Linkage Isomerism in Trimeric and Polymeric 2,3-*cis*-Procyanidins

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Four procyanidin trimers have been isolated and their structures unequivocally established as: epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (5); epicatechin-(4β→6)-epicatechin-(4β-8)-catechin (6); epicatechin-(4β→8)-epicatechin-(4β→6)-catechin (7); and epicatechin-(4β-8)-epicatechin-(4β→8)-epicatechin (13). The presence of the first three trimers in *Pinus taeda* phloem, and the isolation of both epicatechin-(4β→8)-4β-benzylthioepicatechin (9) and epicatechin-(4β→6)-4β-benzylthioepicatechin (10) from partial acid-catalyzed cleavage of the tannins from *Pinus palustris* phloem and *Photinia glabrescens* leaves, shows that linkage isomerism exists in natural oligomeric and polymeric procyanidins. A new system of nomenclature for proanthocyanidins is proposed.

PROCYANIDIN polymers consist of chains of 5,7,3′,4′-tetrahydroxyflavan-3-ol units linked by C(4)–C(6) or C(4)–C(8) bonds.[1] Whereas the procyanidin-B group of dimers are known to exist as pairs of isomers with common flavan-3-ol units, but different interflavanoid linkages,[2,3] the extent of such isomerism in higher molecular weight procyanidins is unknown. A number of trimeric procyanidins have been isolated,[4-7] but no unequivocal proof of their structure has been offered. Large numbers of isomers are possible since the trimeric procyanidins consist of two procyanidin (PC) units each containing three asymmetric centres, a 5,7,3′,4′-tetrahydroxyflavan-3-ol chain-terminating unit containing two chiral centres, and C(4)–C(6) or C(4)–C(8) interflavanoid linkages.

Earlier work by Haslam and his co-workers[4] showed that procyanidin oligomers could be fractionated by column chromatography with ethanol or methanol on Sephadex LH-20. Whereas mono- or di-meric 5,7,3′,4′-tetrahydroxyflavan-3-ols were eluted in earlier fractions, trimers and oligomers were less mobile.[2,4] In this way dimeric procyanidins have been isolated from a variety of plants and a trimer, procyanidin C1, was isolated from horse-chestnut fruits.[4] Similar fractionation of the ethyl acetate-soluble procyanidins of *Pinus taeda* (loblolly pine) phloem yielded (+)-catechin (1) and the major dimeric procyanidins B1 (3) and B7 (4).[8,9] Further Sephadex chromatography yielded fractions containing trimeric flavanoids, whose isolation and structure are fully discussed here. Some preliminary results of this work have been published.[10]

Trimeric Procyanidins of Loblolly Pine.—The major procyanidin (5) was eluted from Sephadex immediately after procyanidin B7 (4), and oligomeric procyanidins with a number-average molecular weight ($\bar{M}_n$) of 1 200 were eluted in later fractions with methanol.[8] Analysis of these fractions by h.p.l.c. showed that the most mobile (and major) procyanidin (5) was essentially homogeneous, whereas the oligomeric fraction contained at least four compounds. Preparative h.p.l.c. afforded pure samples of procyanidins from both fractions, and mass spectrometry of the methyl ethers and g.p.c. of the peracetate derivatives confirmed the triflavanoid constitution of (5) and the two major constituents, (6) and (7), of the oligomeric fraction.

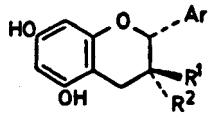
The constitution of each trimer was established by acid-catalysed degradation with toluene-α-thiol.[4] Complete degradation gave 4-benzylthioepicatechin (8) and catechin (1) as the only products, establishing that the PC units possess a 2,3-*cis*-stereochemistry and (+)-catechin (1) as the terminal unit. Partial degradation of the trimers gave, in addition to the above products, procyanidin dimers and dimeric procyanidin benzyl sulphides (see reaction Scheme). In particular, the production of procyanidin B1 (3) from both (5) and (6) enabled the lower interflavanoid linkage (b) to be established as C(4)–C(8), and the formation of procyanidin B7 (4) from (7) indicated a C(4)–C(6) linkage for this trimer. Additionally, trimers (5) and (7) gave the dimeric benzyl sulphide (9) while (6) yielded the chromatographically distinct benzyl sulphide (10). Desulphurization of (9) and (10) with Raney-nickel[4] gave the known procyanidins B2 (11) and B5 (12) respectively, thus establishing the upper (a) interflavanoid linkage of (5) and (7) as C(4)–C(8) and that of (6) as C(4)–C(6).

4-Benzylthioepicatechin (8) when heated in the presence of acid underwent self-condensation to yield both the dimeric procyanidin benzyl sulphides (9) and (10), and polymeric products. However, in the presence of an excess of toluene-α-thiol (phenylmethanethiol) no self-condensation takes place. Furthermore (9) or (10) did not interconvert when heated in acid in the presence of toluene-α-thiol, thus validating this method for establishing the position of the interflavanoid linkages.

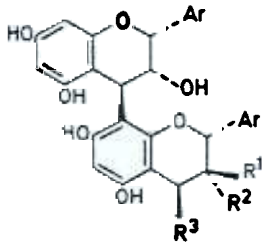
Epicatechin Trimer.—A further trimer (13) was synthesized by reaction of the 2,3-*cis* PC polymers from horse-chestnut fruit or *Photinia* leaves[1] with (−)-epicatechin (2). The trimer (13) was co-eluted with procyanidin B5 (12) on Sephadex LH-20 chromatography, and the two procyanidins were separated by h.p.l.c. The triflavanoid constitution was confirmed by mass spectrometry of the dodecamethyl ether and g.p.c. of the peracetate derivative, and both the interflavanoid

linkages were shown to be C(4)–C(8) from the products of partial acid-catalyzed cleavage with toluene-α-thiol (see Scheme).

