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HETEROGENEITY OF INTERFLAVANOID BOND LOCATION IN LOBLOLLY PINE BARK PROCYANIDINS

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Key Word Index—*Pinus taeda*; Pinaceae; loblolly pine; bark; phloem; procyanidins; condensed tannins interflavanoid linkage; regio-isomerism.

Abstract—Procyanidins B-1, B-3 and B-7 were obtained from *Pinus taeda* phloem in yields of 0.076, 0.021 and 0.034% of unextracted dry wt. Procyanidins B-1 and B-7 were produced in relative yields of 2.4 : 1 by biosynthetically patterned synthesis from catechin and loblolly pine tannins. Partial acid-catalysed thiolytic cleavage of loblolly pine phloem tannins produced (2*R*,3*S*,4*S*)-2,3,-*cis*-3,4-*trans*-3,3',4',5,7-pentahydroxy-4-phenylthioflavan and both (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*trans*-3,3'4',5,7-pentahydroxy-4-[(2*R*,3*S*,4*S*)-2,3-*cis*-3,4-*trans*-3,3',4',5,7-pentahydroxy-4-phenylthioflavan-8-yl]flavan and (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*trans*-3,3',4',5,7-4-[(2*R*,3*S*,4*S*)-2,3-*cis*-3,4-*trans*-3,3',4',5,7-pentahydroxy-4-phenylthioflavan-6-yl]flavan in ratios of 3:1 demonstrating regio-isomerism of the interflavanoid linkage in the polymeric procyanidins of loblolly pine bark.

INTRODUCTION

The C-4–C-8-linked dimeric procyanidins B-1–B-4 reportedly dominate their C-4–C-6-linked pairs B-5 through B-8 by a factor of 8–9 : 1 [1–3]. Based on this high selectivity for substitution at C-8, the polymeric procyanidins are considered to be linear polymers [3–4]. Gupta and Haslam [5] concluded that "The interflavan bonds are formed predominantly but not exclusively between C-4 and C-8 of the various flavan units". However, no evidence was given for the location of the interflavan bond in a dimer thioether obtained from partial cleavage of sorghum tannins [5] and no definite evidence existed for the location of the interflavan bonds in any polymeric procyanidins. Since the shape of the condensed tannins is no doubt of considerable importance, both in regard to their biological properties (i.e. interaction with proteins) and to their industrial application as specialty polymers (i.e. wood adhesives or dispersants), the extent of interflavanoid linkage homogeneity in condensed tannins requires more rigorous proof. A definition of the location(s) of the interflavanoid bond in loblolly pine bark procyanidins was therefore undertaken.

RESULTS AND DISCUSSION

More than one-third of the unextracted dry wt of loblolly pine phloem was soluble in acetone–water (7 : 3). However, the ethyl acetate-soluble portion of this extract amounted to only 2.1% and three dimeric procyanidins were isolated from it in a total yield of only 0.12% of the unextracted phloem dry wt. The majority of the acetone–water extract was condensed tannin together with substantial proportions of carbohydrates [6].

Procyanidin B-1 [(−) - epicatechin - 4,8 - (+) - catechin] (1) (Fig. 1) was obtained in a yield of 0.076% and procyanidin B-3 [(+) - catechin - 4,8 - (+) - catechin] (2) (Fig. 1) was obtained in a yield of 0.024% of the unextracted phloem dry wt. The chromatographic properties of the phenols 1 and 2 and their methylated or acetylated derivatives, ^{1}H and ^{13}C NMR spectra, and optical rotations of the deca-acetates were consistent with reported data [1, 2, 7, 8].

The procyanidin (3) (Fig. 1) obtained in second highest yield (0.034% of unextracted phloem dry wt) had PC R_f values similar to those reported for procyanidins B-5 [(−) - epicatechin - 4,6 - (−) - epicatechin] or B-6 [(+) - catechin - 4,6 - (+) - catechin] [1]. However, the ^{13}C NMR spectra of the phenol and its acetate (Table 1) clearly showed that compound 3 was an epicatechin–catechin dimer. As in the spectrum of procyanidin B-1, the heterocyclic ring carbon shifts of δ 82.0 (C-2) and 67.8 (C-3) demonstrate a catechin unit, while signals at 76.8 (C-2) and 72.0 (C-3) reveal the presence of an epicatechin unit. The chemical shifts for the C-4a carbons of each unit (δ 101.6 and 99.8) show that the epicatechin is the upper unit since the signal for the C-4a carbon of a C-4 substituted catechin unit is shifted downfield to *ca* δ 107 [9] (i.e. procyanidin B-3 has C-4a of δ 106.9). Substitution at C-4 of epicatechin causes no such shift for the C-4a carbon. The epicatechin C-2 carbon signal at δ 76.8 is also consistent with a 2,3 - *cis* - 3,4 - *trans* stereochemistry [2]. Acid-catalysed benzenethiol

