Polymeric Proanthocyanidins: Interflavanoid Linkage Isomerism in (Epicatechin-4)-(epicatechin-4)-catechin Procyanidins

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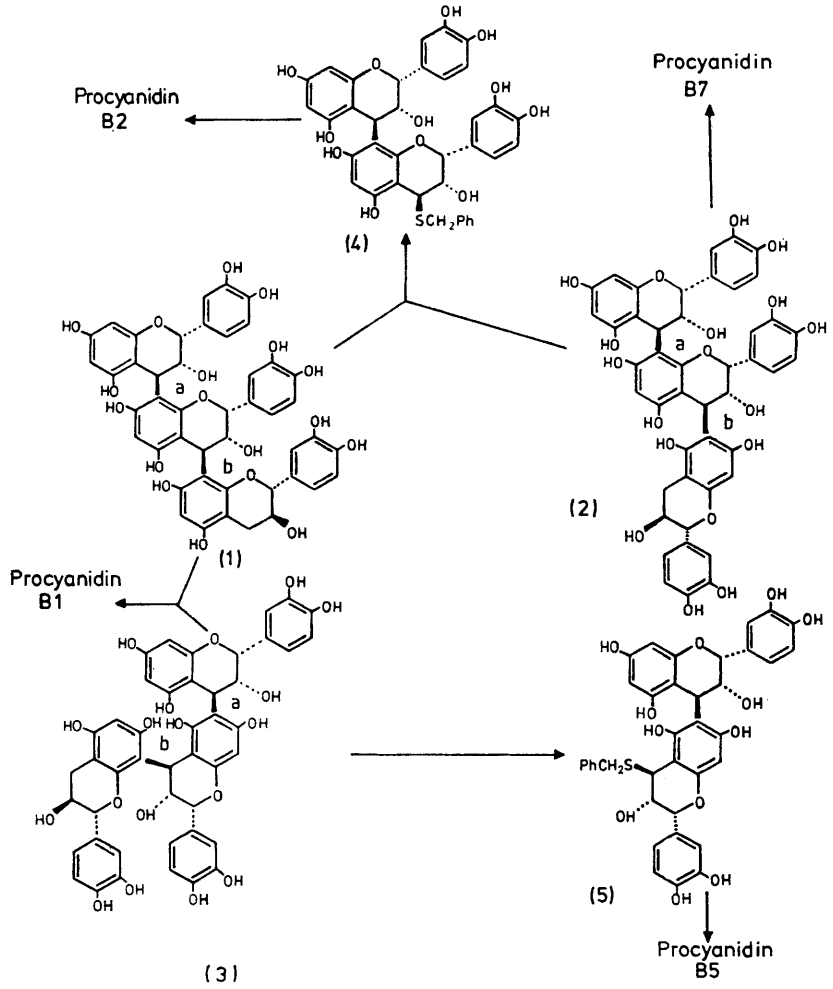
Summary Heterogeneity of the interflavanoid linkages in trimeric procyanidins was demonstrated by the isolation of epicatechin-4,8-epicatechin-4,8-catechin (**1**), epicatechin-4,8-epicatechin-4,6-catechin (**2**), and epicatechin-

4,6-epicatechin-4,8-catechin (**3**) from the phloem of *Pinus taeda* L. where their relative concentrations were 2:1:1 respectively.

PROCYANIDIN trimers have been isolated from a variety of plants,[1-3] but their structures remain unresolved. We have now isolated three configurational isomers of (epicatechin-4)-(epicatechin-4)-catechin from *Pinus taeda* L. (loblolly pine) phloem which exhibit isomerism of the interflavanoid linkages.

Chromatography of the ethyl acetate-soluble fraction of the phloem tannins on Sephadex LH-20 produced crude trimer fractions and further separations by reverse-phase h.p.l.c. (du Pont Zorbax CN column; methanol–water, 3:7 v/v) led to the isolation of three of the five possible configurational isomers of (epicatechin-4)-(epicatechin-4)-catechin: (**1**), (**2**), and (**3**). Mass spectrometry of the dodecamethyl ether ($M^{\cdot+}$ 1034) and gel permeation chromatography of the peracetate derivatives (M_n 1500) confirmed their triflavanoid constitution.‡