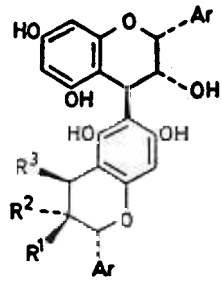
The trimer (13) is probably identical with procyanidin C1, first isolated from horse-chestnuts.[4] The ^{13}C n.m.r. resonances reported[2] for the heterocyclic ring carbons are similar to those obtained for (13).[11] Also, the synthetic trimer is chromatographically indistinguishable (cellulose t.l.c.; h.p.l.c.) from the major natural trimer of horse-chestnuts.



(1) R^1 = OH R^2 = H
(2) R^1 = H, R^2 = OH

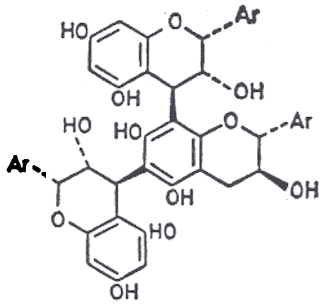


(3) R^1 = OH, R^2 = R^3 = H
(9) R^1 = H, R^2 = OH, R^3 = SCH_2Ph
(11) R^1 = R^3 = H, R^2 = OH
(14) R^1 = H, R^2 = OH, R^3 = $C_6H_2(OH)_3$-2,4,6

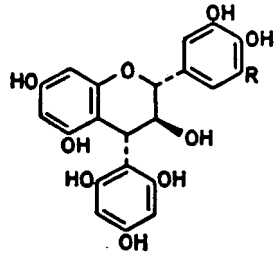


(4) R^1 = OH, R^2 = R^3 = H
(10) R^1 = H, R^2 = OH, R^3 = SCH_2Ph
(12) R^1 = R^3 = H, R^2 = OH
(15) R^1 = H, R^2 = OH, R^3 = $C_6H_2(OH)_3$-2,4,6

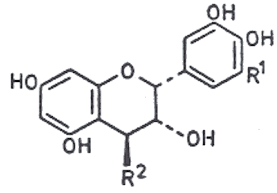
Ar = $C_6H_3(OH)_2$-3,4



(20)



(16) R = H
(17) R = OH



(8) R^1 = H, R^2 = SCH_2Ph
(18) R^1 = H, R^2 = $C_6H_2(OH)_3$-2,4,6
(19) R^1 = OH, R^2 = $C_6H_2(OH)_3$-2,4,6

Dimeric Cleavage Products from Partial Degradation of Procyanidin Polymers.—Treatment of the condensed tannins from *Pinus palustris* (longleaf pine) phloem with toluene-α-thiol provided a convenient synthetic route to larger amounts of the dimeric benzyl sulphides (9) and (10). This enabled the spectroscopic characteristics of the two phenols and their peracetate derivatives to be readily established.

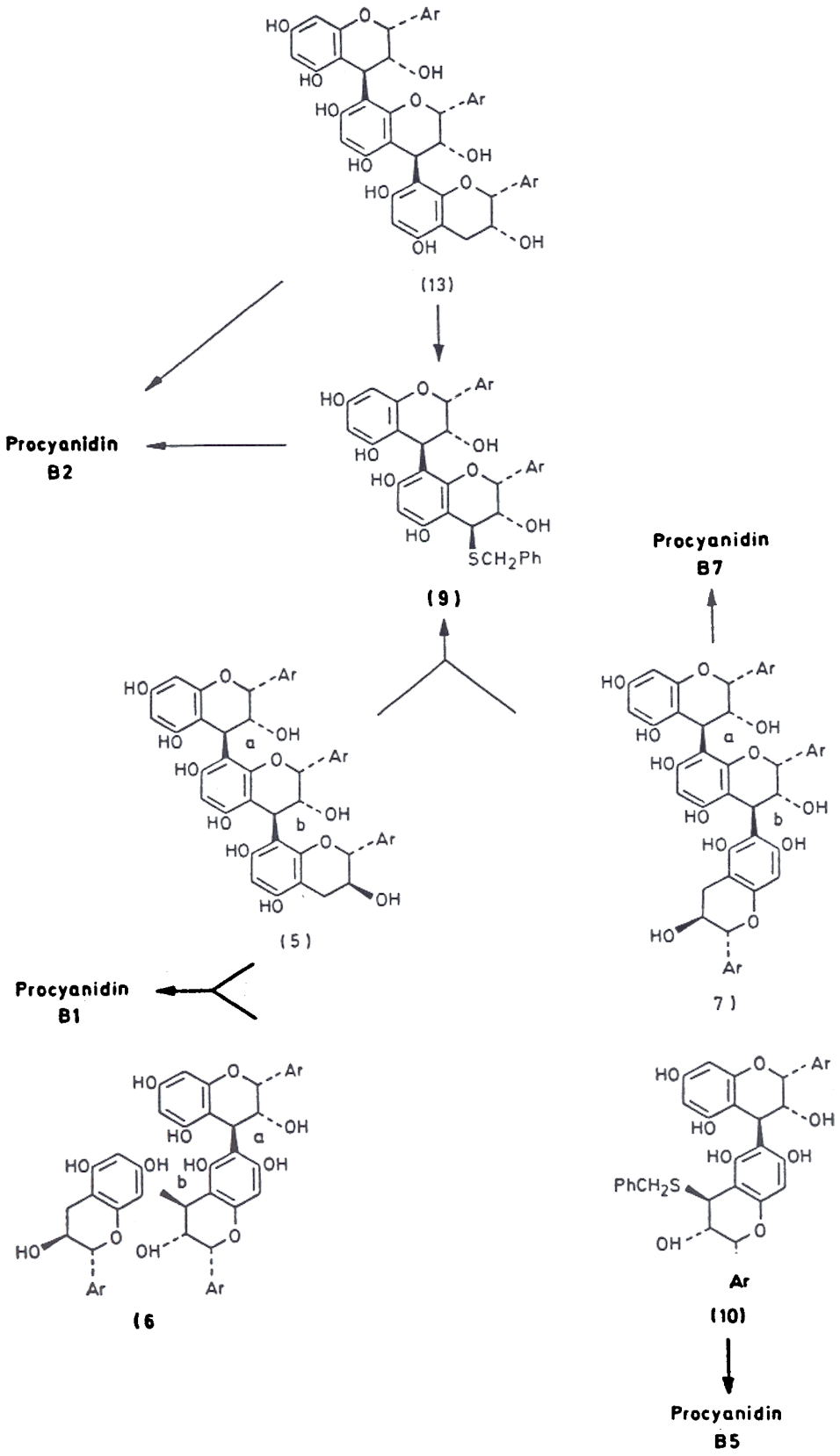
In a similar way treatment of the polymer from *Photinia glabrescens* leaves with phloroglucinol in mild acid provided the equivalent dimeric 4-C-phloroglucinol analogues (14) and (15). These are most useful models for the procyanidin trimers since many of their n.m.r. and chiroptical properties are closely similar.