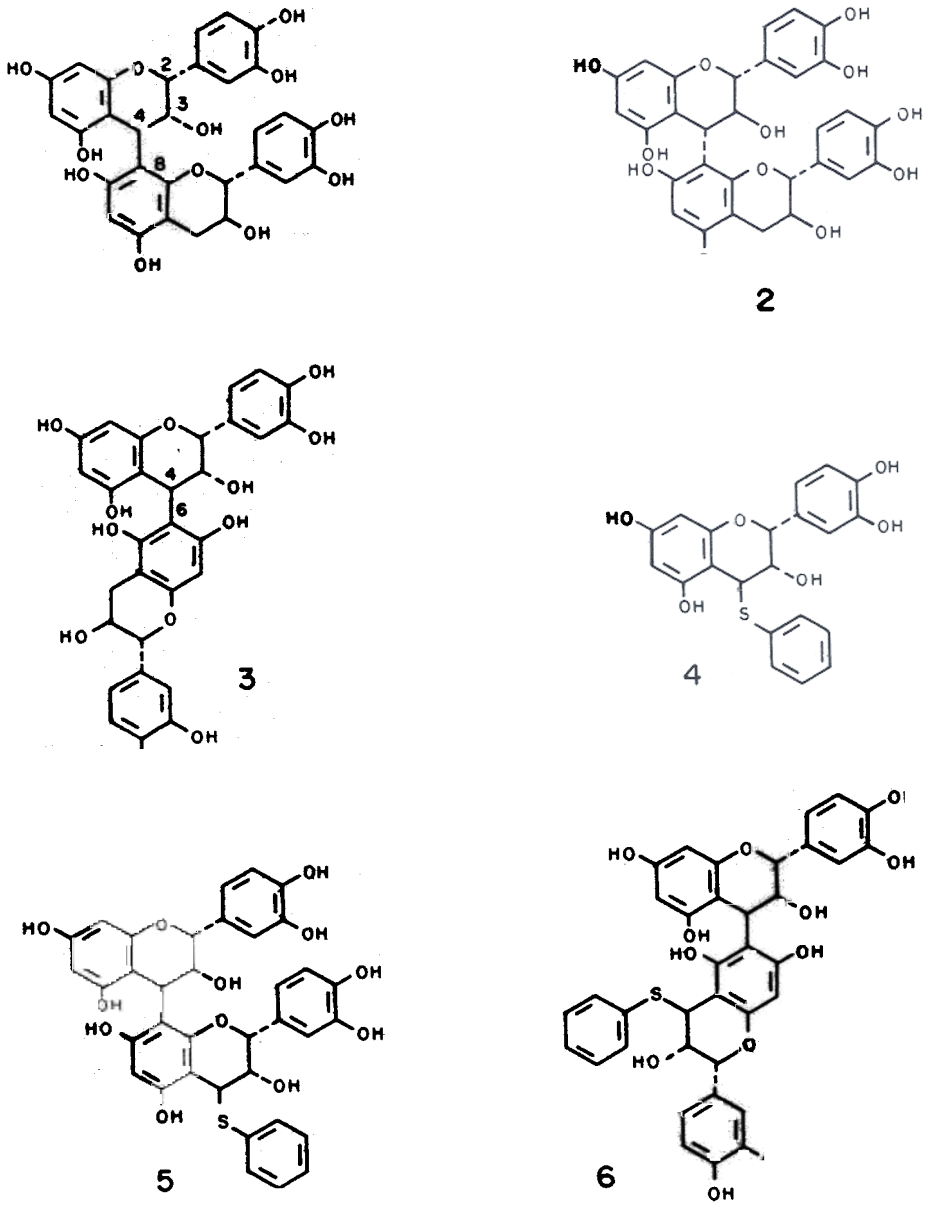

Fig. 1. Procyanidins and flavan thioethers from loblolly pine phloem

degradation and methylation [6, 10] of the ether-soluble product gave (2*R*,3*S*,4*S*)-2,3-*cis*-3,4-*trans*-3 - hydroxy - 3',4',5,7 - tetramethoxy - 4 - phenylthioflavan showing that the upper unit is composed of (−) - epicatechin. Acetylation of the ethyl acetate-soluble thiolysis product gave 3,3'4',5,7 - penta - *O* - acetyl - (+) - catechin establishing the absolute stereochemistry of the lower unit. The chemical shifts of the three A-ring protons at δ 6.59–6.81 in the ^{1}H NMR spectra of the deca-acetate derivative is characteristic of a C-4–C-6 interflavanoid linkage [11]. The strong positive Cotton effect for the 236 nm band of the CD spectrum of the deca-acetate derivative also shows a 4*R* (i.e. 3,4-*trans*) absolute configuration at the interflavanoid linkage [12]. This compound is therefore the procyanidin B-7 [(−)-epicatechin-4,6-(+)-catechin] for which little analytical data existed until now. There must be substantial variation in procyanidin composition among the different species of *Pinus* since Porter [13] and later Yazaki and Hillis [14] reported the procyanidins B-1, B-3, and B-6 in *Pinus radiata* bark, and Thompson *et al.* [1] reported procyanidin B-6 as a major constituent of *Pinus sylvestris* leaves.

