, but no correlation appeared between H-F-2 and H-F-3, thereby confirming the 2,3-*trans* configuration. In the upper subunit of **4**, unfavorable proton dispersion required that a 1D ROESY sequence be used. This experiment was acquired at −30 °C to slow conformational changes in the C-ring which had obscured the results of experiments run at higher temperatures. Irradiation of the C-2 proton produced an ROE at H-C-4 but none at H-C-3, thus confirming the overall relative stereochemistry of **4** to be catechin-3-*O*-β-D-glucopyrano-(4α→8)-catechin-3-*O*-β-D-gluco(2-cinnamoyl)pyranoside. In compound **5**, the C-2 ^{13}C NMR spectral shift of δ 81.8 and the proton doublet of 8.5 Hz indicated 2,3-*trans* configuration of the upper subunit. Again, this was confirmed by use of the 1D ROESY experiment run at −30 °C. Irradiation of H-C-2 produced an ROE at H-C-4 but none at H-C-3. This both verified a 2,3-*trans* configuration of the C-ring and established the α-type interflavanyl linkage. In the lower subunit of **5**, the F-2 carbon resonated at considerably higher field (δ 77.7) than its analog in compound **4**. This suggested a 2,3-*cis* stereochemistry (Porter, 1989). The F-4 proton shifts (δ 2.71, 2.50) and their $^3J_{3\text{-}4}$ couplings (4.5 Hz, 4.5 Hz) were similarly suggestive of a 2,3-*cis* stereochemistry although the F-2 proton doublet of 4.5 Hz was equivocal. A 2D TROESY sequence was used to resolve the discrepancy. It showed a weak ROE correlation between H-F-2 and H_β-F-4 but strong correlations between H-F-2 and H-F-3 as well as between H-F-3 and H_β-F-4. These data are only consistent with a 2,3-*cis* stereochemistry. Compound **5** therefore has the overall relative stereochemistry of catechin-3-*O*-β-D-glucopyrano-(4α→8)-epicatechin-3-*O*-β-D-gluco(6-cinnamoyl)pyranoside. The dimers that form the core of **4** and **5,** procyanidins B-3 and B-4, are commonly reported from plants (Bohm, 1998).

CD spectra of **4** and **5** are nearly identical, particularly in the short wavelength interval between 200 and 220 nm. Both compounds show sharply negative couplets at ca. 215 nm, with $\theta = -2.07 \times 10^5$ and -1.85×10^5, respectively. In 4-arylflavan-3-ols, the sign of the short wavelength Cotton effect is a consistent indicator of upper subunit C4 stereochemistry (Barrett et al., 1979). For the short wavelength couplet, dimers having 2*R*, 3*R*, 4*R* upper subunit stereochemistry, e.g. procyanidins B-1 and B-2, show positive CE's and dimers having 2*R*, 3*S*, 4*S* upper subunit stereochemistry, e.g. procyanidins B-3 and B-4, show negative CE's. The CD

spectra of **4** and **5** are consistent with the catechin-4α→aryl relative stereochemistry that was deduced from NMR spectroscopic data. The sum of the spectral data however do not exclude the possibility of the enantiomer's occurrence.

The five compounds described here, cinnamoyl-glucopyranosides of catechin and catechin-(4α→8)-catechin/epicatechin, are a small subset of a considerable array of related compounds expressed in the young leaves of *I. umbellifera*, an array which numbers at least in the many tens. As is typical of the proanthocyanidin profiles in many plants, including its congener *I. goldmanii* (unpublished data), *I. umbellifera* expresses compounds that are based on similar monomeric structures and have variable stereochemistry. However another layer of complexity, cinnamoyl-glycosylation, is present in these compounds in comparison to the unsubstituted proanthocyanidins found most commonly in land plants.

