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PROANTHOCYANIDIN POLYMERS OF FODDER LEGUMES

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Abstract—The structure of the condensed tannins of the most common fodder legumes is described. The number- ($\bar{M}_n$) and weight-average ($\bar{M}_w$) MW of the polymers have been determined and most legume tannins have an approximately normal distribution of MWs with $\bar{M}_n$ values of 2000–4000.

INTRODUCTION

The proanthocyanidin (PA) polymers (condensed tannins) of herbaceous legumes, especially those with potential as fodder crops, has created much interest [1–4]. Their presence, or absence, has been implicated in the digestability of protein [5–7] and the occurrence of bloat in ruminants [1, 8].

The nature of the PA polymers of herbaceous legumes has been studied [2–4], and MW distribution data derived from ultracentrifuge measurements have been obtained [3]. Utilizing recently developed ^{13}C NMR [9, 10], chiroptical [9], and gel permeation chromatography (GPC), (Porter, L. J. and Williams, V. M., unpublished results) methods, we have re-investigated the PA polymers of herbaceous legumes, with particular emphasis on those used for fodder crops.

RESULTS

The PA polymers were isolated as freeze-dried white, or off-white solids as described elsewhere [3, 9]. All the polymers were studied by ^{13}C NMR except those from *Lotus corniculatus* (root), *Trifolium affine*, *Robinia fertilis*, and *Vicia hirsuta*. However, estimation of the prodelphinidin (PD) to procyanidin (PC) ratios by acid hydrolysis [9], stereochemistry of the units by optical rotation [9], and MW by GPC (Porter, L. J. and Williams, V. M. unpublished results) enabled the properties of these polymers to be defined also. The structural data for each polymer are given in Table 1.

DISCUSSION

The number-average MW ($\bar{M}_n$) and weight-average MW ($\bar{M}_w$) of the PA polymers were estimated from GPC of the acetate derivatives on 500 or 1000 Å μStyragel columns (Porter, L. J. and Williams, V. M., unpublished results). The $\bar{M}_n$ and $\bar{M}_w$ values in Table 1, derived from GPC, are for the phenolic form of the polymer, so that the results may be directly compared with estimates obtained by other methods (the phenols have *ca* 60% of the MW of the peracetates).

Three estimates of $\bar{M}_n$ were made for several of the polymers, from ^{13}C NMR, GPC, and vapour pressure osmometry (VPO, Table 1). It may be seen that all three methods give similar values for $\bar{M}_n$, and confirm that PA polymers have $\bar{M}_n$ values in the range 2000–4000.

The polymers are all polydisperse ($\bar{M}_w/\bar{M}_n > 1$) and as most values of this ratio are *ca* 2 (see Table 1), the MWs possess an approximately normal distribution [11]. A previous survey [3] of some of these polymers estimated MW by ultracentrifugation of the phenols in aqueous solution, and these results are listed in Table 1. Ultracentrifuge measurements yield a *z*-average MW ($\bar{M}_z$), and for a normal distribution, such as we observe here, $\bar{M}_n : \bar{M}_w : \bar{M}_z = 1:2:3$ [12]. Therefore, $\bar{M}_z$ is expected to be about three times $\bar{M}_n$. As the polymers are all polydisperse ultracentrifuge measurements give a range of MWs, from which it is difficult to estimate the distribution of the population of different MW species. However, three of the polymers have a narrow MW range, according to the ultracentrifuge [3], and therefore a direct comparison is possible for the *Lotus pedunculatus* (leaf), *Trifolium repens* and *T. arvense* polymers. These have a mean value of $\bar{M}_z$ of *ca* 7000, 8800, and 8000 respectively (see Table 1). Estimates of $\bar{M}_z$ ($3 \times \bar{M}_n$) obtained from the VPO and NMR data, give values of 7500, 8700, and 6000 respectively, in reasonable agreement with the ultracentrifuge measurements.

The very high MW and extremely polydisperse nature of the tannin from *Onobrychis viciifolia* (Sainfoin) observed earlier [3] was not observed in this study. The ultracentrifuge measurements imply a $\bar{M}_n$ value of 8000–9000. An earlier study reported that $\bar{M}_n$ of sainfoin tannin was too high to estimate by NMR [10]. However, measurements on two subsequent preparations, aided by improved NMR spectra, gave estimates of $\bar{M}_n$ of 2900 (Table 1) for a sample collected in January, and 3600 for a sample

Table 1. Structure and MW of legume condensed tannins

Plant species	Tissue	*cis*:*trans*	PD:PC	$\bar{M}_n$[1]	$\bar{M}_w$[1]	D_p[2]	$\bar{M}_z$[3]	Terminal[4] unit
Coronilla varia L.	Leaf	95:5	71:29	3300[5]	5300	1.6	10100–13200	—
Lotus corniculatus L.	Leaf	92:8	27:73[6]	2000[7]	5300	2.7	—	c
L. corniculatus L.	Root	87:13	22:78[8]	2000	3200	1.6	—	—
L pedunculatus Cav.	Leaf	75:25	70:30	2900[9]	5900	2.0	6800–7100	—
L. pedunculatus Cav.	Root	72:28	75:25	4000	8700	2.2	—	e:c:eg = 10:70:20
L. tenuifolius Presl.	Root	85:15	20:80	2200[10]	4200	1.9	—	e:c = 1:1
Onobrychis vicifolia Scop.	Leaf	90:10	77:23	2100[11]	3300	1.6	$1.7–2.8 \times 10^4$	e:c:eg = 20:40:40
Robinia fertilis Ashe	Leaf	55:45	40:60	2050	3700	1.8	—	—
Trifolium affine L.	Leaf	80:20	50:50	2400	4600	1.9	5800–8700	—
T. arvense L.	Leaf	91:9	53:47	2700[12]	4100	1.5	7900–8200	c
T. repens L.	Flower	65:35	100:0	2050[13]	3050	1.5	8500–9100	g
Vicia hirsuta S. F. Gray	Leaf	90:10	0:100	3600	7700	2.2	—	—
V. sativa L.	Leaf	100:0	0:100	3500[14]	4850	1.4	—	c