The ratio of the procyanidins B-1 : B-7 isolated from the phloem of several *Pinus taeda L.* trees ranged from 1.6–3.3 : 1.0 differing markedly from the 8–9 : 1 ratio of C-4–C-8 and C-4–C-6 pairs reported by Fletcher *et al.* [2]. Biogenetically patterned synthesis of the procyanidins B-1 and B-7 by reaction of loblolly pine tannins with (+)-catechin gave an average relative yield of 2.4 : 1 from four separate syntheses. Reaction of 4-benzylthio-(−)-epicatechin with (+)-catechin in acidic solution gave procyanidin B-1 as the predominant

Table 1. Selected ^{13}C NMR chemical shifts of procyanidins and flavan thioethers from loblolly pine phloem*

Compound	Carbon No.				
	2	3	4	6	8
Phenols					
B-1 (−)-Epicatechin-4,8-	76.5	72.4	36.6	95.9	95.5
(+)-catechin	81.7	67.7	28.0	96.7	108.1
B-3 (+)-Catechin-4,8-	83.3	73.5	38.0	96.6	96.4
(+)-catechin	81.5	69.1	28.3	97.3	108.4
B-7 (−)-Epicatechin-4,6-	76.8	72.0	37.0	96.7	95.9
(+)-catechin	82.0	67.8	28.7	108.3	96.8
(4) (−)-Epicatechin-4-phenylthioether	75.1	70.5	46.2	96.7	95.6
(5) (−)-Epicatechin-4,8-	76.8	72.8	36.7	96.3	95.9
(−)-Epicatechin-4″-phenylthioether	75.1	70.0	46.5	97.4	107.5
(6) (−)-Epicatechin-4,6-	76.9	72.3	37.1	96.2	96.1
(−)-epicatechin-4″-phenylthioether	74.9	70.7	46.2	107.9	96.4
Acetates					
B-7 (−)-Epicatechin-4,6-	73.8	71.0	34.2	108.8	107.6
(+)-catechin	77.9	68.2	29.9	116.6	110.6
(4) (−)-Epicatechin-4-phenylthioether	72.9	70.6	41.7	109.9	107.8
(5) (−)-Epicatechin-4,8-	74.1	71.2	34.4	109.2	107.3
(−)-epicatechin-4″-phenylthioether	73.8	71.1	42.6	111.6	116.5
(6) (−)-Epicatechin-4,6-	73.7	70.6	33.8	108.3	107.2
(−)-epicatechin-4″-phenylthioether	72.6	70.2	42.0	117.6	110.8

*Chemical shift (δ from TMS) of major rotamer measured at 20 MHz with a Varian CFT-20 instrument. B-1, B-3 and B-7 measured in acetone-d_6–D_2O (50:50 v/v), compounds 4, 5a and 6 measured in acetone-d_6 and the acetate derivatives measured in $CDCl_3$.

product in the first few hours of reaction in agreement with the results of Fletcher *et al.* [2]. However, after several days of reaction, the concentration of procyanidin B-Y had increased substantially relative to B-1.

The high relative yield of procyanidin B-7 in *Pinus taeda* caused us to question the exclusive C-4–C-8–linked polymer structure proposed for the condensed tannins [4]. In earlier studies of the condensed tannins in *Pinus taeda* phloem [6], an octa-*O*-methyl-4″-phenylthioprocyanidin B dimer derivative was obtained from partial acid-catalysed thiolytic cleavage and methylation. This isolate was tentatively assigned the structure of a procyanidin B-2 derivative on the basis of its mass spectrum and its desulfurization with W-2 RaNi to an octa-*O*-methylprocyanidin B dimer which gave 3′,4′,5,7,-tetra-*O*-methylepicatechin and methyl-(3′,4′,5,7-tetra-*O*-methylepicatechin-4-yl-thio)acetate after mercaptoacetic acid degradation and methylation [6, 15]. We had, however, no evidence for the location of the interflavanoid linkage.

When larger quantities of this isolate were treated with W-2 RaNi, two products were noted in a ratio of *ca* 3:1. Both were shown to be octa-*O*-methylprocyanidin B dimers by their mass spectra. The major product had TLC chromatographic properties identical with those of octa-*O*-methylprocyanidin B-2. The optical rotation of the major product $[\alpha]_{578}$ + 72.2° was similar to that of octa-*O*-methylprocyanidin B-2 (i.e. + 76.0°). The minor product was identical with octa-*O*-methyl-procyanidin B-5 in its TLC chromatographic properties and its optical rotation $[\alpha]_{578}$ + 130° was similar to that of the synthesized compound (i.e. +137°). Since the acid-catalysed thiolytic cleavage was performed with a large excess of benzenethiol, it is most unlikely that our finding both the C-4–C-8 and C-4–C-6-linked dimeric thioether derivatives would be the result of isomerism of the interflavanoid linkage during the cleavage reaction. Evidence has been obtained showing no isomerism of the interflavanoid bond location in procyanidins reacted under acidic conditions in the presence of excess thiol [11, 16].

As this was the first evidence for the location of the interflavanoid linkages in polymeric procyanidins and both C-4–C-8 and C-4–C-6 bonds were indicated, further proof was sought. Acid-catalysed benzenethiol degradation of a type-C tannin from loblolly pine phloem [6] and isolation of the thioethers as their phenols by chromatography on Sephadex LH-20 [5] gave (2*R*, 3*S*, 4*S*)-2,3-*cis*-3,4-*trans*-3,3′,4′,5,7-pentahydroxy-4-phenylthioflavan (4) and two 4″-phenylthio

