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PROCYANIDIN POLYMERS OF DOUGLAS FIR BARK: STRUCTURE FROM DEGRADATION WITH PHLOROGLUCINOL

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Key Word Index—*Pseudotsuga menziesii*; Pinaceae; Douglas fir; procyanidin; degradation; phloroglucinol-adducts.

Abstract—Reaction of the condensed tannin polymers of Douglas fir inner bark with phloroglucinol yielded catechin, epicatechin, procyanidin B-2, catechin-(4α→2)-phloroglucinol, epicatechin-(4β→2)-phloroglucinol, the novel compound epicatechin-(4α→2)-phloroglucinol and 1,3-di (2,4,6-trihydroxyphenyl)-1-(3,4-di-hydroxyphenyl)-propan-2-ol. Also isolated were epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol, epicatechin-(4β→6)-epicatechin-(4β→2)-phloroglucinol, and three other novel phloroglucinol adducts, catechin-(4α→8)-epicatechin-(4β→2)-phloroglucinol, epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol and epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol. The results suggest that the configuration of the extender units is almost exclusively 2,3-*cis*, while the terminal units are mixed, with 2,3-*cis* slightly predominating. The C-4 to C-8 interflavanoid linkage predominates over the C-4 to C-6 linkage by a 4:1 ratio.

INTRODUCTION

The major timber tree in the U.S. Pacific Northwest is the Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco]. In order to facilitate more efficient bark utilization of this natural resource, we have undertaken a systematic study of its polyphenolic components and have shown the presence of relatively rare flavanoid glycosides [1] and of low-M_r procyanidin oligomers [2]. Polymeric procyanidins have also been reported to be present [3, 4] but have not been closely studied. Major advances in the knowledge of the structure and chemistry of proanthocyanidins, particularly of the low-M_r oligomers [5–8], now allow the gross structure of higher oligomers to be ascertained with IR [9] and ^{13}C NMR [8, 10] spectroscopy. This investigation deals with the degradation of the polymers with phloroglucinol and the identification of the degradation products.

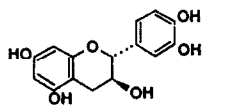
RESULTS AND DISCUSSION

The polymeric fraction was purified on a Sephadex LH-20 column [8] and obtained as an optically active freeze-dried solid which amounted to *ca* 25% of the total weight of the aqueous soluble fraction. The number-average M_r ($\bar{M}_n$) was determined to be 3370 by gel permeation chromatography (GPC) on the acetylated polymers, which have an average chain length of *ca* seven flavanoid units. Reaction of the polymers with 5% hydrochloric acid *t*-butanol gave cyanidin, and calculation based on its optical rotation [8] gave the proportion of 2,3-*cis* to 2,3-*trans* procyanidin units as 17:3, respectively. The predominantly 2,3-*cis* configuration was supported both by its IR spectrum, which showed an absorption band at 795 cm^{-1} [9], and by its ^{13}C NMR spectrum, which showed a large resonance at δ76 that was attributable to the C-2 of the 2,3-*cis* flavan-3-ol extender units [10]. The resonances at δ79 and 81 also indicated that both epicatechin and catechin contributed to the end groups of the polymers.

