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MOLECULAR WEIGHT PROFILES OF PROANTHOCYANIDIN POLYMERS

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Abstract—The MW profiles of proanthocyanidin polymers (condensed tannins) from 32 samples representing a wide range of plant tissues of many different species have been obtained by gel permeation chromatography of the peracetate derivatives. The tannins vary widely in MW, with $\bar{M}_n$ values for the peracetates in the range 1600–5500. The MW profiles vary greatly from those with narrow, rather smooth distributions, to those which are discontinuous.

INTRODUCTION

Proanthocyanidin (PA) polymers (condensed tannins) consist of chains of polyhydroxyflavan-3-ol units linked through C-4–C-6 or C-4–C-8 bonds [1]. They are widely distributed in the plant kingdom and are based on a variety of different flavan-3-ol unit structures [2]. As they possess an oligomeric or polymeric constitution, it is of interest to have some knowledge of the range and magnitude of MW possessed by these natural products.

Whereas ^{13}C NMR [1] or vapour pressure osmometry [1, 3] give an estimate of the number average MW ($\bar{M}_n$) of PA polymers, gel permeation chromatography (GPC) gives both $\bar{M}_n$ and the weight average MW ($\bar{M}_w$). In addition GPC gives a picture of any discontinuities in the distribution of MWs.

The phenolic PA polymers are too polar to be separated on the range of GPC columns currently available, and, therefore, must be derivatized. Two studies of bark tannins used the methyl ethers (formed by repeated treatment with diazomethane) for GPC studies of pine bark tannins [4, 5]. However, methylation of polyhydroxyflavanoids with diazomethane is a somewhat unsatisfactory procedure as it leads to a variable degree of methylation of the phenolic hydroxyls and some aromatic *C*- and *O*-methylation of the aliphatic 3-hydroxyl. We, therefore, chose to acetylate the proanthocyanidins, and some results based on the GPC of the peracetates have already been published in our recent study of legume tannins [3].

This paper reports more details of this approach and presents the GPC profiles of the peracetates of PA polymers isolated from a wide range of plant sources.

RESULTS AND DISCUSSION

PA polymers were isolated from plant tissue as described elsewhere [1, 6], acetylated with acetic anhydride–pyridine, and the peracetates subsequently separated by GPC on μStyragel columns. The eluted PA peracetates were detected with a UV monitor set at 270 nm [the polymer peracetates are characterized by a UV spectrum with maxima at 270 and 280 nm, where procyanidin (PC) polymers have a $E_{1\,\mathrm{cm}}^{1\%}$ of 41, and prodelphinidin (PD) polymers a $E_{1\,\mathrm{cm}}^{1\%}$ of 35]. In this study it was assumed that the PC–PD ratio remained constant throughout the MW profile (i.e. the dependence of *A* on concentration was constant throughout the curve). The MW of a constituent PA peracetate unit is *ca* 500.

The GPC column was calibrated with pure samples of peracetates of polyhydroxyphenols, and mono-, di- and trimeric 5,7,3′,4′-tetrahydroxyflavan-3-ols. This series provided standards up to $\bar{M}_n$ 1500, and higher MW calibrations were performed with polystyrene standards. A typical calibration curve is illustrated in Fig. 1.

A number of different MW profiles were obtained when the polymer peracetates were chromatographed on μStyragel. These are schematically represented in Fig. 2. The most common type of distribution was a smooth and relatively symmetrical curve, like curve (a) in Fig. 2. Such curves were generally encountered in our earlier study of legume tannins [3] when our estimates of $\bar{M}_n$ by GPC were corroborated by ^{13}C NMR and vapour pressure osmometry. As was pointed out earlier this type of curve usually obeys an almost perfect normal distribution where $\bar{M}_w \sim 2\bar{M}_n$.

Earlier GPC experiments were performed on 500 Å (10^4 MW exclusion limit, equivalent to a 20 unit PA chain) or 1000 Å (5×10^4 MW exclusion limit, equivalent to a 100 PA unit chain) μStyragel columns. However, a number of polymers contained material which was excluded from either column, as indicated by the separation producing a MW profile similar to Fig. 2, curve (b). Consequently later measurements were all performed on 1000 and 10 000 Å (5×10^5 MW exclusion limit, equivalent to a 1000 unit PA chain) columns connected in series. This combination also had the advantage of improved resolution over a single column. All polymers so far encountered were satisfactorily separated on this column combination.