- (epicatechin)$_2$ isomers. The dimer thioether obtained in higher yield was shown to be (2*R*,3*R*,4*R*) - 2,3 - *cis* - 3,4 - *trans* - 3,3',4',5,7 - pentahydroxy - 4 - [(2*R*,3*S*,4*S*) - 2,3 - *cis* - 3,4 - *trans* - 3,3',4',5,7 - pentahydroxy - 4 - phenylthioflavan - 8- yl]flavan (**5**) on the basis of the ^{13}C NMR spectra of the phenol and its acetate derivative (Table 1), the mass spectrum of its methylated derivative, and the essential identify of the RaNi desulfurization products of the phenol and the methyl ether with procyanidin B-2 and octa-*O*-methylprocyanidin B-2 (i.e. $[\alpha]_{578}$ + 74° and identical TLC R_f). The dimer thioether obtained in lower yield was shown to be (2*R*,3*R*,4*R*) - 2,3 - *cis* - 3,4 - *trans* - 3,3',4',5,7 - pentahydroxy - 4 - [(2*R*,3*S*,4*S*) - 2,3 - *cis* - 3,4 - *trans* - 3,3'4,5,7 - pentahydroxy - 4 - phenylthioflavan - 6 - yl]flavan (**6**) also on the basis of ^{13}C NMR spectra of the phenol and acetate (Table 1), the mass spectrum of the methyl ether and essential identity of the RaNi desulfurization products of the phenol and its methyl ether with procyanidin B-5 and octa-*O*-methylprocyanidin B-5 (i.e. $[\alpha]_{578}$ +138°) and identical TLC R_f.

Acid-catalysed thiolytic cleavage of tannin-C [6] for 20 hr gave 4-phenylthio-(−)-epicatechin (**4**), 4″-phenylthio-(−)-epicatechin-4,8-(−)-epicatechin (**5**) and 4″ - phenylthio - (−) - epicatechin - 4,6 - (−)-epicatechin (**6**) in relative yields of 10 : 3.6 : 1 and 43% of the tannin was recovered as a water-soluble polymer. When the residual tannin was subjected to an additional 20 hr of cleavage, products **4**, **5** and **6** were obtained in relative yields of 8 : 2 : 1. Partial degradation of tannin-B [6] from loblolly pine phloem also gave the two isomeric dimer thioethers **5** and **6** but in relative proportions of *ca* 4.5 : 1 after 20 hr of reaction. These results show conclusively that both C-4–C-8 and C-4–C-6 interflavanoid linkages are present in the dimeric and polymeric procyanidins of loblolly pine phloem and that the proportion of C-4–C-6 linkages is quite high.

The above results are also consistent with the recent isolation of three trimeric procyanidins from loblolly pine phloem which were shown to be: (−) - epicatechin - 4,8 - (−) - epicatechin - 4,8 - (+) - catechin; (−) - epicatechin - 4,8 - (−) - epicatechin - 4,6-(+)-catechin; and (−)-epicatechin-4,6-(−)-epicatechin-4,8-(+)-catechin [11, 16]. The all C-4–C-8-linked linear polymer structures proposed by Haslam [4] for condensed tannins such as those from sorghum, do not correspond structurally to the tannins in *Pinus taeda* bark. Isomerism of the location of the interflavanoid bonds has now been shown in dimeric, trimeric and polymeric procyanidins of loblolly pine. While it is tempting to postulate a branched polymer structure, additional evidence must be obtained, and we have not yet found doubly substituted trimers analogous to the (fisetinidol)$_2$-catechin trimeric procyanidins described by Botha *et al.* [17].

EXPERIMENTAL

Isolation of dimeric procyanidins from phloem. A loblolly pine tree (28 yr old, 24 cm diameter) was cut from the Evangeline District of the Kisatchie National Forest in central Louisiana. Outer bark was removed with a draw knife and the phloem was stripped from the xylem cambium and frozen immediately to retard enzymic browning. Extraction of the fresh phloem (1100 g fr. wt) with Me_2CO–H_2O (7 : 3), gave an average yield of 36% of the unextracted phloem dry wt as a red-brown powder (137 g). The dried extract was dispersed in H_2O (2 l.) and extracted (×3) with *n*-hexane (1.2 l. total) to remove 0.3% of the unextracted phloem dry wt as a light-yellow oil (1.5 g). Subsequent extraction (×5) with EtOAc (2.5.1 total) gave 2.1% of the unextracted phloem dry wt as a yellow-orange powder (7.8 g). During extraction with EtOAc, a red-brown ppt separated which was recovered by filtration (3.7 g). The crude H_2O-soluble tannin was freeze-dried to obtain a red-brown powder (117 g) that was separated (3 g portions) by cellulose CC into a carbohydrate fraction (1.8 g) and type-B and type-C tannins (0.7 and 0.4 g) [6].

The EtOAc-soluble extract (4 g portions) was applied to a Sephadex LH-20 column (100 × 2.5 cm) and eluted with EtOH (360 fractions, 10 ml). *Ca* 50% of the EtOAc extract was eluted in fractions 20–70 and PC (2-BuOH–HOAc–H_2O, 14 : 1 : 5 and 6% HOAc) showed that it was a mixture of compounds that migrated to high R_f in both solvents. The combined fractions were freeze-dried from *t*-BuOH to obtain a pale-yellow powder that was reserved for future study. Fractions 71–100 contained (+)-catechin [PC, TLC, acetate mmp 130–131°, $[\alpha]_D$ + 36° ($CHCl_3$; *c* 0.1)].