3. Experimental

3.1. General

Optical rotations were measured with a Perkin-Elmer 343 polarimeter. UV spectra were obtained using a Cary Conc 50 UV–vis spectrophotometer (Varian), whereas CD spectra were recorded with an AVIV 62A DS spectrophotometer. HRFAB MS were acquired on a Finnigan MAT 95 spectrometer in glycerol/MeOH, and HRMALDI MS were obtained on an Applied Biosystems Voyager DE-STR instrument in MeOH/CHCA. ESI-MS were acquired on a MicroMass Quattro instrument in the positive ion mode by infusion in MeOH. NMR spectra were recorded on a Varian iNOVA 500 spectrometer at 26 °C (unless otherwise noted). Solvents used were acetone-d_6 (compounds **1**–**3**) and methanol-d_4 (compounds **2a**, **4**, **5**). The center line of each solvent multiplet was used as internal reference, 2.05 and 3.31 ppm respectively in proton spectra, 29.9 and 49.2 ppm respectively in ^{13}C spectra. ^{1}H–^{1}H correlations were observed with gradient-selected double-quantum filter COSY sequences. One-bond ^{1}H–^{13}C correlations were observed with phase-cycled HMQC and gradient-selected HSQC sequences. Multiple-bond ^{1}H–^{13}C correlations were observed with phase-cycled and gradient-selected HMBC sequences. ROE correlations were observed using gradient-selected 1D ROESY and 2D TROESY sequences (5 KHz spectral window, 38 dB spin-lock power, 50 μs spin-lock pulse width and a mix time of 600 ms). Flash chromatography was run on a 1.9×20 cm column using 'Baker' Silica Gel 40 μm Flash Chromatography Packing (JT Baker). Solid phase extraction (SPE) was performed on a manually-packed 1.9×30 cm column loaded with Bakerbond Octadecyl (C_{18}) 40 μm Prep LC Packing (JT Baker). HPLC was carried out using a Hitachi L6200A pump equipped with an L4500 diode-array detector and a 10×250 mm Microsorb (5 μm) semi-prep. column (Varian), with a guard column (Varian) of proprietary material.

3.2. Plant material

The collection site is the Barro Colorado Nature Monument (BCNM, 79° 50′ W, 9° 10′ N) longitude), Republic of Panama, managed by the Smithsonian Tropical Research Institute (STRI). Young leaves (105 g), at 5–80% of full expansion, were gathered from several *I. umbellifera* trees in November of 2001. Identification was made by Dr. P.D. Coley. Vouchers are maintained at the BCNM Herbarium (#5345 and #11889) and at the STRI Herbarium, Panama (#5379 and #6748).

3.3. Extraction and isolation

Extraction of the leaves was begun within hours of collection. Leaves were macerated with a Waring blender followed by a Polytron (Kinematica, Switzerland) in 380 ml of 95% EtOH (ca. 80% EtOH including leaf water). The suspension was stored at −50 °C until shipped to the University of Utah on dry ice where it was stored at −80 °C. In our lab, the suspension was filtered and the marc was extracted a second time H_2O–EtOH (1:1, 400 ml). EtOH was removed from the extract under reduced pressure, and, the aqueous phase (ca. 250 ml) was defatted with ligroin and extracted three times with equal volumes of first Et_2O and then EtOAc. The remaining aqueous phase was divided into three portions which were applied to an ODS SPE column, washed with four column-volumes (ca. 500 ml total) of water and eluted with one column volume of MeOH. The MeOH elutions (ca. 375 ml) were combined. The Et_2O, EtOAc and MeOH fractions were dried under vacuum to yield extracts 0.36, 1.44 and 2.98 g, respectively. A 55 mg portion of the Et_2O fraction was subjected to separation on silica gel using an isocratic elution of MeOH–CH_2Cl_2 (1:9, ca. 300 ml) which gave 23 mg of **3**. A 300 mg portion of the EtOAc fraction was similarly subjected to separation by flash chromatography on silica gel (MeOH/CH_2Cl_2 (12:88, 300 ml) which gave 38 mg of **1** and 34 mg of **2**. Compounds **4** and **5** were purified from 1.2 g of the MeOH eluate by semi-preparative ODS HPLC using a linear gradient elution consisting of MeOH and 20 mM phosphate buffer at pH 2.1 and proceeding from 48 to 68% MeOH in 50 min. at a flow rate of 3.5 ml/min. Compounds **4** and **5** were retained 21 and 32 min, respectively. Fractions were pooled, concentrated and desalted using the same column but run with an isocratic elution of 35 and 40% aqueous MeOH for **4** and **5**, respectively. Concentration followed by lyophilization yielded 17 mg of **4** and 21 mg of **5**.

*3.3.1. (+)-Catechin-3-O-β-D-gluco(2-cinnamoyl)-pyranoside (**1**)*

Yellowish powder, $[\alpha]_D^{22}$ (MeOH) −39.0 (*c* 0.013 g/ml); CD (MeOH) $[\theta]_{282}$−3020, $[\theta]_{237}$ +2 910; UV λ_{max}^{MeOH} nm (log ϵ) 279 (4.78), 236sh (4.0), 222sh (4.3); ^{1}H and ^{13}C NMR: see Table 1; HR-FAbmS *m/z* 583.1792 amu, $[M+H]^+$, calc. for $C_{30}H_{31}O_{12}$, −2.0 mmu error.