Separations were initially performed in chloroform. However, it was subsequently noted that when results using this eluting solvent were compared with those obtained in THF, the $\bar{M}_n$ and $\bar{M}_w$ values were lower in chloroform than THF. Deviations were especially serious at higher MW. From this it was concluded that chloro-

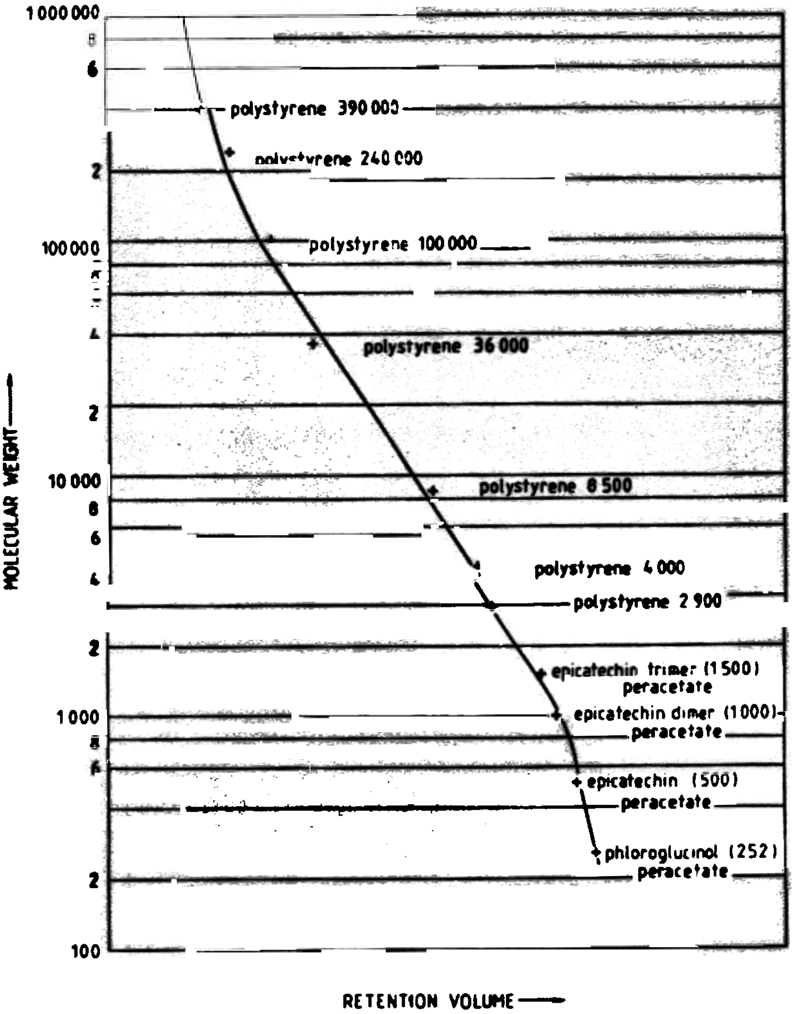

Fig. 1. Typical calibration curve for a 10^4 and 10^3 A μStyragel column combination using THF as solvent.

form was insufficiently polar for true GPC conditions to operate, and consequently THF was used for all analyses.

Values of $\bar{M}_n$ and $\bar{M}_w$, calculated from the GPC curves, are summarized in Table 1. As may be seen the values of $\bar{M}_n$ vary very widely. In addition, the GPC profiles vary considerably in shape, which may be classified into five types. These are: (1) Those with both an approximately symmetrical distribution and roughly obeying a normal MW distribution. These have curves similar to (a) in Fig. 2. (2) Those with a very narrow MW range and correspondingly narrow symmetrical peak, such as curve (c) in Fig. 2. (3) Those with a symmetrical distribution for the majority of PA species, but with some resolution of lower MW species. This type is represented by curve (d) in Fig. 2. (4) Those with a basically normal distribution of species, but with a low, but significant, proportion of higher MW species. These appear as a distinct hump eluted immediately prior to the main curve, i.e. curve (e) in Fig. 2. This is the second most common type of distribution. (5) Those with two distinct peaks comprising higher and lower MW components. These typically look like curve (f) in Fig. 2.

The type of curve produced by each PA polymer is listed in Table 1, according to the above classification, along with its $\bar{M}_n$ and $\bar{M}_w$ values. It is evident, on the basis of these data, that PA polymers and oligomers exist over an extremely wide MW range, from dimers through the species with MWs of several hundred thousand, containing hundreds of PA units. However, tannins containing molecules of extremely high MW are evidently fairly uncommon, and the majority contain molecules of MW mostly in the range 2000–20 000 (4–40 units).

It is evident that plants are capable of synthesizing PA polymers over an extremely wide range of MW. The actual MW range produced is species dependent, and such factors as seasonal variation and tissue maturity appear to have, by comparison, little influence. For instance, the remarkably narrow MW range displayed by barley tannins, which contain virtually only dimers and trimers, remains essentially invariant throughout the growth season [7]. Other polymers, such as those from *Aesculus carnea*, *Cydonia oblonga*, and *Vicia sativa*, have been