All three triflavanoids yielded catechin and 4-benzylthioepicatechin on acid-catalysed cleavage[1] with phenylmethanethiol, thus establishing catechin as the terminal unit and epicatechin as the chain extenders. The configurations of the interflavanoid linkages (Scheme, a and b) were established by partial cleavage with phenylmethanethiol and isolation of the dimeric products. Compounds (**1**) and (**3**) gave epicatechin-4,8-catechin (procyanidin B1) establishing the lower interflavanoid linkage as C(4)–C(8), while (**2**) yielded epicatechin-4,6-catechin (procyanidin B7) showing that the lower linkage of (**2**) is C(4)–C(6). Both (**1**) and (**2**) gave an identical dimeric sulphide (**4**) which yielded epicatechin-4,8-epicatechin (procyanidin B2) on treatment with Raney-nickel, while the trimer (**3**) yielded a chromatographically different dimeric sulphide (**5**) which was converted into epicatechin-4,6-epicatechin (procyanidin B5) on reaction with Raney-nickel, thus establishing the configuration of the upper linkages (a) of the trimers. Independent experiments showed that (**4**) and (**5**) are not interconvertible by intermolecular rearrangement under the con-



SCHEME. Method for establishing the configuration of the upper (a) and lower (b) interflavanoid linkages in trimeric procyanidins.

‡ Structures of all new compounds were supported by satisfactory microanalytical and spectroscopic data for the phenols or their peracetates.

ditions used, thus establishing the validity of partial acid-catalysed thiolytic cleavage for determining the configuration of interflavanoid linkages in oligomeric and polymeric procyanidins.

The three isomers (**1**), (**2**), and (**3**) are major oligomeric procyanidins of *Pinus taeda* phloem, their combined yield being about 0·8% on a dried weight basis. As expected,[7] (**1**) is the major trimer, but (**2**) and (**3**) are present in unexpectedly high relative concentrations (2 : 1 : 1 respectively). The same three trimers were also obtained in similar proportions from the reaction of tannins from *Pinus taeda*[6] or *Photinia glabrescens*[4] with catechin in acidic solutions. The high degree of heterogeneity of the interflavanoid linkages in both the synthetic and natural trimers indicates the occurrence of C(4)–C(6) linkages in polymeric procyanidins, and therefore the proposed linear helical structure for polymeric procyanidins[7] will need reappraisal.

TABLE. ^{13}C N.m.r. chemical shifts and specific rotations for procyanidin trimers.

Compound	Unit	Chemical shifts[a] C(2)	C(3)	C(4)	C(6)	C(8)	$[\alpha]_{578}^{20}$/°
(**1**)	Upper	76·7	72·9	36·9	96·6	96·1	
	Middle	76·7	71·7	36·9	97·2	106·9	+74 (*c* 0·141, H_2O)
	Lower	81·4	67·5	28·2	97·2	108·0	
(**2**)	Upper	76·6	73·1	36·9	96·4	96·1	
	Middle	76·6	71·2	37·3	97·5	107·4	+207 (*c* 0·112, H_2O)
	Lower	82·0	68·0	28·9	108·5	96·5	
(**3**)	Upper	76·4	72·5	36·9	96·8	96·1	
	Middle	76·8	72·0	37·1	107·1	96·4	+106 (*c* 0·186, H_2O)
	Lower	81·5	67·9	28·3	96·8	107·1	

[a] In p.p.m. recorded on a Varian FT-80A instrument at 30 °C in [$^2H_6$]acetone–H_2O at 20 MHz. The chemical shifts (p.p.m.) of the unsubstituted A-ring carbon atoms of the lower flavan unit of procyanidin B1 and B2 are 97·0 and 97·3 respectively and of procyanidin B5 and B7 C8 are 96·9 and 96·7 respectively.

The structures of (**1**), (**2**), and (**3**) were also supported by ^{13}C n.m.r. data (Table). The ratios of the areas of the terminal catechin C(3) signal to that of the corresponding signals of the epicatechin chain extenders were 1 : 2 in all three compounds thereby confirming their (epicatechin-4)-(epicatechin-4)-catechin constitution.[4] A branched structure, such as those of fisetinidol-fisetinidol-catechin isomers,[5] would have a spectrum with all four C(6)–C(8) resonances displaying *meta–meta* coupling, whereas only one of the pairs of resonances displays such coupling in the observed spectra. Comparison of the spectra of the pairs of dimeric procyanidins B1/B7 and B2/B5 shows that C(8) is more shielded than C(6), resulting in a chemical shift difference of about 0·5 p.p.m. The observed chemical shifts for the unsubstituted A-ring carbon atoms of the middle and lower units have the values similar to that predicted from the dimers (see Table).

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