Benzylthiol has been used commonly to cleave interflavanoid linkages in elucidating the structure of proanthocyanidins [5–7]. However, this reagent has an objectionable odour that severely limits its general use, particularly on a preparative scale. Therefore, phloroglucinol was investigated as an alternative cleaving reagent in this structural study of Douglas fir polymers. Reaction of the polymers with phloroglucinol in 1% hydrochloric acid in ethanol at ambient temperatures under nitrogen for 0.5 hr yielded catechin (**1**) and epicatechin (**2**) in the ratio 10:13, and a small amount of procyanidin B2 (**3**). The most significant product was epicatechin-(4β→2)-phloroglucinol (**4**), preponderant to catechin-(4α→2)-phloroglucinol (**5**) in the ratio of *ca* 21:1. Another product (**6**), isolated in much lower yield, gave an $[M-H]^-$ peak at *m/z* 413 in the negative-ion spectra in fast atom bombardment mass-spectrometry (FABMS), indicating it to be of the same chemical constitution as **4** and **5**. This was corroborated by its ^{13}C NMR spectrum, which was clearly identifiable with a flavanoid-phloroglucinol condensation product and similar to the spectra of **4** and **5**. One diagnostic feature used to distinguish the C-4 substituted derivatives of catechin and epicatechin was the relative position of C-2 resonances in the ^{13}C NMR spectra. Substitution at C-4 of the flavan-3-ol caused a large downfield shift (~5 ppm) to the C-3 resonance and a smaller downfield shift (1–2 ppm) to the C-2 resonance. But because of the normally axial conformation of the C-4 substituent [11] in the 2,3-*cis*-3-4-*trans* derivative, the shift (~3 ppm) to the C-2 resonance was upfield relative to the unsubstituted flavan-3-ol. This upfield shift was caused by 1,3-diaxial interactions, which resulted in large differences in the C-2 resonances in C-4 substituted catechin and epicatechin [10]. In the ^{13}C NMR spectrum

of (**6**), the C-2 resonance was observed at δ80.9 and the C-3 resonance at 72.7, 1.0 and 5 ppm downfield, respectively, from C-2 and C-3 resonances of epicatechin (Table 1). The absence of the γ-gauche effect indicated that **6** was of the unusual 2,3-*cis*-3,4-*cis*-phloroglucinol adduct. The observed proton–proton couplings (4.5 Hz) between H-3 and H-4 were consistent with published values for 2,3-*cis*-3,4-*cis* flavans [12, 13]. Additional supporting evidence for this 3,4-*cis* configuration was available from its chiroptical properties; the CD and optical rotation, $[\alpha]_{589}$ $-64°$ (MeOH; *c* 0.07), were of opposite signs to those of epicatechin-(4β→2)-phloroglucinol (**4**). The 2,3-*cis*-3,4-*cis* product (**6**) represented the first exception to the reported stereospecificity of the reaction at C-4 in 2,3-*cis* flavans [14]; therefore the reaction at this site was not unlike the 2,3-*trans* isomers.

Another low-M_r product (**7**) was two mass units higher than the phloroglucinol adducts of flavan-3-ols, with an $[M-H]^-$ peak at *m/z* 415 in negative-ion FAB mass spectra. The characteristic chemical shifts in the heterocyclic ring carbons of flavanoids were no longer apparent in the ^{13}CNMR spectrum of **7**. In their place were oxygenated carbon δ77.6 and two higher field aliphatic carbon resonances at δ46.4 and 30.4. The ring carbon resonances of both catechol and phloroglucinol moieties were still readily evident, indicating that a phloroglucinol moiety had been added at the C-2 of the flavan nucleus, with subsequent opening of the pyran ring. Thus compound **7** was 1,3-di-(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-propan-2-ol, which has also been isolated from the reaction of loblolly pine bark tannins with phloroglucinol under alkaline conditions [15].



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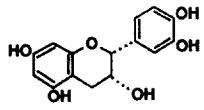

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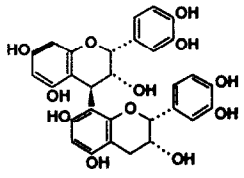

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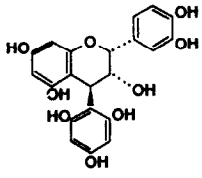

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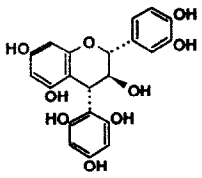

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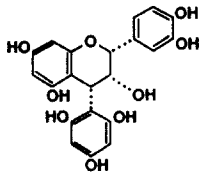

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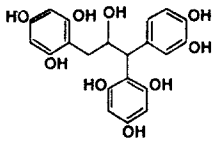

