Direct Proof of a Homogeneous Polyflavan-3-ol Structure for Polymeric Proanthocyanidins

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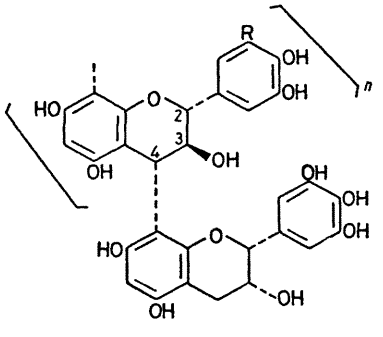
Summary A combination of ^{13}C n.m.r., chiroptical, and chemical degradation evidence proves that polymeric proanthocyanidins consist exclusively of repeating flavan-3-ol units.

RECENT work has elucidated the chemistry of dimeric procyanidins,[1,2] but little is known of the corresponding polymers which, quantitatively, are of greater significance in most plants.[2] Current techniques enable the isolation and purification of the polymers in an undegraded state[3] and the structural homogeneity of proanthocyanidins so isolated is now demonstrated.

Ribes sanguineum leaves yield a polymer which earlier degradation evidence showed to consist largely of monomer units derived from the (+)-gallocatechin carbocation,[4] with some (+)-catechin units and a (−)-epigallocatechin terminal group, (**1**). The polymer was quantitatively cleaved[5] with phenylmethanethiol[6] to yield the 4-sulphides of catechin and gallocatechin, plus (−)-epigallocatechin; these products were analysed by g.l.c. as trimethylsilyl ethers to yield a monomer:terminal group ratio of 21:1, implying a mean M.W. of about 6600. Ultracentrifuge measurements[3] indicated a wide M.W. distribution with a mean M.W. of about 7000.



(**1**) R = H or OH

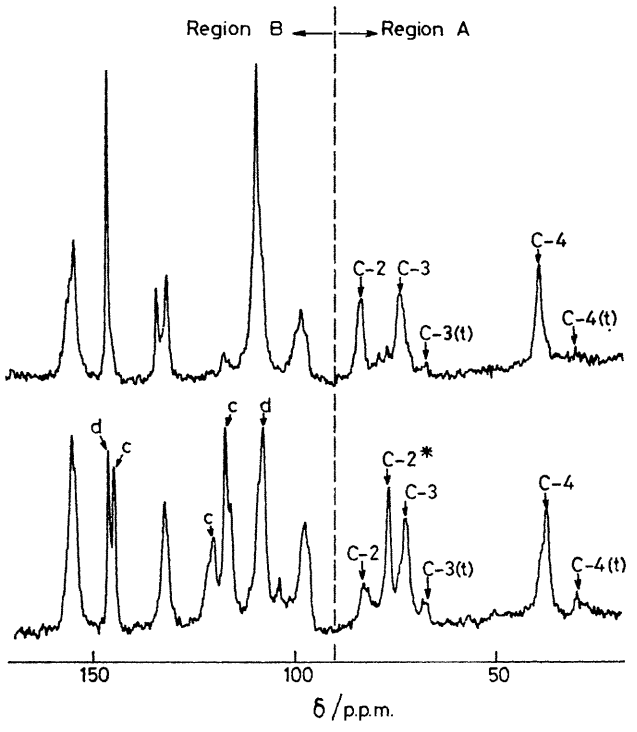


FIGURE. ^{13}C (20 MHz) Fourier transform n.m.r. spectra of proanthocyanidin polymers in H_2O–D_2O at 50 °C recorded relative to external Me_4Si and corrected for magnetic susceptibility. Upper trace *Ribes sanguineum* polymer, lower trace *Pinus radiata* polymer. Assignments are based on the spectra of the model[2] compounds (**2**) and (**3**) measured under the same conditions. C_2*: signal from 2,3-*cis*-3,4-*trans* monomer; (t): terminal unit resonances; c: signals from 3′,4′-dihydroxyphenyl ring; d: signals from 3′,4′,5′-trihydroxyphenyl ring.

This general structure for the *Ribes* polymer was vindicated by its ^{13}C n.m.r. spectrum (Figure) as all observed resonances were consistent with a 4—8 (or 6)[2] linked polyflavan-3-ol structure (**1**). Broadening of the resonances is due to the high M.W. and also the diversity of unresolved chemical shifts. The spectrum may be considered in two regions: region A 30—90 p.p.m. and region B 90—160 p.p.m.

Region A signals arise from heterocyclic ring carbons and observed shifts are dictated by the ring stereochemistry.[2] Furthermore, when observed, C-3 of the flavan-3-ol terminal group occurs at 65—66 p.p.m., well separated from the C-3 signal of the monomer units (72—73 p.p.m., see Figure). The mean M.W. of the *Ribes* polymer was calculated from the ratio of areas of the C-3 signals giving a monomer:terminal group ratio of 18:1 (*i.e.* mean M.W. *ca.* 5900), in reasonable agreement with the earlier values.† Estimation of M.W. by ^{13}C n.m.r. spectroscopy is controlled by the observable limit of the terminal unit C-3 signal (*i.e.* M.W. <8000).