Both the C(4)–C(8) linked isomers, (9) and (14), have been isolated previously.[2,4,12] However, earlier studies did not provide an unequivocal proof of structure.

Configuration of the Interflavanoid Linkages.—The relative configuration of the interflavanoid linkages of compounds (9), (10), (14), and (15), and of the trimers (5)—(7) and (13), deduced from the products of chemical degradation, are supported by the ^{13}C n.m.r. chemical shifts of the heterocyclic ring carbons,[2,11] and the ^1H n.m.r. chemical shifts of H-6 and -8 of a number of procyanidin peracetates, including the above compounds, given in the Table.

The ^1H n.m.r. spectra of all compounds are complicated by conformational isomerism.[2] However, the population of conformers is normally unequal, so that the resonances characteristic of the major rotational isomer may be readily assigned.

The ^1H n.m.r. of the peracetates of the C(4)–C(6) linked procyanidins B7 (4) and B5 (12) are similar. Thus, the AB quartet of the upper flavan-3-ol unit is centred near



SCHEME. Cleavage of procyanidin trimers with toluene-α-thiol

δ 6.7, whereas in the C(4)–C(8) linked procyanidins B1 (3) and B2 (11), the A-ring protons of the major conformational isomer are shifted upfield by *ca.* 0.5 p.p.m. and are centred on 6.1 p.p.m.[2] The rotamer populations for the two C(4)–C(6) linked dimers (procyanidin B7 and B5) are nearly equal, while those of the C(4)–C(8) linked procyanidins B1 and B2 are present in proportions of *ca.* 2 : 1. In addition, the signals for H-4 of the upper unit of both B-5 and B-7 peracetates appear as a pair of signals of near-equal intensity at δ 4.15 and 4.32 (procyanidin B5) and δ 4.35 and 4.45 (procyanidin B7). In contrast, the H-4 signals for the C(4)–C(8) linked procyanidin peracetates appear at δ 4.48 (procyanidin B1) and δ 4.45 (procyanidin B2). Thus, procyanidins with a C(4)–C(6) interflavanoid bond are readily distinguished from those with a C(4)–C(8) bond on the basis of the ^{1}H n.m.r. spectra of the peracetate derivatives.

The A-ring ^{1}H n.m.r. shifts (δ) of flavan-3-ol peracetates in $CDCl_3$

Compound	Flavan unit [a]	6-H	8-H	Rotamer population
(+)-Catechin (1) [b]		6.59	6.67	
(−)-Epicatechin (2) [b]		6.55	6.69	
(18) [b]		6.60	6.77	
(8) [b]		6.45	6.60	
Procyanidin B1 (3) [b]	U	5.99	6.30	2.5 : 1
	L	6.67		
Procyanidin B7 (4) [c]	U	6.63	6.69	1 : 1
	L		6.80	
Procyanidin B2 (11) [b]	U	5.98	6.23	2 : 1
	L	6.65		
Procyanidin B5 (12) [b]	U	6.60	6.73	1.1 : 1
	L		6.61	
(9) [b]	U	6.00	6.26	2.7 : 1
	L	6.62		
(10) [b]	U	6.52	6.72	2 : 1
	L		6.63	
(14) [b]	U	6.04	6.27	5.1 : 1
	L	6.65		
(15) [b]	U	6.63	6.63	3 : 1
	L		6.83	
(5) [b]	U	5.96	6.25	*d*
(6) [b]	U	6.53	6.58	*d*
(7) [b]	U	6.06	6.29	*d*
(13) [b]	U	5.94	6.25	*d*

[a] U = upper, L = lower.

[b] Monomers. [c] Major comformer. [d] Highest field AB quartet. [e] Not possible to calculate since the trimers exist as an equilibrium mixture of four conformational isomers.