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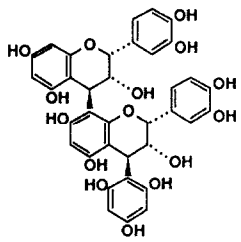

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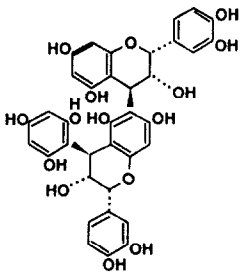

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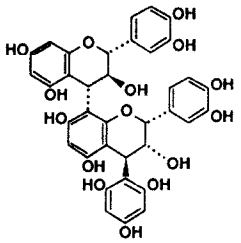

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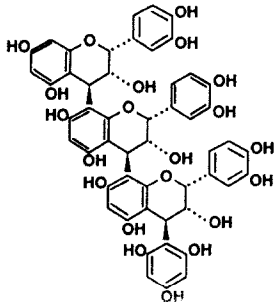

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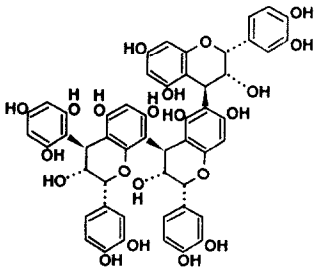

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Before the interflavanoid linkages in the polymer could be assessed, some oligomeric procyanidin-phloroglucinol adducts had to be characterized. Three dimeric procyanidin phloroglucinol adducts, (**8**–**10**), and two trimeric adducts, (**11**, **12**), were isolated and their chemical constitutions elucidated. Compound **8** was isolated in most significant yield among this group, and negative-ion FABMS gave an $[M-H]^-$ peak at *m/z* 701, which is consistent with the constitution of a procyanidin dimer

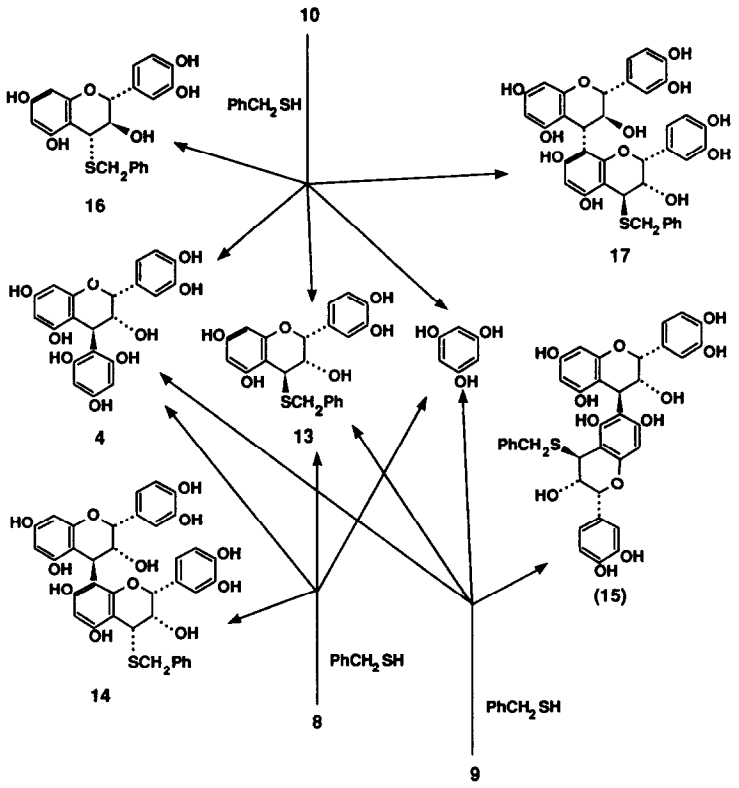
Table 1. ^{13}C NMR chemical shifts of heterocyclic carbons of phloroglucinol adducts in MeOH-d_4