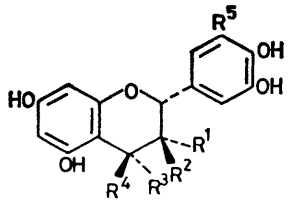
As shown by the *Pinus* polymer spectrum (Figure) the C-2 signal, region A, for a monomer with 2,3-*trans*-3,4-*trans*-stereochemistry is at 83 p.p.m., well separated from C-2 (77 p.p.m.) for the 2,3-*cis*-3,4-*trans*-monomer. The ratio of areas of the C-2 signals yields the relative proportion of monomers with each stereochemistry in mixed polymers (Table). These ratios are compared with values calculated from $[\phi]_{578}$ measurements for each polymer, these values being independent of chiral effects evident in the o.r.d. curve at lower wavelengths arising from the polymer conformation.[5] Polymers with sterically homogeneous monomers have the same $[\phi]_{578}$ values as the model compounds (**2**) or (**3**), Table. Therefore the mole fraction, X, of 2,3-*cis*-3,4-*trans* monomers may be calculated from the relation:[7] $[\phi]_{polymer} = 450X - 690(1 - X)$ to give the values in the Table.

TABLE. Composition of proanthocyanidin polymers.

Proanthocyanidin	$[\phi]_{578}$	Mole fraction of 2,3-*cis*-isomer: From rotation	From ^{13}C n.m.r.	Ratio, prodelphinidin:procyanidin: Acid degradation	Thiol degradation	^{13}C n.m.r.	Mean M.W.: ^{13}C n.m.r.
(**2**)	+450	1·0	1·0	—	—	—	—
(**3**)	−690	0	0	—	—	—	—
Sainfoin[a,b]	+345	0·91	0·87	81:19	83:17	81:19	e
Lotus[a,b]	+255	0·83	0·75	77:23	80:20	82:18	e
Ribes[a,b]	−692	0	0	87:13	93:7	91:9	5900
Siebel[a,c]	+436	0·99	1·0	20:80	22:78	10:90	e
Beaujolais[a,c]	+390	0·95	0·91	20:80	18:82	13:87	4900
Cyathea[a,b]	+455	1·00	1·0	41:59	38:62	40:60	e
Dicksonia[a,b]	+425	0·98	1·0	18:82	5:95	13:87	2900
Pinus radiata[a,d]	+217	0·80	0·76	50:50	49:51	48:52	1800
Rose hips[a,c]	+111	0·70	0·72	10:90	13:87	13:87	6400

[a] Polymer. [b] Isolated from leaves. [c] Isolated from unripe fruit. [d] Isolated from phloem. [e] C_3(t) Signal not observed.

† The ^{13}C n.m.r. spectra were run on a Varian FT-80A spectrometer at 20 MHz and 50 °C, using a 16 K data table to minimise digitisation. Differential nuclear Overhauser (n.O.e.) or T_1 effects would not affect the reliability of the ^{13}C n.m.r. data, since peaks with similar relaxation behaviour were selected for comparisons. T_1 (by inversion-recovery) and n.O.e. (by gated-decoupling) experiments were run on a solution containing (by weight) 13% *Pinus radiata* tannin, 68% H_2O, and 19% D_2O. For the peaks at 146 and 145 p.p.m., T_1 = 1·7 s and η = 0·8, while for the aliphatic carbons (including those in terminal units) T_1 = 0·06 s and η = 0·6. Any variations within the two groups were inside experimental uncertainties. Low n.O.e. values are expected for molecules of this size, even if the relaxation mechanism is entirely dipolar.[8] Weak signals and long pulse delays ($>4T_1$) made the general use of gated decoupling impractical, so the data shown in the Table were obtained with 45° pulses at 0·2 s intervals, during continuous decoupling. Gated decoupling experiments on the *Pinus radiata* tannin showed no significant differences in the values for M.W., fraction of *cis*-isomer, or prodelphinidin:procyanidin ratio.



(2) R^1 = OH, R^2 = R^3 = H; R^4 = 2,4,6-trihydroxyphenyl; R^5 = H or OH

(3) R^1 = R^4 = H; R^2 = OH; R^3 = 2,4,6-trihydroxyphenyl; R^5 = H or OH

Region B provides the ratio of prodelphinidin to procyanidin units from the relative peak areas of the 146 p.p.m. (C-3′ and C-5′ of the 3′,4′,5′-trihydroxyphenyl ring) and 145 p.p.m. signals (C-3′ and C-4′ of the 3′,4′-dihydroxyphenyl ring, see Figure). The ratios calculated by ^{13}C n.m.r. spectroscopy are compared (Table) with those determined by two independent degradation methods: (i) the delphinidin to cyanidin ratio by the method of Bate-Smith;[8] and (ii) the ratio of 4-sulphides as determined by g.l.c.[5]

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