Phytochemistry, Vol 23, No. 6, pp. 1309–1311, 1984.
Printed in Great Britain.

0031–9422/84 $3 00+0 00

MOLECULAR WEIGHT AVERAGES AND ^{13}C NMR INTENSITIES PROVIDE EVIDENCE FOR BRANCHING IN PROANTHOCYANIDIN POLYMERS

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(*Received* 14 *October* 1983)

Key Word Index—*Chaenomeles chinensis*; Rosaceae; condensed tannins; proanthocyanidin polymers; branching.

Abstract—Molecular weight distributions for several proanthocyanidin polymers (condensed tannins) have previously been shown to be characterized by $DP_w/DP_n > 2$, where DP denotes the degree of polymerization. Molecular weight ratios of the size observed experimentally are expected if a few percent of the units form trifunctional branch points. An independent assessment of the extent of branching can be obtained from analysis of intensities of resonances in the ^{13}C NMR spectra. Similar branch contents are deduced from DP_w/DP_n and from the spectral analysis. In the case of the Chinese quince (*Chaenomeles chinensis*) polymer, about 3% of the epicatechin units form trifunctional branch points.

INTRODUCTION

Recent measurements [1] of number- and weight-average molecular weights for proanthocyanidin polymers (condensed tannins) from several plants yield $DP_w/DP_n > 2$, where DP_n and DP_w denote the number- and weight-average degree of polymerization, respectively. Such large ratios demand the molecular weight distribution be broader than that defined by the 'most probable' distribution for a linear polymer [2, 3]. One possible source of the broadening would be the presence of branch points. Here we provide an estimation of the effect of a small number of trifunctional branch points on the ratio of the weight- to number-average molecular weight in proanthocyanidin polymers. A model described by Stockmayer [4] and Flory [5] is employed for the analysis based on DP_w/DP_n. The model considers polymerization of N_0 trifunctional units, $\mathrm{A}\!-\!\!<^{\mathrm{A}}_{\mathrm{A}}$, and L_0 bifunctional units, A–A. The trifunctional units are used here merely as a device for introduction of a few branch points into predominantly linear polymers. The theory shows molecular weight ratios observed [1] with proanthocyanidin polymers could be obtained if 1–4% of the units form trifunctional branch points. An independent assessment of the degree of branching, based on an analysis of the intensities of ^{13}C NMR resonances [6], is consistent with a branch content in this range.

THEORETICAL DISTRIBUTION FUNCTIONS LEADING TO DP_w AND DP_n

In the customary treatment of branched macromolecules, a trifunctional unit becomes a trifunctional branch point only if all three of its A's condense with As on other units. If it contains a single unreacted A, a trifunctional unit simply propagates a linear chain, and it terminates the chain if two of its As remain unreacted. Following the notation adopted earlier [5], the fraction of the As on trifunctional units is denoted by ρ.

$$\rho = 3N_0/(3N_0 + 2L_0) \tag{1}$$

The probability that a given A has reacted with another A is denoted by p. This probability is assumed to hold for all A. Intramolecular condensation, giving rise to cyclic products, is ignored here. The probability that all three As on a given trifunctional unit have reacted is given by p^3, and the fraction of units which are trifunctional is $N_0/(N_0 + L_0)$. Consequently the fraction of units forming trifunctional branch points in the polymer, denoted B, is

$$\begin{aligned} B &= p^3 N_0/(N_0 + L_0) \\ &= 2\rho p^3/(3-\rho). \end{aligned} \tag{2}$$