Compound	Unit	C-2	C-3	C-4
4		77.1	73.0	37.1
5		84.2	73.4	38.5
6		80.9	72.7	36.4
8	Upper	77.0	73.4	37.3
	Lower	76.9	72.4	37.2
9	Upper	77.2	73.1	37.6
	Lower	77.4	72.9	37.4
10	Upper	83.5	73.1	39.0
	Lower	72.3	72.9	37.2
11	Upper	77.1	73.4	37.5
	Middle	77.1	72.8	37.5
	Lower	77.1	72.4	37.6
12	Upper	77.4	73.0	37.6
	Middle	77.3	73.0	37.6
	Lower	77.1	72.9	37.8

adduct. The ^{13}C NMR spectrum also showed dual signals for C-2 (δ77.0, 76.9), C-3 (δ73.4, 72.4), and C-4 (δ37.2, 37.3) in the heterocyclic carbon region, which is characteristic of two procyanidin units with the 2,3-*cis* configuration. Confirmation of the structure and the nature of the interflavanoid linkage was made by partial degradation of **8** with benzylthiol (Scheme 1). This reaction generated phloroglucinol, epicatechin-(4β→S)-benzylthioether (**13**), epicatechin-(4β→2)-phloroglucinol (**4**) and epicatechin-(4β→8)-epicatechin-(4β→S)-benzylthioether (**14**). The identity of these products established **8** as epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol.

Compound **9** was shown by an $[M-H]^-$ ion at *m/z* 701 in negative-ion FABMS to be another procyanidin dimer-phloroglucinol adduct. The ^{13}C NMR spectrum of **9** also showed pairs of signals for C-2 (δ73.1, 72.9), C-3 (δ77.2, 77.4) and C-4 (δ37.6, 37.4), and it closely reassembled that of **8**, with both flavanoid units possessing the 2,3-*cis* configuration. Compound **9** therefore had to be the regioisomer of **8** with the less common C-4 to C-6 interflavanoid linkage. Confirmation of this structure was made by partial cleavage of **9** with benzylthiol, which yielded degradation products identical with those for **8**, with one important difference: the presence of epicatechin-(4β→6)-epicatechin-(4β→S)-benzylthioether (**15**) instead of the isomeric C-4 to C-8 linked benzylthioether (**14**). Thus **9** was epicatechin-(4β→6)-epicatechin-(4β→2)-phloroglucinol.

Compound **10** was yet another procyanidin dimer-phloroglucinol adduct, shown by the $[M-H]^-$ peak at *m/z* 701 in negative-ion FABMS. The ^{13}C NMR spectrum of the compound was consistent with the chemical



Scheme 1. Partial cleavage of procyanidin-phloroglucinol adducts with benzylthiol.

constitution of **8** and **9**, with one major difference: C-2 resonance had shifted downfield to 83.5, indicating that one flavanoid unit had the 2,3-*trans* configuration. The nature of the interflavanoid linkage and the sequence of the flavanoid units in **10** was elucidated by partial cleavage with benzylthiol. Examination of the thiolytic-degradation products on 2D TLC revealed epicatechin-(4β→S)-benzylthioether (**13**) and catechin-(4α→S)-benzylthioether (**16**) in a ratio of *ca* 1:6, as estimated by relative spot intensity after visualizing with vanillin–hydrochloric acid. The large difference in the yield of the thioethers in the early stages of degradation was consistent with the locations of the units, the catechin unit at the top end of the molecule being more readily liberated than the inner epicatechin unit. The increase in spot intensity of epicatechin-(4β→S)-benzylthioether (**13**) was apparent as the reaction progressed. In addition to phloroglucinol, epicatechin-(4β→8)-phloroglucinol (**4**) and catechin-(4α→8)-epicatechin-(4β→S)-benzylthioether (**17**) were also identified, the latter compound by yielding catechin-(4α→8)-epicatechin in its reaction with Raney nickel. Compound **10** was therefore established as catechin-(4α→8)-epicatechin-(4β→2)-phloroglucinol.