Comparison of the A-ring proton shifts for the upper unit of the acetate derivatives of the dimer sulphides (9) and (10) (*i.e.* AB quartets centred on δ 6.13 and 6.62 respectively) and of the phloroglucinol adducts (14) and (15) (*i.e.* AB quartets centred on δ 6.16 and 6.63) are consistent with the earlier assignment of C(4)–C(8) and C(4)–C(6) interflavanoid linkages for the two pairs of compounds. In a similar way, the upfield shift of the A-ring protons of the upper unit of the trimeric procyanidins (5), (7), and (13) corroborates the assignment of a C(4)–C(8) interflavanoid linkage for the upper bond (a), and the normal shift of the upper unit A-ring protons in the trimer (6) indicates a C(4)–C(6) interflavanoid linkage.

The c.d. spectra of proanthocyanidins are largely determined by the interaction of the A-ring chromophores,[13,14] analogous to other diphenylmethanes with fixed geometry.[15,16] This effect facilitates the direct assignment of the absolute configuration at C(4), and thus the interflavanoid linkage. Similarly the sign of the long-wavelength specific rotation of proanthocyanidins is also determined by this interaction,* the observed strong positive rotation at 578 nm for the dimeric procyanidins (9), (10), (14), and (15), and the trimers (5)—(7), and (13), and for the peracetate derivatives, all being consistent with the assigned absolute configuration at C(4). This is also corroborated by the c.d. spectrum, where it has been established that for 4-phenyl substituted flavan-3-ol units with a 2*R* configuration, a 4β-configuration is associated with a positive c.d. band near 230 n.m.[14] As expected, such a positive band is observed for all the procyanidin peracetate derivatives isolated in this study.

Proanthocyanidin Nomenclature: Some Proposals.—Currently procyanidin molecules are named according to a trivial system initiated by Weinges [18] and extended by Haslam.[4] However, the isolation of proanthocyanidin dimers with mixed oxidation pattern, and the current isolation of trimers of known absolute configuration, highlights the need for a workable system of nomenclature for these oligomers. Roux [23] has recently used a method for naming proanthocyanidin dimers based on I.U.P.A.C. rules, which uses flavan as the basic ring system and (*RS*) nomenclature for indicating the absolute configuration. However, the (*RS*) system becomes excessively cumbersome (and misleading) when extended to trimers. For instance, in trimer (5) the absolute configuration of C(4) at bond (a) is 4*R*, but that at bond (b) is 4*S*.

This nomenclatural problem can be avoided by naming proanthocyanidin oligomers in an analogous manner to oligo- and poly-saccharides. In the latter case chiral units, with various structures, are attached through an asymmetric centre [C(1)], to various other positions on neighbouring units. Thus C(4) of proanthocyanidins is equivalent to C(1) of sugars. This leads to a system for proanthocyanidin nomenclature which has the following features.

(1) It defines the basic structural units in terms of the familiar monomeric flavan-3-ols. Further, the names catechin, epicatechin, gallocatechin, afzelechin *etc.* are

* It has been demonstrated that the $[\alpha]_{578}$ values for proanthocyanidin polymers obey a simple additive relationship depending on the ratio of 2,3-*cis*- to 2,3-*trans*-units only, and independent of B-ring oxidation pattern.[1] This has been extended [17] to the pairs of phloroglucinol derivatives (16) and (17), possessing 2,3-*trans*-stereochemistry, and (18) and (19), possessing 2,3-*cis*-stereochemistry. These pairs have the *same* specific rotation at 578 nm, −200° and +122° respectively, even though their B-ring oxidation pattern is different. Therefore, for proanthocyanidins containing units with a 2*R*-configuration, a *pseudo*-axially oriented (β) substituent at C(4) is associated with a strong *positive* specific rotation at 578 nm, and a *pseudo*-equatorial (4α) substituent with a strong *negative* rotation. This rule is obeyed by all dimeric procyanidins and their acetate and methyl ether derivatives,[2,4,18] and the known prodelphinidins [18,19,20] and propelargonidins.[21,22]

reserved for units with a 2*R*-configuration, and thus the absolute configuration of C(3) is automatically defined. Flavan-3-ol units with a 2*S*-configuration, which are known to occur naturally,[24] are distinguished by the *enantio*-prefix.[25] Thus (+)-epicatechin is *ent*-epicatechin.

(2) The interflavanoid linkage is indicated the same way as in carbohydrates, the bond and its direction contained in brackets.

(3) The configuration of the interflavanoid bond at C(4) is indicated by the (αβ) nomenclature,[25] thus avoiding an extra set of brackets, and the ambiguities of the (*RS*) system.

The proposed system assumes that all flavanoid structures are drawn in the normal way.

The procyanidins are now readily named, procyanidin B2 (11) becomes epicatechin-(4β→8)-epicatechin, and procyanidin B3 catechin-(4α→8)-catechin. This method has the advantage that branched structures, such as (20), are also readily named: epicatechin-(4β→6)-catechin-(8→4β)-epicatechin.

The structures of the four trimers are now established unequivocally as: epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (5); epicatechin-(4β→6)-epicatechin-(4β→8)-catechin (6); epicatechin-(4β→8)-epicatechin-(4β→6)-catechin (7); and epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (13).

Linkage Isomerism in Procyanidin Polymers.—Isolation of the trimeric procyanidin fraction by gel filtration and subsequent analysis by h.p.l.c. showed that the major trimers were present in loblolly pine phloem in the relative proportions of 2 : 1 : 1 respectively. They were formed in similar proportions when synthesized from the polymer. Similarly the polymer, when treated with toluene-α-thiol, yielded the C(4)–C(8) and C(4)–C(6) dimers, (9) and (10), in a ratio of *ca.* 3 : 1. Therefore, both experiments imply that the ratio of C(4)–C(8) to C(4)–C(6) linkages, between *procyanidin* units, is *ca.* 3 : 1 in the original polymer. Similar results were obtained from the degradation of longleaf pine phloem and *Photinia* leaf tannins with toluene-α-thiol. In contrast, only small or negligible amounts of the C(4)–C(6) isomer was formed from the polymers isolated from *Vicia sativa* leaves and the fruits of blueberry or horsechestnut.