A given polymer molecule contains n trifunctional units and l bifunctional units. The number- and weight-average degrees of polymerization are [4, 5]:

$$DP_n = \frac{\Sigma\Sigma\,(n+l)N_{n,l}}{\Sigma\Sigma\,N_{n,l}} \tag{3}$$

$$DP_w = \frac{\Sigma\Sigma\,(n+l)W_{n,l}}{\Sigma\Sigma\,W_{n,l}} \tag{4}$$

The mole- and weight-fraction of n, l-mer are denoted, respectively, by $N_{n,l}$ and $W_{n,l}$. They are calculated as

$$N_{n,l} = (2/p)\,(1-p)^2\,[3\,(1-p)-\rho]^{-1}\,\zeta^n\,\eta^l\,\omega'_{n,l} \tag{5}$$

$$W_{n,l} = [3\,(1-p)-\rho]\,(3-\rho)^{-1}\,(n+l)N_{n,l} \tag{6}$$

$$\zeta = \rho p\,(1-p) \tag{7}$$

$$\eta = p\,(1-\rho) \tag{8}$$

$$\omega'_{n,l} = 3\,(l+2n)!\,[l!\,n!\,(n+2)!]^{-1} \tag{9}$$

Summations in equations 3 and 4 extend from $l = 0$ to $l = \infty$, with n independently running from 0 to ∞, the combination $n = l = 0$ being excepted. Evaluation of the double sums is extremely tedious for very large polymers. Present interest, however, is in comparatively small polymers containing only a few branch points. These conditions arise when p and ρ are both small. Summations indicated in equations 3 and 4 can then be adequately approximated by increasing n and l until $\Sigma N_{n,l}$ and $\Sigma W_{n,l}$ approach one. Numerical evaluation of the double sums under these circumstances is well within the capabilities of typical microcomputers.

RESULTS AND DISCUSSION

Illustrative numerical results for various p are depicted in Fig. 1. Calculations performed with $\rho = 0$ yield the familiar result [2, 3] for linear polymers having the most probable distribution. Here $DP_n = 1/(1-p)$ and $DP_w/DP_n = 1+p$. Consequently the line denoted '0.00' in Fig. 1 will approach $DP_w/DP_n = 2$ in the limit where DP_n becomes infinite. This line never rises above 2.

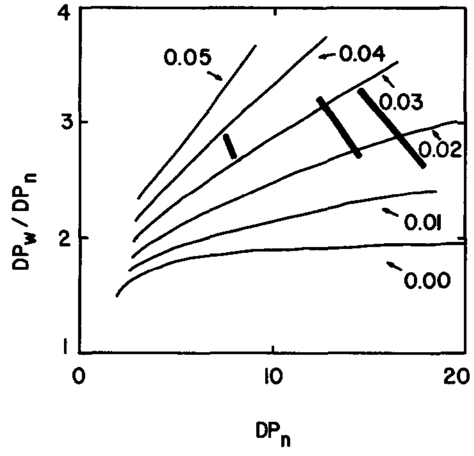

Fig. 1. Ratio of DP_w to DP_n, as a function of DP_n. The fraction of units forming trifunctional branch points is noted for each curve. Short heavy lines denote experimental results for three proanthocyanidin polymers (see text). Reading from left to right, the short heavy lines are for proanthocyanidin polymers obtained from *Watsonia*, Chinese quince and quince. Molecular weights are reported in Table 1.

Addition of a few trifunctional branch points, achieved by letting ρ rise slightly above zero, markedly broadens the molecular weight distribution. The trifunctional branch points produce a dramatic increase in DP_w/DP_n at any DP_n. Short heavy lines in Fig. 1 denote experimentally observed ranges for DP_n and DP_w/DP_n for proanthocyanidin polymers obtained from Chinese quince, quince, and *Watsonia*. Molecular weight measurements were obtained using ^{13}C NMR or vapor pressure osmometry (for DP_n) and low-angle laser light scattering (for DP_w). The data is summarized in Table 1. Figure 1 shows that a possible explanation for the large DP_w/DP_n might be that the polymers are not linear, but instead have 1–4 % of their units present as trifunctional branch points.

The ^{13}C NMR chemical shifts of proanthocyanidin polymers containing 2, 3-*cis* units are well understood [7].