Compound **11** gave an $[M-H]^-$ peak at *m/z* 989 in the negative-ion spectra of FABMS, suggesting it to be a trimer procyanidin phloroglucinol adduct. The ^{13}C NMR spectrum of **11** was also consistent with this constitution, in which all the C-3 resonances (72.4, 72.8 and 73.4) of the three pyran rings of the flavanoid units were well resolved. The C-2 (δ77.1) and C-4 (δ37.6–37.5) resonances were not resolved for each flavanoid unit, but the relatively upfield position of these resonances indicated that the flavanoid units were all of the 2,3-*cis* configuration. Support for this structure and mode of interflavanoid linkages was obtained from degradation reactions with benzylthiol in which phloroglucinol, epicatechin-(4β→2)-phloroglucinol (**4**), epicatechin-(4β→S)-benzylthioether (**13**) and epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol (**8**) were identified. Thus compound **11** was epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol.

Compound **12** also gave an $[M-H]^-$ ion peak at *m/z* 989, indicating it to be another phloroglucinol adduct of a trimeric procyanidin. The ^{13}C NMR spectrum of **12** was consistent with this constitution and similar to the spectrum of **11** in many respects. However, the three resonances attributed to C-2 (δ77.4, 77.3, 77.1) and C-4 (δ37.8, 37.6, 37.6) of the pyran ring were well resolved, while C-3 resonances (δ72.9–73.0) were not. It was clear from both the FABMS and ^{13}C NMR data that **11** and **12** were regioisomers. Degradation of **12** with benzylthiol gave products that differed from those of **11** in only one important entity, the presence of epicatechin-(4β→6)-epicatechin-(4β→S)-benzylthioether (**15**) instead of its C-4 to C-8 linkage isomer. This thioether was readily generated in comparable yield to epicatechin-(4β→S)-benzylthioether (**13**), indicating that the C-4 to C-6 linked biflavanoid fragment was located at the top end of the molecule. Hence **12** was epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol.

Derivation of the structure of Douglas fir procyanidins

The acid-catalysed cleavage of the polymers with phloroglucinol yielded exclusively flavanoid units having a phloroglucinol A-ring and a catechol B-ring hydroxylation pattern with a preponderance of units in the 2,3-*cis* configuration. While the terminating catechin and epicatechin units were isolated in the ratio of 10:13, the ratio of catechin-(4α→2)-phloroglucinol (**5**) to epicatechin-(4β→2)-phloroglucinol (**4**), representative of the proportion of 2,3-*trans* and 2,3-*cis* extender units, was 1:21. This indicates that the 2,3-*trans* units were present mostly as terminating units in the polymers. The proportion of the C-4 to C-6 linkage to the C-4 to C-8 linkage in the polymers could be estimated directly from the fragmented products that retained some of these bondings. Thus from the yields of the phloroglucinol adducts (**8**–**10**), the proportion of both C-4 to C-8 and C-4 to C-6 linkages was *ca* 21:5, the ratio being the sum of the yields of **8** and **10** to the yield of **9**. Interestingly, the same ratio was obtained by computing the proportion of the bondings in the higher oligomeric adducts **11** and **12**, the yields of each product being taken into account. These estimates were made with the assumption that phloroglucinol, under the given conditions, cleaved the C-4 to C-8 and C-4 to C-6 interflavanoid linkages with equal facility.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were obtained on samples dissolved in MeOH-d_4. Specific rotations were measured in MeOH at 589 nm at ambient temp. IR and FABMS were obtained as previously reported [1, 2]. Analytical and semi-prep. TLC was performed with Schleicher and Schuell cellulose-coated plates developed with *t*-BuOH–HOAc–H_2O (3:1:1, solvent A) and HOAc–H_2O (3:47, solvent B).