Another interesting observation was that the yields of the two dimers were time dependent. For example, the degradation of longleaf tannin led initially to the rapid formation of compound (9), reaching a maximum concentration in 3—4 h, while the C(4)–C(6) isomer was initially formed in low yield and reached a maximum concentration after 18—20 h. These observations are partly explained by the fact that the C(4)–C(6) bond is cleaved at a slower rate than the C(4)–C(8) bond in acid solution,[26] but fails to explain the *increase* in the *absolute* concentration of compound (10) after a long reaction period. However, this behaviour may be rationalised if it is assumed that the C(4)–C(6) linkages are initially inaccessible to attack.

These observations suggest that the polymers are of two types: the first group includes those from horsechestnut, *etc.* where the interflavanoid linkages are almost exclusively C(4)–C(8), and are linear or threadlike structures, as proposed by Haslam and his co-workers,[2] and are rapidly cleaved by acidic toluene-α-thiol. The second group include those of the *Photinia*, *etc*, and contain a high proportion of C(4)–C(6) linkages, and are likely to be highly branched globular structures. Thiolytic degradation of these latter polymers is much slower, and proceeds initially by cleavage of the peripheral units, mostly linked C(4)–C(8), before the internal C(4)–C(6) bonds become accessible for attack.

The existence of at least two different polymer structures based on the same PC units is most significant since it implies that their biosynthesis must be under different enzymatic control. Further experiments are in progress, using high-field ^{13}C n.m.r. spectroscopy, to explore the above proposal.

EXPERIMENTAL

Mass spectra were obtained with an A.E.I. MS30. ^{1}H N.m.r. spectra were recorded at 80 MHz and ^{13}C n.m.r. at 20 MHz with a Varian FT-80A spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and circular dichroism measurements on a Jasco Model ORD/UV-5 instrument in methanol. High-performance liquid chromatography (h.p.l.c.) was carried out on Waters μBondapak C-18, Waters 500Å μStyragel, and du Pont Zorbax CN columns using a Waters Associates ALCGPC 244 unit. The μStyragel gel permeation chromatography (g.p.c.) column was standardised with flavan-3-ol and oligomeric procyanidin acetates, and low molecular weight polystyrene standards (solvent, chloroform or tetrahydrofuran).

Cellulose t.l.c. analyses were performed on Schleicher and Schüll F1440 sheets in the solvent systems (A) t-butyl alcohol–acetic acid–water (3 : 1 : 1, v/v) and (B) 6% acetic acid (v/v).

Thiolysis of Procyanidins: General Analytical Procedure.—A sample of procyanidin (5 mg) and toluene-α-thiol (0.2 ml) were dissolved in ethanol (3 ml) in a vial, the contents flushed with nitrogen for 2 min, glacial acetic acid (0.1 ml) added, and the vial sealed with a silicone rubber septum. The sealed vial was placed on a boiling water-bath and the products periodically sampled by syringe. The progress of the reaction was monitored by two-dimensional t.l.c. on cellulose using solvents (A) and (B).

Desulphurization was achieved by treating a small sample of the reaction products with W-2 Raney-nickel in ethanol.

Extraction of Loblolly Pine Phloem.—The phloem (1 100 g wet weight) collected from a 28 year-old tree was extracted with acetone–water (7 : 3 v/v), and the extract, on concentration and freeze-drying yielded a red-brown solid (137 g, 30% yield based on dried weight). The solid was redissolved in water (1 500 ml), and the aqueous solution was washed with light petroleum (3 × 300 ml) and extracted with ethyl acetate (6 × 300 ml) to yield a solid (7.8 g). The ethyl acetate-soluble fraction (4-g portions) was chromatographed on Sephadex LH-20 (2.5 × 100 cm column), eluted with ethanol, and the eluate collected in

15-ml fractions. The dimeric procyanidins B1, B3 (minor component), and B7 were present up to fraction 275.

Epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (5). This compound was obtained in 2.5% crude yield from fractions 288—320. The trimer (5) was purified by reverse phase h.p.l.c. on a 4.6-mm × 25-cm nitrile column (solvent, methanol–water, 7 : 3 v/v; flow-rate, 1.2 ml/min, retention volume 6.6 ml) to yield the *procyanidin* as a white powder, after freeze-drying (Found: C, 61.9; H, 4.8. $C_{45}H_{38}O_{18}$·0.5 H_2O requires C, 61.7; H, 4.5%).* $[\alpha]_{578}$ +74° (*c* 0.14 in H_2O); R_F (A) 0.23, R_F (B) 0.58.

The dodecamethyl ether was obtained by methylation of the phenol (5) with ethereal diazomethane in methanol and purified by t.l.c. [chloroform–methanol (40 : 1 v/v); silica; R_F 0.4], *m/e* 1 034 (M^+) 855, 837.

The *peracetate* (acetic anhydride–pyridine) of (5) was purified by t.l.c. [benzene–acetone (8 : 2, v/v); silica; R_F 0.32] to yield a white amorphous solid (Found: C, 60.3; H, 4.6. $C_{75}H_{68}O_{33}$ requires C, 60.2; H, 4.6%); $[\alpha]_{578}$ +95° (*c* 0.51 in acetone); c.d. maxima $\Delta\varepsilon_{225}$ +9.6 (shoulder), $\Delta\varepsilon_{244}$ +5.5; g.p.c. gave $\bar{M}_n$ 1 500.