Procyanidin B-1 (**1**) was obtained in fractions 165–195 (PC, 2-BAW and 6% HOAc, R_f 0.35 and 0.55) and the combined fractions were obtained in a yield of 3.6% of the EtOAc extract or 0.076% of the unextracted phloem dry wt. After further purification by reverse phase HPLC (Zorbax CN, 25 cm × 9.4 mm, MeOH–H_2O, 3 : 7, 5 ml/min), it was obtained as a white amorphous powder. (Found C, 58.9; H, 5.0. Calc. for $C_{30}H_{26}O_{12}\cdot 2H_2O$: C, 58.6; H, 4.9%.) ^{13}C NMR showed an epicatechin–catechin dimer (Table 1) and chemical shifts consistent with those reported for procyanidin B-1 [2]. Benzenethiol degradation followed by methylation gave 3',4',5,7 - tetra - *O* - methyl - (+) - catechin and 3',4',5,7 - tetra - *O* - methyl - 4 - phenylthio - (−) - epicatechin (TLC, ^1H NMR). Methylation of **1** (CH_2N_2) gave a product isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8:2, R_f 0.33); EIMS, *m/z* (rel. int.); 690 $[M]^+$ (69), 672 (17), 511 (26), 496 (30), 479 (53), 344 (47), 331 (55), 327 (51), 299 (85), 180 (33), and 151 (100). Acetylation of **1** (Ac_2O–C_5H_5N, 1 : 1) gave the deca-acetate (found C, 59.5; H, 4.7, calc. for $C_{50}H_{46}O_{22}$: C, 60.1; H, 4.7%) which was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.51); $[\alpha]_{578}$ + 109° (Me_2CO; *c* 0.5), lit. [8] $[\alpha]_{578}$ + 110.7°. ^1H NMR (100 MHz, $CDCl_3$): δ 1.85–2.38 (30H, *m*, ROAc), 2.4–3.4 (2H, *m*, H-4 catechin), 4.34–4.43 (1H, *d*, *J* = 9 Hz, H-2 catechin), 4.47 (1H, *d*, *J* = 2 Hz, H-4 epicatechin), 4.98–5.40 (1H, *m*, H-3 catechin), 5.22 (1H, *d*, *J* = 2 Hz, H-3 epicatechin), 4.98–5.40 (1H, *m*, H-3 catechin), 5.22 (1H, *d*, *J* = 2 Hz, H-3 epicatechin), 5.51 (1H, *s*, H-2 epicatechin), 6.06 and 6.35 (2H, *d* + *d*, *J* = 2 Hz, epicatechin Ar–H), 6.74 (1H, *s*, H-6 catechin), 6.93–7.32 (6H, *m*, B-ring Ar–H) [7].

Procyanidin B-3 (**2**) was obtained in fractions 196–230 together with small impurities of the procyanidin B-1. The combined fraction was separated a second time on Sephadex LH-20 eluting with EtOH to obtain a pure isolate (PC, 2-BAW and 6% HOAc, R_f 0.35 and 0.50) in a yield of 1.0% of the EtOAc extract or 0.021% of the unextracted phloem dry wt. Reverse phase HPLC gave a white amorphous powder. (Found C, 59.4; H, 4.7. Calc. for $C_{30}H_{26}O_{12}\cdot 1.5H_2O$: C, 59.5; H, 4.8%.) ^{13}C NMR showed a catechin–catechin dimer (Table 1) and chemical shifts consistent with reported data [2]. Acetylation of **2** gave the deca-acetate (found C, 60.0, H, 4.8, calc. for $C_{50}H_{46}O_{22}$: C,

60.1: H, 4.7%) which was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.35); $[\alpha]_{578}$ − 134° (Me_2CO; *c* 1.0), lit. [8] $[\alpha]_{578}$ − 133°. ^1H NMR (100 MHz, $CDCl_3$): δ 1.65–2.35 (30H, *m*, RO–Ac), 2.50–2.92 (2H, *m*, H-4 catechin lower), 4.47, 4.57 (1H, *d*, *J* = 10 Hz, H-4 catechin upper), 4.73, 4.83 (1H, *d*, *J* = 10 Hz, H-2 catechin upper), 4.9–5.1 (2H, *m*, H-2 and H-3 catechin lower), 5.56, 5.66, 5.76 (1H, *t*, H-3 catechin upper), 6.52 (3/4H, *d*, *J* = 2 Hz, H-6 catechin upper, major rotamer), 6.54 (1/4H, *s*, H-6 catechin lower, minor rotamer), 6.64 (3/4H, *s*, catechin lower, major rotamer), 6.72 (1/4H, *d*, *J* = 2 Hz, H-6 catechin upper, minor rotamer), 6.80 (1/4H, *d*, *J* = 2 Hz, H-8 catechin upper, minor rotamer), 6.94 (3/4H, *d*, *J* = 2 Hz, H-8 catechin upper, major rotamer), 7.04–7.28 (6H, *m*, B-ring Ar–H) [7].