Extraction and isolation of polymers. Fresh inner bark (1 kg) of a 120-year-old Douglas fir was extracted exhaustively with MeOH and the combined extract concentrated on a rotatory evaporator under red. pres. The residual extract was diluted with H_2O and the resulting aq. soln extracted with 4 vol. hexane, then with 5 vol. EtOAc. Freeze-drying this extract yielded a fluffy brown solid (48 g), a portion of which (40 g) was applied to a Sephadex LH-20 column (5 × 25 cm). The column was washed with MeOH–H_2O (1:1 v/v) until the washings were almost colourless. The polymer fraction was then eluted from the column with Me_2CO–H_2O (3:2), the Me_2CO rotatory evaporated and the residual aq. soln freeze-dried. Procyanidin polymers were yielded as a light-brown fluffy solid (9.0 g), $[\alpha]_{589}$ +100.4° (MeOH; *c* 0.14), Mn 3370 (GPC of acetates). ^{13}C NMR gave broad peaks in the general regions (ppm): 29, 37, 67, 68, 73, 77, 79, 82, 84, 97, 98, 101, 102, 107, 115, 116, 119, 131, 145 and 154–158. IR ν_{max} (cm^{-1}): 3423, 1610, 1522, 1443, 1359, 1284, 1250, 1202, 1110, 1062, 822, 795, 782, 768.

Reaction of the polymer with phloroglucinol. The polymer (6.0 g) and phloroglucinol (4.0 g) were stirred in 1% HCl in EtOH (45 ml) until complete dissolution of all solids. The solution was left standing for 30 min at ambient temp., then concd to *ca* half vol. with a stream of N_2. The reaction mixture was applied to a Sephadex LH-20 column and eluted with EtOH. Fractions collected in 10-ml test tubes were monitored by UV (280 nm) and by cellulose TLC with solvents A and B. Where necessary, the fractions were combined and further purified by rechromatography on Sephadex LH-20 with EtOH–H_2O (3:17).

Partial cleavage with benzylthiol. A mixture of the sample (2 mg), benzylthiol (1 drop) and HOAc (1 drop) in EtOH (2 ml) were heated in a sealed tube under N_2 at 95°C for 1 hr. The reaction mixture was examined by 2D TLC on cellulose with solvents A and B and visualized with vanillin–HCl spray. 12 TLC plates were used for semi-preparative scale. The products

were located under UV and the spots scraped off and eluted with Me_2CO. The extract was dried under a stream of N_2, and the residue was dissolved in EtOH and treated with a small amount of Raney nickel and a drop of HOAc.

(+)-*Catechin* (**1**), (−)-*epicatechin* (**2**), *and epicatechin-(4β→8)-epicatechin* (**3**). Obtained as freeze-dried powders (145, 194, 40 mg, respectively). Specific rotations, FABMS, ^{1}H and ^{13}C NMR were identical to authentic samples [1, 2].

Epicatechin-(4β→2)-phloroglucinol (**4**). Obtained as a freeze-dried solid (1154 mg), $[\alpha]_{589}$ +121° (MeOH; *c* 0.28), R_f 0.50 (A), 0.60 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 413. ^{13}C NMR (ppm): 37.0, 72.9, 77.0, 95.8, 96.1, 96.4, 101.5, 107.4, 115.1, 115.9, 119.2, 132.5, 145.3, 145.6, 157.5, 157.6, 158.1 and 158.1. ^{1}H NMR (δ): 4.98 (1H, *s*), 4.54 (1H, *s*), 5.08 (1H, *s*), 5.90 (2H, *br s*), 5.97 (1H, *s*), 6.03 (1H, *s*), 6.73 (2H, *m*), 6.92 (1H, *s*).

Catechin-(4α→2)-phloroglucinol (**5**). Obtained as a freeze-dried solid (54 mg), $[\alpha]_{589}$ −188° (MeOH; *c* 0.17), R_f 0.53 (A), 0.62 (B). FABMS gave an $[M-H]^-$ ion peak at m/z 413. ^{13}C NMR (ppm): 38.5, 73.4, 84.2, 96.2, 97.5, 106.7, 107.8, 116.0, 116.1, 121.0, 132.3, 146.0, 146.3, 157.2, 157.3, 157.8, 158.0, 158.5, 158.7. ^{1}H NMR (δ): 4.4–4.53 (3H, *m*), 5.8–6.0 (4H, *m*) and 6.7–7.0 (3H, *m*).