Degradation of compound (5) with toluene-α-thiol gave catechin (1), 4β-benzylthioepicatechin (8), procyanidin B1 (3), and (9), and, on treatment with Raney-nickel, catechin (1), epicatechin (2), and the procyanidins B1 (3) and B2 (11). All products were identified by cochromatography with authentic compounds (t.l.c. and h.p.l.c.).

Isolation of the trimers (6) *and* (7). Further elution of the Sephadex LH-20 column separation of the loblolly phloem procyanidins with methanol yielded a fraction (390 mg) which a previous investigation had shown to consist largely of tri- and tetra-meric procyanidins.[8] Reverse-phase h.p.l.c. on a nitrile column [same conditions as for the isolation of (5)] showed that the fraction contained two major compounds with retention volumes of 6.7 and 10.8 ml. However, these compounds were more conveniently synthesized as follows.

Synthesis of the trimers (5), (6), *and* (7). The water-soluble tannin from loblolly pine phloem (25 g) was treated with (+)-catechin (5 g) in dioxan–water (1 : 1, v/v; 100 ml) containing concentrated hydrochloric acid (5 ml). The reaction mixture was stirred for 48 h under argon and then poured into water (300 ml) and extracted with ethyl acetate (6 × 300 ml). Evaporation of the ethyl acetate extract gave a solid (6.7 g) which on chromatography on Sephadex LH-20, with ethanol, gave initially (+)-catechin, followed by procyanidin B1 {$[\alpha]_{578}$ +104° (*c* 0.43, H_2O) after purification by h.p.l.c. on a nitrile column and drying over P_2O_5, lit.[4] value $[\alpha]_{578}$ +31° (*c* 0.8 in EtOH)} and procyanidin B7 {$[\alpha]_{578}$ +142° (*c* 0.10 in H_2O) purified similarly} up to fraction 270. Fractions 270—320 contained the trimer (5), which was shown to be identical with the natural product by reverse-phase h.p.l.c. (Zorbax nitrile, methanol–water, 3 : 7 v/v and μBondapak C-18, 3.9 mm × 30 cm column, methanol–water–acetic acid, 20 : 79 : 1 v/v, 1.2 ml/min. retention volume 7.0 ml), and by co-chromatography on cellulose t.l.c. and by degradation with toluene-α-thiol.

Fractions 320—380 contained a mixture of procyanidins, the major components (6) and (7) being present in nearly equal proportions. These were separated by reversed-phase h.p.l.c. on a nitrile column, methanol–water (7 : 3 v/v), and the resulting fractions were freeze-dried to yield the following.

Epicatechin-(4β→6)-epicatechin-(4β→8)-catechin (6), retention volume 6.7 ml, an off-white amorphous *solid* (Found: C, 60.3. H, 4.6. $C_{45}H_{38}O_{18}$·1.5H_2O requires C, 60.5; H, 4.6%), $[\alpha]_{578}$ +106° (*c* 0.19 in H_2O); R_F (A) 0.30, R_F (B) 0.60. Formed a dodecamethyl ether, *m/e* 1 034 (M^+), 855, and 837.

The peracetate was prepared and purified as for compound (5) to give an amorphous *solid*, t.l.c. R_F 0.28 (Found: C, 59.3; H, 4.4. $C_{75}H_{68}O_{33}$. H_2O requires C, 59.4; H, 4.7%), $[\alpha]_{578}$ +94.5° (*c* 0.15 in acetone); c.d. maxima $\Delta\varepsilon_{234}$ +12.8, $\Delta\varepsilon_{262}$ +6.2; g.p.c. M_n 1 500.

Treatment of compound (6) with toluene-α-thiol yielded catechin (1), 4β-benzylthioepicatechin (8), procyanidin B1 (3), and compound (10). Reaction with Raney-nickel yielded, additionally, epicatechin (2) and procyanidin B5 (12).

Epicatechin-(4β→8)-epicatechin-(4β→6)-catechin (7), retention volume 10.8 ml; an off-white amorphous *solid* (Found: C, 61.6; H, 5.0. $C_{45}H_{38}O_{18}$. 0.5 H_2O requires C, 61.7; H, 4.5%), $[\alpha]_{578}$ +207° (*c* 0.11 in H_2O); R_F (A) 0.37, R_F (B) 0.47. Formed a dodecamethyl ether, *m/e* 1 034 (M^+), 855, and 837.

The peracetate was prepared and purified as for compound (5) to yield an amorphous *solid*, t.l.c. R_F 0.34 (Found: C, 59.7; H, 4.6. $C_{75}H_{68}O_{33}$·0.5H_2O requires C, 59.8; H, 4.6%), $[\alpha]_{578}$ +110° (*c* 0.16 in acetone); c.d. maxima $\Delta\varepsilon_{230}$ +17.4, $\Delta\varepsilon_{264}$ +7.2; g.p.c. M_n 1 500.

Treatment of the trimer (7) with toluene-α-thiol yielded catechin (1), 4β-benzylthioepicatechin (8), procyanidin B7 (4), and compound (9). Reaction with Raney-nickel yielded, additionally, epicatechin (2) and procyanidin B2 (11).

Relative proportions of trimers in loblolly pine phloem. A portion of the crude ethyl acetate fraction was dispersed in acetone–water (1 : 1, v/v) and filtered twice through a millipore filter. A sample (180 mg) in acetone–water (0.5 ml) was separated by gel-filtration on a Sephadex G-50 column (2.5 × 150 cm) pre-swollen in acetone–water (1 : 1, v/v). The eluate was collected as 20-ml fractions, the acetone removed under reduced pressure at 20 °C, and the procyanidins monitored in each fraction by cellulose t.l.c. and reverse-phase h.p.l.c. on a 3.0-mm × 25-cm Waters μBondapak C-18 column (solvent methanol–water–acetic acid 20 : 79 : 1, v/v, flow rate 1.2 ml/min). Numbering from the void volume, the trimers were present in fractions 5—7, dimers 8—13, and (+)-catechin 13—17. The three major trimers (5), (6), and (7), which had retention volumes of 6.9, 9.0, and 16.6 ml respectively, were in the relative proportions of 2 : 1 : 1.