*3.3.2. (+)-Catechin-3-O-β-D-gluco(6-cinnamoyl)-pyranoside (**2**)*

Pinkish powder, $[\alpha]_D^{22}$ (MeOH) +44.1 (*c* 0.012 g/ml), CD (MeOH) $[\theta]_{289}$ 14 850, $[\theta]_{235}$ -5820, UV λ_{max}^{MeOH} nm (log ϵ) 279 (4.3), 236sh (4.1), 222sh (4.4); ^{1}H and ^{13}C NMR data, see Table 1; HR-FAbmS *m/z* 605.1631 amu, $[M+Na]^+$, calc. for $C_{30}H_{30}NaO_{12}$, +0.4 mmu error.

*3.3.3. (+)-Catechin-3-O-β-D-gluco(2,6-bis-cinnamoyl)-pyranoside (**3**)*

Yellowish powder, $[\alpha]_D^{22}$ (MeOH) +34.5 (*c* 0.035 g/ml); CD (MeOH) $[\theta]_{263}$ 7710, $[\theta]_{235}$ −6370; UV nm (log ϵ) 279 (4.7), 223sh (4.7); ^{1}H and ^{13}C NMR data, see Table 1; HR-FAbmS *m/z* 713.2259 amu, $[M+H]^+$, calc. for $C_{39}H_{37}O_{13}$, +2.4 mmu error.

*3.3.4. Preparation of 5,7,3′,4′-tetra-O-methylcatechin-3-O-β-D-glucose (**2a**): methylation/deacylation of **1–3***

A subsequent extraction and CC purification of plant material yielded **1** (100 mg), **2** (120 mg), and **3** (95 mg). These were individually dissolved in MeOH (3 ml) and treated with an excess of a diazomethane in diethyl ether (15 ml) 3× for 24 h at 22 °C, conditions which led to the simultaneous methylation and deacylation of **1**–**3**. Purification by semi-preparative ODS HPLC yielded 35, 42 and 25 mg respectively of the deacylated tetra-methyl ethers of **1**–**3** (**2a**): White powder, CD (MeOH) $[\theta]_{280}$ −2440; UV λ_{max}^{MeOH} nm (log ϵ) 279 (3.85), 230sh (4.52), $[\alpha]_D^{22}$ (MeOH) −6.0 (*c* 0.009 g/ml); for ^{1}H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z* obsd. $[M+Na]^+$ 531.3 amu, calculated for $C_{25}H_{32}O_{11}Na$.

3.3.5. Preparation of 5,7,3′,4′-tetra-O-methylcatechin (TMC) from (+)-catechin

(+)-Catechin (20 mg, Sigma) was dissolved in MeOH (1 ml) and treated with excess diazomethane in diethyl ether (15 ml) for 16 h at 22 °C. TMC was purified by semi-prep ODS HPLC. CD (MeOH) $[\theta]_{280}$ −1760; UV λ_{max}^{MeOH} nm (log ϵ) 279 (3.9); 230sh (4.5).

*3.3.6. Preparation of 5,7,3′,4′-tetra-O-methylcatechin (TMC) from deacylated tetramethyl **1***

Of deacyl-**1** (35 mg, prepared as above, Section 3.3.4) was dissolved in MeOH and refluxed in 20 ml of 0.5 N HCl/MeOH for 3 h. TMC was purified by semi-prep ODS HPLC. CD (MeOH) $[\theta]_{279}$ −2640; ESI-MS *m/z* 347.3 amu, $[M+H]^+$, calc. for $C_{19}H_{22}O_6$.

*3.3.7. Catechin-3-O-β-D-glucopyrano-(4α→8)-catechin-3-O-β-D-gluco(2-cinnamoyl)pyranoside (**4**)*

White powder, $[\alpha]_D^{22}$ (MeOH) −56.3 (*c* 0.0103 g/ml); CD (MeOH) $[\theta]_{237}$ −73 700, $[\theta]_{237}$ −207 800, UV (CH_3OH) λ_{max} nm (log ϵ) 280 (4.5) 236sh (4.3); ^{1}H and ^{13}C NMR data, see Table 2; HR-MALDIMS *m/z* 1055.2792 amu, $[M+Na]^+$, calc. for $C_{51}H_{52}O_{23}Na$, −0.5 mmu error.

*3.3.8. Catechin-3-O-β-D-glucopyrano-(4α→8)-epicatechin-3-O-β-D-gluco(6-cinnamoyl)pyranoside (**5**)*

White powder, $[\alpha]_D^{22}$ (MeOH) −76.7 (*c* 0.0113 g/ml); CD (MeOH) $[\theta]_{237}$ −69 600, $[\theta]_{215}$ −185 400; UV (CH_3OH) λ_{max} nm (log ϵ) 280 (4.4) 236sh (4.4); ^{1}H and ^{13}C NMR data, see Table 2; HR-MALDIMS *m/z* 1055.2844 amu, $[M+Na]^+$, calc. for $C_{51}H_{52}O_{23}$, +4.7 mmu error.