[1]By GPC of the acetate derivatives.
[2]Dispersitivity = $\bar{M}_w/\bar{M}_n$.
[3]From ref. [3].
[4]c = Catechin; e = epicatechin; eg = epigallocatechin; g = gallocatechin.
[5]$\bar{M}_n$ = 3600 (NMR).
[6]Variable results depending on the plant source; ratio as high as 50:50.
[7]$\bar{M}_n$ = 2800(NMR); 3000(VPO).
[8]Variable results depending on the plant source; ratio as high as 10:90.
[9]$\bar{M}_n$ = 2500(NMR); 2500(VPO).
[10]$\bar{M}_n$ = 3000 (NMR).
[11]$\bar{M}_n$ = 2900(NMR); 2600 (VPO).
[12]$\bar{M}_n$ = 2000 (NMR).
[13]$\bar{M}_n$ = 2900 (NMR); 2200 (VPO).
[14]$\bar{M}_n$ = 3200 (NMR).

collected in March. Moreover, $\bar{M}_w/\bar{M}_n$) is about the same as for the other legume tannins (Table 1), and hence the dispersivity of sainfoin tannin is similar.

The earlier observation that sainfoin tannin was less effective at precipitating blood protein [3], and in binding receptor molecules to the surface of *Rhizobia* (Jones, WT., unpublished data) than other legume tannins with a similar PD:PC ratio and stereochemistry, is not readily explicable. The most plausible explanation at this stage is that the MW of sainfoin tannin may vary considerably on a seasonal and cultivar basis, and the particular sample used in the binding studies did, indeed, have a much higher MW. An alternative explanation could be that sainfoin tannin has an unusual tertiary structure.

There is little pattern to the stereochemistry or oxidation pattern of the constituent flavan-3-ol units of the PA polymers, which range from pure PC to pure PD polymers. Units with 2,3-*cis* stereochemistry predominate, as is apparently normal in the majority of plants [10]. The polymer from *Trifolium repens* flowers is novel; it is one of only two pure PD polymers which have been encountered so far.

The finding of PA polymers in *Vicia* does not support the earlier view [13] that they are rare or absent in the Vicieae. While they were reported to be absent from *V. cracca* [4], both *V. hirsuta* and *V. sativa* contain PC polymers, the latter being a particularly rich source of tannin. In addition a PA polymer has been reported to be present in the seed coat of *V. faba* [2].

Bate-Smith [2] reported that *Lathyrus pratensis* is a rich source of PA polymers, and it has been considered that this is the only species in this genus containing tannin [14]. On the basis of an intense vanillin reaction on the crushed leaves [1], we anticipated that *Lathyrus latifolius* would also be a rich source of polymer. However, only a small yield of PD polymer was obtained, being present at only 2% of the concentration observed in *V. sativa* leaves. It was concluded that the intense vanillin test was due to a high concentration of ethyl acetate soluble flavan-3-ols.

PA polymers isolated from the roots and leaves of *L. corniculatus* vary considerably in their PD:PC ratio, and such differences are also manifest between different varieties of this plant. It is widely accepted that the tetraploid variety of *L. corniculatus* is a comparatively recently developed segmented allopolyploid [15] and shows great variability in a large number of morphological characters. The observation that the PD:PC ratio is varietally dependent probably shows that flavonoid biosynthesis is a further variable character. However, the observation that polymers isolated from the leaves and roots of the same plant show a similar structural variation, suggests that the biosynthesis of tannins may be under different control in the two areas of the plant. Two independent processes (a) light-mediated and occurring in the apical meristem and (b) nutritional and occurring in the root system, have been identified in mutants of *L. pedunculatus*. Biosynthesis of PA in leaves (alone) was controlled by light quality, and in the roots by stressing the plants by applying conditions of nitrogen deficiency (Jones, W. T. and Ross, M.D., unpublished data). Although in *L. pedunculatus* the PD:PC ratio of the root and leaf tannin is essentially invariant, the above observations support the occurrence of two independent biosynthetic routes for PA synthesis in *L. corniculatus* hybrids.

EXPERIMENTAL

The proanthocyanidin polymers were extracted and analysed as described elsewhere [3, 9]. GPC was performed on Waters Associates 500 and 1000 Å μStyragel columns, solvent tetrahydrofuran, on a Waters ALCGPC 244 unit, using a UV detector set at 270 nm. The columns were standardized against peracetates of known mono- and oligomeric flavan-3-ols and polystyrene standards (Pressure Chemicals Co.). VPO was performed in $CHCl_3$ on the tannin peracetates by the Microanalytical Service, University of Otago, New Zealand.

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