Synthesis of Procyanidins B2 (11), B 5 (12) *and the Trimer* (13).—Reaction of *Aesculus hippocastanum* or *Photinia glabrescens* tannins with (−)-epicatechin (2) under conditions similar to those used for loblolly pine phloem, and separation of the crude product by Sephadex LH-20 chromatography, followed by purification by h.p.l.c. on a du Pont nitrile column in methanol–water (3 : 7, v/v) led to the isolation of the following: procyanidin B2 (11), retention volume 5.6 ml, $[\alpha]_{578}$ +25.7° (*c* 0.37 in water) [lit.,[4] +15.2° (*c* 1.2 in ethanol)], procyanidin B5(12), retention volume 14.0 ml, R_F(A) 0.38 R_F(B) 0.33, $[\alpha]_{578}$ +102° (*c* 0.10 in water) [lit.,[4] +119° (*c* 1.35 in methanol], and the trimer, epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (13), retention volume 7.8 ml as a white *solid* (Found: C, 60.3; H, 4.8. $C_{45}H_{38}O_{18}$. 1.5 H_2O requires C, 60.3; H, 4.6%), $[\alpha]_{578}$ +92° (*c* 0.19 in water), R_F(A) 0.35, R_F(B) 0.50. It

* Microanalyses were performed after the procyanidins had been dried in an aluminium capsule, as described for proanthocyanidin polymers.[1]

formed a dodecamethyl ether, *m/e* 1 034 (M^+), 855, and 837.

The peracetate was prepared and purified as for the trimer (5) to give an amorphous *solid*, t.l.c. R_F 0.30 (Found: C, 60.3; H, 4.9. $C_{75}H_{88}O_{33}$ requires C, 60.2; H, 4.5%), $[\alpha]_{578}$ +51.6° (*c* 0.38 in acetone); c.d. maxima $\Delta\nu_{222}$ +8.5 (shoulder), $\Delta\varepsilon_{260}$ +4.9; g.p.c. M_n 1 500.

Treatment of compound (13) with toluene-α-thiol yielded epicatechin (2), 4β-benzylthioepicatechin (8), procyanidin B2 (11), and compound (9). Reaction with Raney-nickel gave the epicatechin (2) and procyanidin B2 (11) as the only products.

Synthesis of the Dimeric Benzylthio-compounds (9) *and* 10).—Reaction of the purified tannin from *Pinus palustris* phloem (6 g) with toluene-α-thiol (6 ml) and acetic acid (2 ml) in ethanol (60 ml) in sealed tubes under nitrogen on a water-bath for 24 h yielded a red-brown oil, after evaporation under nitrogen. The oil was poured into water (250 ml) and extracted with diethyl ether (5 × 100 ml). The dried solution was evaporated under nitrogen and the resulting oil chromatographed on Sephadex LH-20 (2.5 × 50-cm column) eluted with chloroform–ethanol (4 : 1, v/v) and collected as 20-ml fractions. Fractions 40—80 contained 4β-benzylthioepicatechin (1.2 g) together with a small amount of catechin. Re-chromatography on Sephadex LH-20 gave 4β-benzylthioepicatechin {(8), identified by t.l.c., ^{13}C n.m.r., ^1H n.m.r., $[\alpha]_{578}$ −17.7° (*c* 0.51 in methanol) [lit.,[4] $[\alpha]_{578}$ −21.1° (*c* 1.2 in ethanol)]}. Fractions 90—115 contained catechin (2, 100 mg). After elution of catechin the solvent was changed to chloroform–ethanol (5 : 2, v/v). Fractions 151—195 contained the following.

Epicatechin-(4β→8)-4β-benzylthioepicatechin (9), which, after rechromatography on Sephadex, was obtained as a light tan *solid*, 278 mg (Found: C, 63.4; H, 5.1; S, 4.2. $C_{37}H_{32}O_{12}S$ requires C, 63.4; H, 4.6; S, 4.6%), $[\alpha]_{578}$ +41.0° (*c* 0.14 in methanol) {lit.,[12] $[\alpha]_{578}$ +64.1° (*c* 0.53 in ethanol)}, R_F(A) 0.75, R_F(B) 0.60.

The phenol (9) formed an octamethyl ether, $[\alpha]_{578}$ +99.4° (*c* 0.18 in chloroform), *m/e* 812(M^+), 689 (M^+ − SCH_2Ph), and 672.

Acetylation gave the deca-acetate as a white *solid* (Found: C, 60.9; H, 4.7; S, 2.7. $C_{57}H_{52}O_{22}S$ requires C, 61.1; H, 4.7; S, 2.9%), $[\alpha]_{578}$ +40.9° (*c* 0.10 in chloroform) {lit.,[12] value $[\alpha]_{578}$ +34.4 (*c* 0.27 in chloroform)}; c.d. maxima $\Delta\varepsilon_{225}$ +6.8 (shoulder), $\Delta\varepsilon_{266}$ +5.5.

The phenol (9) gave procyanidin B2 (11) on desulphurization with Raney-nickel, and degradation with toluene-α-thiol yielded 4β-benzylthioepicatechin (8) as the only product.