Procyanidin B-7 (3). Fractions 231–270 contained one compound (PC, 2-BAW and 6% HOAc, R_f 0.45 and 0.40) and the combined fractions were recovered in a yield of 1.6% of the EtOAc extract or 0.034% of the unextracted phloem dry wt. Reverse phase HPLC gave an off-white amorphous powder; (found C, 59.0; H, 5.0; $C_{30}H_{26}O_{12}\cdot 1.5H_2O$ requires C, 59.5; H, 4.8%); $[\alpha]_{578}$ + 161° (MeOH; *c* 0.5). ^{13}C NMR showed an epicatechin–catechin dimer (Table 1). Acid-catalysed benzenethiol degradation and methylation of the Et_2O-soluble product gave 3',4',5,7 - tetra - *O* - methyl - 4 - phenylthio - (−) - epicatechin: mmp 208°, lit. [6, 11] 208–210°; $[\alpha]_D$ + 8.9° ($CHCl_3$; *c* 1.5), lit. [6, 10] $[\alpha]_D$ + 8.6 and 8.8°. Acetylation of the EtOAc-soluble product gave 3,3',4',5,7 - penta - *O* - acetyl - (+) - catechin: $[\alpha]_D$ + 37.8° ($CHCl_3$; *c* 1.5), lit. [1] $[\alpha]_D$ + 39.7°. Acetylation of 3 gave the deca-acetate; (found C, 60.2; H, 4.7, $C_{50}H_{46}O_{22}$ requires C, 60.1; H, 4.7%); $[\alpha]_{578}$ + 123° (Me_2CO; *c* 0.5) that was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.45). ^1H NMR (100 MHz, $CDCl_3$) showed evidence of restricted rotation about the interflavanoid bond but the spectrum could be interpreted as: δ 1.95 and 1.98 (3H and 3H, *s* + *s*, aliph.–OAc), 2.10–2.46 (24H, *m*, ArOAc), 2.50–3.00 (2H, *m*, H-4, catechin), 4.35–4.45 (1H, *d*, H-4 epicatechin), 5.24–5.26 (2H, *m*, H-3 catechin and epicatechin), 5.35 (1H, *d*, H-2, catechin), 5.50 (1H, *d*, H-2 epicatechin), 6.59–6.81 (3H, *m*, A-ring ArH), 7.20–7.34 (6H, *m*, B-ring ArH). ^{13}C NMR of the deca-acetate was consistent for a peracetate of an epicatechin–catechin dimer (Table 1). CD of the deca–acetate showed ΔE_{236} + 45.5, ΔE_{269} + 1.6 and ΔE_{279} + 2.21/mol·cm.

Biogenetically patterned syntheses. The crude H_2O-soluble tannin from loblolly pine phloem (25 g) and (+)-catechin (5 g) were dissolved in 150 ml *p*-dioxane–H_2O (2 : 1), the soln purged with Ar gas and HCl (5.5 ml) added slowly. After 48 hr at 25° under a slow flow of Ar gas in an Al-foil covered flask, the soln was diluted with H_2O (100 ml) and extracted with EtOAc (×6, 1 l. total) to recover a light tan EtOAc-soluble product (7.0 g). The product was applied to a Sephadex LH-20 column (100 × 2.5 cm) and eluted with EtOH to give fractions containing the procyanidins B-1 and B-7 as described above. The combined fractions containing B-1 and B-7 were separated further by reverse phase HPLC (Zorbax CN, 25 cm × 9.4 mm; MeOH–H_2O, 3 : 7, 5 ml/min) and the relative yields of procyanidins B-1 and B-7 determined by measurement of the relative peak areas (UV detector, 254 nm). The chromatographic and spectral properties of the synthesized procyanidins B-1 and B-7 and their acetate derivatives were all consistent with data obtained from the natural products described above. The condensed tannins were also reacted with (−)-epicatechin under similar conditions and the procyanidins B-2 and B-5 were isolated from the EtOAc-soluble product by chromatography on Sephadex LH-20 as described above.

Procyanidin B-2 was isolated as a white amorphous powder after additional purification by reverse phase HPLC: (PC, 2-BAW and 6% HOAc, R_f 0.36 and 0.65). (Found C, 59.6; H, 5.0. Calc. for $C_{30}H_{26}O_{12}\cdot 1.5\ H_2O$: C, 59.5; H, 4.8%.) ^{13}C NMR chemical shifts were consistent with reported data [2] for procyanidin B-2. Methylation and prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.35) gave a white amorphous powder by pptn from *n*-hexane; (found C, 65.9; H, 6.2, calc. for $C_{38}H_{42}O_{12}$: C, 66.1; H, 6.1%); $[\alpha]_{578}$ + 76° ($CHCl_3$; *c* 0.67) for which EIMS showed *m/z*, $[M]^+$ 690 and fragmentation typical of an octa-*O*-methylprocyanidin B dimer. Acetylation gave the deca-acetate; (found C, 60.3; H, 4.7, calc. for $C_{50}H_{46}O_{22}$: C, 60.1; H, 4.7%); $[\alpha]_{578}$ + 54° (Me_2CO; *c* 1.4), lit. [7] $[\alpha]_{578}$ + 47° which was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.45). ^1H NMR was consistent with reported spectral data for the deca-acetate of procyanidin B-2 [7].

Procyanidin B-5 was isolated as an off-white amorphous powder after further purification by reverse phase HPLC: (PC, 2-BAW and 6% HOAc, R_f 0.41 and 0.35). (Found C, 59.3; H, 5.0. Calc. for $C_{30}H_{26}O_{12}\cdot 1.5H_2O$: C, 59.5; H, 4.8%.) ^{13}C NMR shifts were consistent with data reported for the procyanidin B-5 [2]. Methylation and prep. TLC gave an off-white amorphous powder by pptn from *n*-hexane; (found C, 65.9; H, 6.2, calc. for $C_{38}H_{42}O_{12}$: C, 66.1; H, 6.1%); $[\alpha]_{578}$ + 137° ($CHCl_3$; *c* 0.20) for which EIMS showed *m/z* $[M]^+$ 690 and fragmentation typical of an octa-*O*-methyl procyanidin B dimer. Acetylation gave the deca-acetate; (found C, 60.1; H, 4.7, calc. for $C_{50}H_{46}O_{22}$; C, 60.1; H, 4.7%); $[\alpha]_{578}$ + 79.5° (Me_2CO; *c* 1.4) which was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.45).