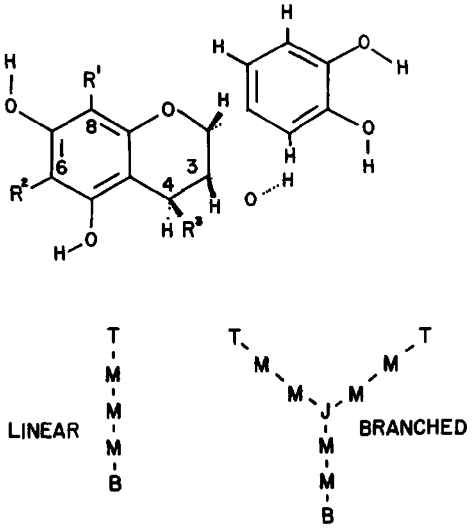

Fig. 2. Schematic proanthocyanidin polymers. Symbols used are: J = unit substituted at C-4, C-6, and C-8 ($R^1 = R^2 = R^3$ = epicatechin); T = unit substituted at C-4 ($R^1 = R^2$ = H, R^3 = epicatechin); B = C-6 or C-8 substituted epicatechin unit (R^1 or R^2 = epicatechin, R^2 or R^1 = H, R^3 = H; and M = unit substituted at C-4 and either C-6 or C-8 (R^1 or R^2 = epicatechin, R^2 or R^1 = H, R^3 = epicatechin).

Table 1. Degrees of polymerization of proanthocyanidin polymers*

	DP_n		DP_w		GPC data§		
Source	NMR†	VPO‡	LALLS‡	DP_w/DP_n	DP_n	DP_w	DP_w/DP_n
Chinese quince	12.2	14.3	38.5	2.7–3.2	9.3	24.2	2.6
Quince	14.2	17.8	46.2	2.6–3.3	9.9	42.9	4.6‖
Watsonia	7.5	8.0	21.4	2.7–2.9	5.0	12.0	2.4

*From ref. [1].

†Estimated as described in ref. [8].

‡Obtained for the phenolic polymer in methanol using vapor phase osmometry or low-angle laser light scattering.

§Obtained for polymer peracetate derivatives in tetrahydrofuran on μ Styragel columns using polystyrene standards. This procedure tends to underestimate DP_n and DP_w because proanthocyanidin peracetate chains have a lower radius of gyration/mass ratio than polystyrene.

‖Unusual distribution according to GPC. The polymer shows a high molecular weight shoulder.

From such spectra an independent estimate of branch points may be obtained. In particular, the polymer from Chinese quince (*Chaenomeles chinensis*) fruit, which consists entirely of epicatechin units, may be considered to consist of four types of units, denoted by T, M, J and B in Fig. 2. In particular, J is the trifunctional branch point (defined above), and such branching increases the ratio of T units to B units, as shown in Fig. 2. This ratio may be used to estimate the number of J units, as follows: the T units, both 4 → 6 and 4 → 8 linked, give rise to a unique C-8 resonance at δ96.1 [7]. Using the DP_n values for Chinese quince polymer in Table 1, it may be calculated that the signal at δ96.1 would account for 7–8 % of the C-6/C-8 resonance region (centered on δ97.5 [6]) if no J units are present, or 15–17 % if one J unit was present [6]. The observed [6] intensity of δ96.1 is approximately 12 % of the C-6/C-8 resonance. Precision of this estimate is limited because the T unit signal is not fully resolved from the bulk C-6/C-8 signal at the field (62.5 MHz) used in the experiment. Nevertheless, the implied fraction of units constituting trifunctional branch points ($p = 0.03$–0.04) is in reasonable agreement with the values ($p = 0.02$–0.03) calculated from the molecular weight ratios.

While only a few per cent of the units form trifunctional branch points, they produce a significant broadening of the molecular weight distribution. Branching to this extent should also have an appreciable influence on various configuration-dependent physical properties, particularly for samples of high molecular weight.

Acknowledgement—Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research

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