Epicatechin-(4β→6)-4β-benzylthioepicatechin (10) was obtained on further elution with chloroform–ethanol (5 : 2, v/v) in crude form in fractions 205—260; re-chromatography yielded the pure phenol (212 mg) as a light tan amorphous *powder* (Found: C, 63.8; H, 4.7; S, 4.2. $C_{37}H_{32}O_{12}S$ requires C, 63.4; H, 4.6; S, 4.6%) $[\alpha]_{578}$ +65.4° (*c* 0.15 in methanol), R_F(A) 0.75, R_F(B) 0.38.

The phenol (10) formed an octamethyl ether, $[\alpha]_{578}$ +135° (*c* 0.18 in chloroform), *m/e* 812 (M^+), 689 (M^+ − SCH_2Ph), and 672.

Acetylation gave the deca-acetate as a white *solid* (Found: C, 61.2; H, 5.1; S, 3.0. $C_{57}H_{52}O_{22}S$ requires C, 61.1; H, 4.7; S, 2.9%), $[\alpha]_{578}$ +64.1° (*c* 0.16 in chloroform); c.d. maxima $\Delta\varepsilon_{230}$ +17.9, $\Delta\varepsilon_{266}$ +4.4.

The phenol (10) gave procyanidin B5 (12) on desulphurization with Raney nickel and 4β-benzylthioepicatechin (8) as the only product on reaction with toluene-α-thiol.

Synthesis of the Phloroglucinol Adducts (14) *and* (15).—Treatment of *Photinia glabrescens* polymer (10 g) with phloroglucinol (3.3 g) in acidic dioxan–water (1 : 1, v/v; 100 ml) for 60 h, as described elsewhere,[16] yielded 10 g of crude phenols. Chromatography on Sephadex LH-20 (5 × 100 cm column, in ethanol, 20 ml fractions), yielded phloroglucinol [fractions 15—25, R_F(A) 0.79, R_F(B) 0.66], epicatechin-(4β→2),phloroglucinol (17) [fractions 25—50, R_F(A) 0.51, R_F(B) 0.58], and, in fractions 40—100, the following.

Epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol (14) was obtained in a crude yield of 1.8 g. Purification by h.p.l.c. on du Pont Zorbax nitrile column (12 mm × 20 cm), solvent methanol–water (20 : 80, v/v), flow rate 4 ml/min, retention volume 50 ml, yielded, after evaporation and freeze-drying the phenol as a white *solid* (Found: C, 59.6; H, 5.0. $C_{36}H_{30}O_{15}\cdot H_2O$ requires C, 60.0; H, 4.5%), $[\alpha]_{578}$ +210° (*c* 0.20 in H_2O) [lit.,[3] +129.5° (*c* 0.4 in methanol)], R_F(A) 0.36 R_F(B), 0.56. Formed an undecamethyl ether, *m/e* 856 (M^+), 645, and 627.

The peracetate was prepared as for trimer (5) to give an amorphous *solid* (Found: C, 59.9; H, 4.8. $C_{62}H_{56}O_{28}$ requires C, 59.6; H, 4.5%), $[\alpha]_{578}$ +99.7° (*c* 0.33 in acetone); c.d. maxima $\Delta\varepsilon_{225}$ +9.2 (shoulder), $\Delta\varepsilon_{266}$ +1.6.

Treatment of compound (14) with toluene-α-thiol yielded phloroglucinol, 4β-benzylthioepicatechin (8), epicatechin-(4β→2)-phloroglucinol (17) and compound (9). Reaction with Raney-nickel gave, additionally, epicatechin (2) and procyanidin B2 (11).

Epicatechin-(4β→6)-epicatechin-(4β→2)-phloroglucinol (15).—H.p.l.c. (du Pont Zorbax nitrile packing; 12 mm × 20 cm column; solvent methanol–water–acetic acid, 20 : 79 : 1, v/v; flow rate 3 ml/min) of fractions 70—180 (containing 1 g of crude phenols from the *Photinia* reaction) revealed a complex mixture, with two major components, retention volume 31.2 ml, R_F(A) 0.14 R_F(B) 0.40, and retention volume 44.1 ml, R_F(A) 0.38, R_F(B) 0.40. Preparative h.p.l.c. under the above conditions yielded the latter compound (11 mg), which was shown to be the phenol (15) isolated as an off-white *solid* (Found: C, 60.6; H, 4.4. $C_{36}H_{30}O_{15}\cdot 0.5H_2O$ requires C, 60.8; H, 4.4%), $[\alpha]_{578}$ +104° (*c* 0.1 in water). It formed an undecamethyl ether, *m/e* 856 (M^+), 645, and 627.

A further portion of the fractions from the *Photinia* reaction containing compound (15) was acetylated and the crude product (1.1 g) was fractionated by t.l.c. (silica, benzene–acetone, 8.2 v/v). The position of the peracetate of (15) (t.l.c., silica, benzene–acetone, 8 : 2, v/v, R_F 0.5), was determined by acetylation of a small amount of purified material from h.p.l.c. Repeated fractionation yielded the peracetate of compound (15) as a white amorphous *solid* (Found: C, 59.4; H, 4.5. $C_{62}H_{56}O_{28}$ requires C, 59.6; H, 4.5%), $[\alpha]_{578}$ +127° (*c* 0.74 in acetone); c.d. maxima $\Delta\varepsilon_{230}$ +14.2, $\Delta\varepsilon_{266}$ +2.9.

Treatment of the phenol (15) with toluene-α-thiol as for compound (5) yielded phloroglucinol, 4β-benzylthioepicatechin (8), epicatechin-(4β→2)-phloroglucinol (17), and compound (10). Reaction with Raney-nickel gave, additionally, epicatechin (2) and procyanidin B5 (12).

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