(+)-Catechin (15 mg) and 4-thiobenzylepicatechin (20 mg) were dissolved in EtOH (2 ml) and HCl (1 drop) was added while the soln was purged with Ar gas. The reaction tube was sealed with a rubber septum closure and the reaction at 25° was monitored by withdrawing samples (3 μl) by syringe and examining products by cellulose 2D-TLC developed with TBA (*t*-BuOH–HOAc–H_2O, 3 : 1 : 1), and 6% HOAc. Proportions were estimated by visual comparison of relative spot intensity after spraying the plates with vanillin–HCl and warming the plates. Products of the reaction were evaluated over periods of 15 min to several days.

Octa-O-methyl procyanidins B-2 and B-5 from tannin cleavage. Acid-catalysed benzenethiol degradation of loblolly pine phloem tannin followed by methylation gave an octa-*O*-methyl-4"-phenylthioprocyanidin B dimer isolate that was partially described earlier [6]. Additional quantities (100 mg) of this isolate were treated with W-2 RaNi in EtOH at 25° for 1.5 hr. Two products were isolated by prep. TLC. The major product (55.3 mg) had TLC chromatographic properties identical with those of octa-*O*-methylprocyanidin B-2 (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.35) and was isolated as a white amorphous powder by pptn from *n*-hexane; $[\alpha]_{578}$ + 72° ($CHCl_3$; *c* 0.76); EIMS $[M]^+$ 690 and fragmentation ions typical of an octa-*O*-methylprocyanidin B dimer. The minor product (18.1 mg) had TLC chromatographic properties identical with those of octa-*O*-methylprocyanidin B-5 (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.31) and was isolated as a white amorphous powder by pptn from *n*-hexane: $[\alpha]_{578}$ + 130° ($CHCl_3$; *c* 0.16); EIMS, $[M]^+$ 690 and fragmentation ions typical of an octa-*O*-methylprocyanidin B dimer.

Thiolysis products from tannin C. Tannin C (3.0 g), which was isolated as described earlier [6], was dissolved in EtOH (50 ml), and benzenethiol (3.5 ml) and HOAc (1.5 ml) were added while the soln was purged with Ar gas. The soln was transferred to Ar-purged vials and sealed. After 20 hr in a

100° steam bath, the product was reduced to an oil under a stream of N_2. The product was transferred to 250 ml H_2O with a small amount of EtOH and extracted with Et_2O (×5, 1.5 l. total). The Et_2O was removed under a stream of N_2 and the oil applied to a Sephadex LH-20 column (30 × 2.5 cm) with a small amount of EtOH. The column was eluted with $CHCl_3$–EtOH (4 : 1), collecting 20 ml fractions. The residual H_2O-soluble polymer was freeze-dried to obtain a light-brown powder (1.3 g).

(2R,3S,4S) - 2,3 - cis - 3,4 - trans - 3,3',4',5,7 - *pentahydroxy* - 4 - *phenylthioflavan* (**4**). Fractions 51–70 contained primarily one compound (cellulose 2D-TLC, TBA and 6% HOAc, R_f 0.77 and 0.43). The combined fraction (1.1 g) was purified further by chromatography on Sephadex LH-20 by eluting with Me_2CO to obtain a white amorphous powder; (found C, 63.4; H, 5.1; S, 7.8%, $C_{21}H_{18}O_6S$ requires C, 63.3; H, 4.5; S, 8.0); $[\alpha]_{578}$ −21.6° (Me_2CO; *c* 1.4). ^{13}C NMR chemical shifts were consistent with the 4-phenylthioether of epicatechin (Table 1). Methylation of **4** and prep. TLC (Si gel, C_6H_6–Me_2CO, 9 : 1, R_f 0.57) gave a product that crystallized as small needles from Me_2CO: mp 208°, lit. [6, 10] 208–210°; (found C, 66.2; H, 5.7; S, 7.1, calc. for $C_{25}H_{26}O_6S$: C, 66.2; H, 5.7; S, 7.1%); $[\alpha]_D$ +8.9° ($CHCl_3$; *c* 0.20); lit. [6, 10] $[\alpha]_D$ +8.6 and 8.8°. Acetylation of **4** gave the penta-acetate; (found C, 61.5; H, 4.7; S, 5.3, $C_{31}H_{28}O_{11}S$ requires C, 61.2; H, 4.6; S, 5.3%); $[\alpha]_{578}$ +88.5° ($CHCl_3$; *c* 1.1) which was isolated by prep. TLC (Si gel, C_6H_6–Me_2CO, 9 : 1, R_f 0.48). ^1H NMR (60 MHz, $CDCl_3$): δ 1.87, 2.12, 2.28 (15H, *m*, –OAc), 4.51 (1H, *d*, H-4), 5.18 (1H, *d*, H-3), 6.57 (1H, *s*, H-2), 6.62 (2H, *d*, A-ring ArH), 7.18–7.60 (16H, *m*, B-ring + phenyl ArH). Fractions 102–150 contained catechin (120 mg). After elution of the catechin, the solvent was changed to $CHCl_3$–EtOH (2.5 : 1).