Epicatechin-(4α→2)-phloroglucinol (**6**). Isolated as a freeze-dried off-white solid (28 mg), $[\alpha]_{589}$ +64° (MeOH; *c* 0.08), R_f 0.58 (A), 0.35 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 413. ^{13}C NMR (ppm): 36.4, 72.7, 80.9, 96.4, 96.6, 97.3, 97.5, 102.4, 106.0, 115.2, 115.9, 119.3, 132.3, 145.8, 145.9, 157.6, 157.7, 158.1, 158.7 and 159.3. ^{1}H NMR (δ): 4.10 (1H, *d*, J = 4.5 Hz), 4.7–5.1 obscured by –OH peak, 5.80 (1H, *d*, J = 2.2 Hz), 5.82 (1H, *d*, J = 2.4 Hz), 5.98 (1H, *d*, J = 2.4 Hz), 5.99 (1H, *d*, J = 2.2 Hz), 6.76 (1H, *d*, J = 8.2 Hz), 6.84 (H, *dd*, J = 1.6, 8.2 Hz), and 7.04 (1H, *d*, J = 1.6 Hz).

1,3-*di*-(2,4,6-*trihydroxyphenyl*)-3-(3,4-*dihydroxyphenyl*)-*propan*-2-*ol* (**7**). Isolated as a freeze-dried solid (77 mg), $[\alpha]_{589}$ −6.3° (MeOH; *c* 0.17), R_f 0.59 (A) and 0.56 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 415. ^{13}C NMR (ppm): 30.4, 46.4, 77.6, 96.1, 106.6, 107.5, 115.9, 116.8, 120.6, 135.5, 144.0, 145.7 157.7, 158.0, 158.2 and 158.5. ^{1}H NMR (δ): 2.45 (1H, *dd*, J = 14.5, 10.2 Hz), 2.88 (1H, *dd*, J = 1.3, 14.5 Hz), 4.58 (1H, *m*), 4.74 (1H, *d*, J = 3.6 Hz), 5.8–5.92 (4H, *m*), 6.6–6.74 (3H, *m*).

Epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol (**8**). Isolated as a freeze-dried solid (537 mg), $[\alpha]_{589}$ +144° (MeOH; *c* 0.12), R_f 0.38 (A) and 0.56 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 701. ^{13}C NMR (ppm): 37.2, 37.3, 72.4, 73.4, 76.9, 77.0, 96.2–96.8, 99.8, 101.9, 107.1, 108.1, 115.2, 115.4, 115.8, 115.9, 132.6, 145.3, 145.4, 145.7, 154.8, 157.0, 157.1, 157.7 and 158.2. ^{1}H NMR (δ): 4.02 (1H, *br s*), 4.06 (1H, *br s*), 4.62 (1H, *br s*), 4.72 (1H, *br s*), 5.08 (1H, *br s*), 5.23 (1H, *br s*), 5.85–5.62 (*m*), 6.7–7.2 (6H, *m*). Reaction with benzylthiol gave phloroglucinol, epicatechin-(4β→S)-benzylthioether (**13**), epicatechin-(4β→2)-phloroglucinol (**4**) and epicatechin-(4β→8)-epicatechin-(4β→S)-benzylthioether (**14**).

Epicatechin-(4β→6)-epicatechin-(4β→2)-phloroglucinol (**9**). Isolated as a freeze-dried solid (137 mg), $[\alpha]_{589}$ +174° (MeOH; *c* 0.17), R_f 0.36 (A), 0.43 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 701. ^{13}C NMR (ppm): 37.4, 37.6, 72.9, 73.1, 77.2, 77.4, 96.2–96.8, 99.8, 101.9, 107.1, 108.1, 115.2, 115.4, 115.8, 115.9, 119.2, 119.7, 132.4, 132.5, 145.5–145.8, 155.7–159.4. ^{1}H NMR (δ): 3.91 (1H, *s*), 3.94 (1H, *s*), 4.41 (1H, *br s*), 4.57 (1H, *s*), 4.82 (1H, *s*, partially obscured by OH), 5.0 (1H, *br s*), 5.6–6.2 (*m*), 6.7–6.95 (6H, *m*). Treatment with benzylthiol yielded phloroglucinol, epicatechin-(4β→S)-benzylthioether (**13**), epicatechin-(4β→2)-phloroglucinol (**4**) and epicatechin-(4β→6)-epicatechin-(4β→S)-benzylthioether (**15**).