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References

Bae, Y.S., Burger, J.F.W., Steynberg, J.P., Ferreira, D., Hemingway, R.W., 1994. Flavan and procyanidin glycosides from the bark of blackjack oak. Phytochemistry 35, 473–478.

Barrett, M.W., Klyne, W., Scopes, P.M., Fletcher, A.C., Porter, L.J., Haslam, E., 1994. Plant proanthocyanidins. Part 6. Chiroptical studies. Part 95. Circular dichroism of procyanidins. Journal of the Chemical Society, Perkin Transactions 1, 2375–2377.

Bohm, B.A., 1998. Introduction to the Flavanoids. Harwood Academic Publishers, Australia.

De Mello, J.P., Petereit, F., Nahrstedt, A., 1996. Flavan-3-ols and prodelphinidins from *Stryphnodendron adstringens*. Phytochemistry 41, 807–813.

Dokotsch, R.W., Mikhail, A.A., Chatterji, S.K., 1973. Structure of the water-soluble feeding stimulant for *Scolytus multistriatus*: a revision. Phytochemistry 12, 1153–1155.

Harborne, J.B., 1997. Flavonoid patterns in the genus *Inga*. In: Pennington, T.D. (Ed.), The Genus Inga Botany. The Royal Botanic Gardens, Kew, p. 74.

Hwang, B.Y., Kim, H.S., Lee, J.H., Hong, Y.S., Ro, J.S., 2001. Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. Journal of Natural Products 64, 82–84.

Kite, G.C., 1997. Non-protein amino acid chemistry. In: Pennington, T.D. (Ed.), The Genus Inga Botany. The Royal Botanic Gardens, Kew, p. 59.

Kolodziej, H., Bonefeld, M., Burger, J.F.W., Brandt, E.V., Ferreira, D.,

1991. Structure and conformational analysis of a procyanidin 3-O rhamnoside from *Erythroxylum novagranatense*. Phytochemistry 30, 1255–1258.

Kursar, T.A., Coley, P.D., 2003. Convergence in defense syndromes of young leaves in tropical rainforests. Biochemical Systematics and Ecology 31, 929–949.

Lewis, J.R., Davis, A.L., Cai, Y., Davies, A.P., Wilkins, J.P.G., Pennington, M., 1998. Theaflavate B, isotheaflavin-3′-O-gallate and neotheaflavin-3-O-gallate: three polyphenolic pigments from black tea. Phytochemistry 49, 2511–2519.

Malan, E., 1991. Derivatives of dextro catechin-5-gallate from the bark of *Acacia nilotica*. Phytochemistry 30, 2737–2740.

Morimoto, S., Nonaka, G-I., Nishioka, I., 1985. Tannins and related compounds. XXXIX. Procyanidin C-glucosides and an acylated flavan-3-ol glucoside from the barks of *Cinnamomum cassia* Blume and *C. obtusifolium* Nees. Chemical and Pharmaceutical Bulletin 34, 643–649.

Murakami, T., Wada, H., Tanaka, N., Kuraishi, T., Saiki, Y., Chen, C.M., 1985. Chemical and chemotaxonomical studies of Filices. 56. Constituents of the davalliaceous ferns. 1. Yakugaku Zasshi 105, 649–654.

Nonaka, G-I., Miwa, N., Nishioka, I., 1982. Stilbene glycoside gallates and proanthocyanidins from *Polygonum multiflorum*. Phytochemistry 21, 429–432.

Porter, L.J.. In: Dey, P.M., Harborne, J.B. (Eds.), Methods in Plant Biochemistry, Vol. 1, Plant Phenolics. Academic Press, London, pp. 389–419.

Pennington, T.D., 1997. The Genus Inga Botany. The Royal Botanic Gardens, Kew.

Tschesche, R., Braun, T.M., Sassen, W.V., 1980. Symplocoside: a flavanol glycoside from *Symplocos uniflora*. Phytochemistry 19, 1825–1829.

Van Rensburg, H., Steynberg, P.J., Burger, J.F.W., van Heerden, P.S., Ferreira, D., 1999. Circular dichroic properties of flavan-3-ols. Journal of Chemical Research (S) 450–451.

Zhang, B., Nonaka, G-I., Nishioka, I., 1988. Potentillanin: a biflavanoid and procyanidin glycoside from *Potentilla viscosa*. Phytochemistry 27, 3277–3280.