(2R,3R,4R) - 2,3 - cis - 3,4 - trans - 3,3',4',5,7 - *pentahydroxy* - 4 - [(2R,3S,4S) - 2,3 - cis - 3,4 - trans - 3,3',4',5,7 - *pentahydroxy* - 4 - *phenylthioflavan* - 8 - *yl*]*flavan* (**5**). Fractions 156–188 contained primarily one compound (cellulose 2D-TLC, TBA, and 6% HOAc, R_f 0.60 and 0.55). The combined fraction (366 mg) was purified further on Sephadex LH-20 eluting with Me_2CO to yield an amorphous white powder; (found C, 61.2; H, 5.0; S, 4.3, $C_{36}H_{30}O_{12}S \cdot H_2O$ requires C, 61.4; H, 4.6; S, 4.6%); $[\alpha]_{578}$ +49° (Me_2CO; *c* 1.5). ^{13}C NMR chemical shifts (Table 1) were consistent with 4"-phenylthioprocyanidin B-2. Desulfurization with W-2 RaNi gave a product identical with procyanidin B-2 by cellulose 2D-TLC. Methylation of **5** and prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.51) gave a white amorphous powder by pptn from *n*-hexane; (found C, 66.0; H, 6.0; S, 3.9; $C_{44}H_{46}O_{12}S$ requires C, 66.2; H, 5.75; S, 4.0%); $[\alpha]_{578}$ +94.5° ($CHCl_3$; *c* 0.66). EIMS *m/z* (rel. int.): 689 $[M-SPh]^+$ (19), 509 (14), 327 (16), 303 (23), 299 (15), 191 (49), 180 (38), 167 (31), 165 (26), 151 (100), 110 (36), 109 (24). Desulfurization with W-2 RaNi gave a product identical with octa - *O* - methylprocyanidin B-2 in both TLC chromatographic properties (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.35) and an optical rotation of $[\alpha]_{578}$ +74° ($CHCl_3$; *c* 0.33). Acetylation of **5** and prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.73) gave the deca-acetate; (found C, 60.5; H, 4.6; S, 3.0; $C_{56}H_{50}O_{22}S$ requires C, 60.7; H, 4.5; and S, 2.9%); $[\alpha]_{578}$ +110° ($CHCl_3$; *c* 0.60). ^{13}C NMR chemical shifts (Table 1) were consistent with those expected of the peracetate of an (epicatechin)$_2$ derivative. ^1H NMR (100 MHz, $CDCl_3$). δ 5.96 and 6.22 (2H, *dd*, H-6 and H-8 upper epicatechin unit) and 6.66 (1H, *s*, H-6 of lower epicatechin unit) consistent with a C-4–C-8 interflavanoid linkage [11].

(2R,3R,4R) - 2,3 - cis - 3,4 - trans - 3,3',4',5,7 - *pentahydroxy* - 4 - [(2R,3S,4S) - 2,3 - cis - 3,4 - trans - 3,3',4',5,7 - *pentahydroxy* - 4 - *phenylthioflavan* - 6 - *yl*]*flavan* (**6**). Fractions 190–230 contained one compound (cellulose 2D-TLC, TBA and 6% HOAc, R_f 0.68 and 0.37). The combined fraction (105 mg) was recovered as an off-white powder; (found C, 61.2; H, 5.0; S, 4.4%, $C_{36}H_{30}O_{12}S \cdot 1H_2O$ requires C, 61.4; H, 4.6; S, 4.6%); $[\alpha]_{578}$ +111° (Me_2CO; *c* 0.34). ^{13}C NMR shifts (Table 1) were consistent with those expected of 4"-phenylthioprocyanidin B-5. Desulfurization of **6** gave a product identical with procyanidin B-5 by cellulose 2D-TLC. Methylation of **6** and prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.50) gave an off-white amorphous powder by pptn from *n*-hexane; (found C, 66.0; H, 5.9; S, 3.8, $C_{44}H_{46}O_{12}S$ requires C, 66.1; H, 5.8; S, 4.0%); $[\alpha]_{578}$ + 140° ($CHCl_3$; *c* 0.21). EIMS, *m/z* (rel. int.): 689 $[M-SPh]^+$ (15), 509 (9.3), 327 (12), 303 (18), 299 (17), 191 (39), 181 (25), 180 (32), 179 (18), 167 (26), 165 (21), 151 (100), 110 (27), 109 (15). Desulfurization with W-2 RaNi gave a product identical with octa-*O*-methylprocyanidin B-5 in TLC chromatographic properties (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.31) and the optical rotation of the desulfurization product was $[\alpha]_{578}$ + 138° ($CHCl_3$; *c* 0.25). Acetylation of **6** and prep. TLC (Si gel, C_6H_6–Me_2CO, 8 : 2, R_f 0.70) gave the deca-acetate; (found C, 60.5; H, 4.6; S, 3.1, $C_{56}H_{50}O_{22}S$ requires C, 60.7; H, 4.5; S, 2.9%); $[\alpha]_{578}$ + 127° ($CHCl_3$; *c* 0.70). ^{13}C NMR chemical shifts (Table 1) were consistent with the peracetate of an (epicatechin)$_2$ derivative. ^1H NMR (100 MHz, $CDCl_3$): δ 6.48–6.70 (3H, *m*, H-6 and H-8 of epicatechin units) was consistent with a C-4–C-6 interflavanoid linkage [11].

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