Catechin-(4α→8)-epicatechin-(4β→2)-phloroglucinol (**10**). Isolated as a freeze-dried solid (33 mg), $[\alpha]_{589}$ −31.4° (MeOH; *c* 0.15), R_f 0.40 (A), 0.66 (B). FABMS gave an $[M-H]^-$ ion peak at *m/z* 701. ^{13}C NMR (ppm): 37.2, 39.0, 72.9, 73.1, 77.3, 83.5, 96.2–97.2, 100.6, 107.1, 107.5, 107.6, 115.0, 116.0, 116.1, 116.4, 118.7, 121.3, 132.2, 132.9, 145.3, 145.8, 146.4, 115.6, 156.9, 157.1, 157.3, 157.4, 157.8, 158.4. Reaction with benzylthiol gave phloroglucinol, epicatechin-(4β→2)-phloroglucinol (**4**), epicatechin-(4β→S)-benzylthioether (**13**), catechin-(4α→S)-benzylthioether (**16**) and catechin-(4β→8)-epicatechin-(4β→S)-benzylthioether (**17**). The latter compound yielded procyanidin B4 on treatment with Raney nickel.

Epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol (**11**). Isolated as a freeze-dried solid (74 mg), $[\alpha]_{589}$ +142° (MeOH; *c* 0.12), R_f 0.30 (A), 0.55 (B). ^{13}C NMR (ppm): 37.4, 37.4, 37.5, 72.4, 72.8, 73.4, 77.1 (*br s*), 96.2–97.6, 101.0, 101.2, 102.6, 107.6 (b), 107.9, 115.0–116.0, 118.8–119.3, 132.7 (b), 145.3–145.9, 154.9, 156.8–158.3. ^{1}H NMR (δ): 4.03 (1H, *br s*), 4.06 (1H, *br s*), 4.11 (1H, *br s*), 4.6–4.8 (3H, *m*), 5.09 (1H, *br s*), 5.25 (1H, *br s*), 5.27 (1H, *br s*), 5.8–6.3 (*m*), 6.6–7.3 (*m*). Reaction with benzylthiol gave phloroglucinol, epicatechin-(4β→2)-phloroglucinol (**4**), epicatechin-(4β→S)-benzylthioether (**13**), and epicatechin-(4β→8)-epicatechin-(4β→S)-benzylthioether (**14**).

Epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol (**12**). Isolated as a freeze-dried solid (47 mg), $[\alpha]_{589}$ +151° (MeOH; *c* 0.12), R_f 0.53 (A), 0.48 (B). ^{13}C NMR (ppm): 37.4, 37.6 (× 2), 72.9, 73.0 (× 2), 77.1, 77.3 (× 2), 96.2–97.3, 99.6, 100.7, 101.2, 107–108.4, 115.1–116.0, 118.9–119.6, 132.4, 132.5 (× 2), 145.4–145.8, 155.7–159.7. ^{1}H NMR (δ): 3.91 (1H, *br s*), 3.94 (1H, *br s*), 4.00 (1H, *br s*), 4.4–4.6 (*m*), 4.8–5.2 (obscured by –OH signal), 5.8–6.2 (*m*), 6.6–7.2 (*m*). Reaction with benzylthiol gave phloroglucinol, epicatechin-(4β→2)-phloroglucinol (**4**), epicatechin-(4β→S)-benzylthioether (**13**) and epicatechin-(4β→6)-epicatechin-(4β→S)-benzylthioether (**15**).

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