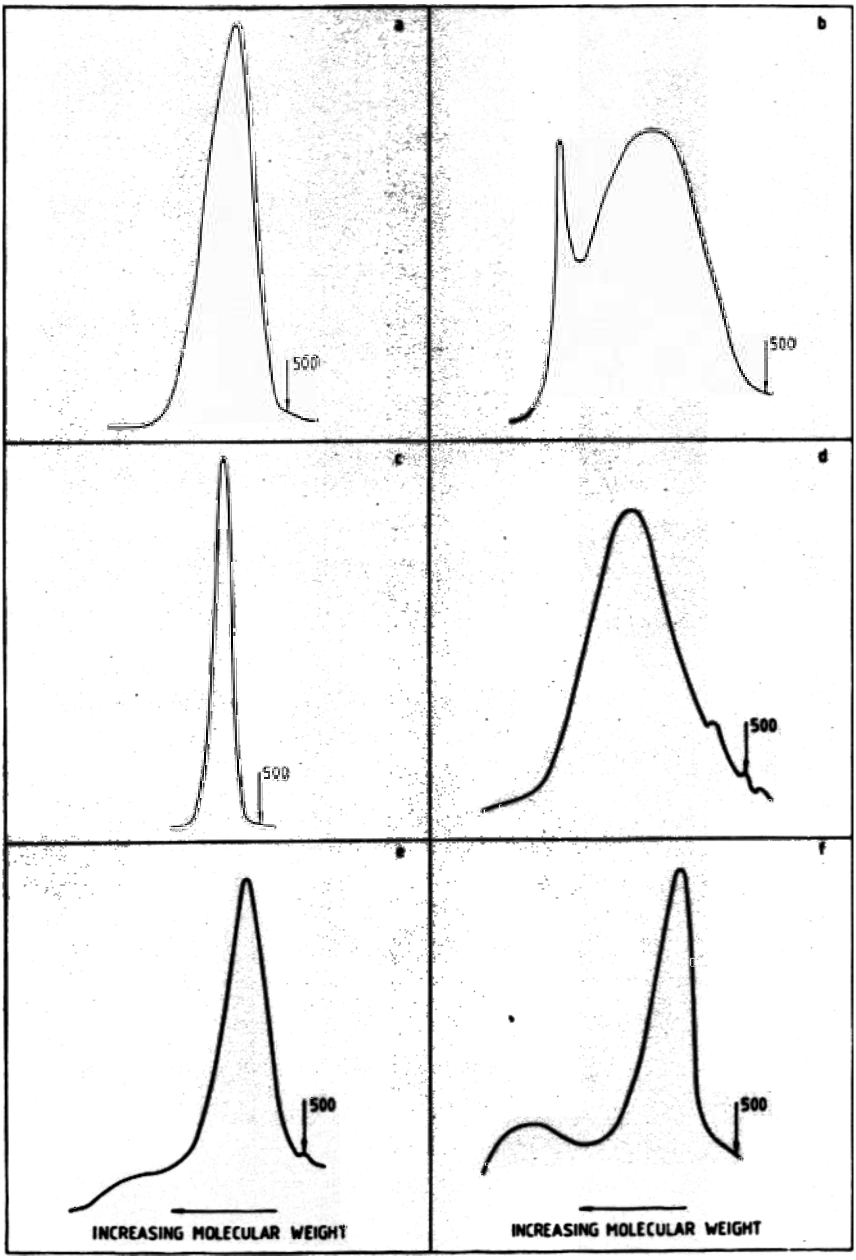

Fig. 2. Curves representing the types of MW distributions observed for tannin peracetates.

sampled over several seasons, and the observed composition ($\overline{M}_n$, stereochemistry, etc.) has remained remarkably constant.

The most intriguing result is the observation of a double-humped distribution for three tannins [see Fig. 2, curve (f)]. Whether the explanation is the existence of two classes of PA polymer with distinctly different properties cannot be answered on current evidence.

EXPERIMENTAL

The GPC analyses were carried out on a Waters model 6000 A pump unit using a model 450 variable wavelength detector, and Waters 10^3 and 10^4 A μStyragel columns connected in series.

The tannins were acetylated with pyridine–Ac_2O (1:1), and after standing overnight the reaction mixture was poured into H_2O and the ppted peracetate collected by suction filtration and washed thoroughly with H_2O. The product was dried in a vacuum desiccator.

For analysis, 2–5 mg tannin peracetate was dissolved in 1 ml freshly redistilled THF, millipore filtered, and 10 μl injected. This loading may be increased *ca* 100 times before significant decrease in resolution occurred. A flow rate of 1 ml/min was normally used when the system had a resolution of *ca* 9000 theoretical plates. Each analysis took *ca* 25 min to complete.

Table 1. MW of proanthocyanidin polymers

Plant species	Organ	$\bar{M}_n$	$\bar{M}_w$	Dp
Polymers with type (a) curves				
Aesculus carnea	fruit	4200	8500	2.02
Astelia fragrans	inflorescence	3500	5500	1.57
Agathis australis	bark	2200	3200	1.45
Agathis australis	leaf	3600	5500	1.53
Crataegus oxyacantha	berry (unripe)	2700	3700	1.37
Photinia glabrescens	leaf	5400	11 900	2.20
Rhopalostylis sapida	fruit	5100	9000	1.76
Chaenomeles chinensis	fruit (unripe)	4600	12 000	2.61
Vicia sativa	leaf	5400	12 700	2.35
Trifolium repens	flower	3100	5000	1.61
Watsonia pyramidata	leaf	2800	4300	1.54
Zantedeschia aethiopica	fruit	3700	5900	1.59
Polymers with type (c) curves				
Phoenix canariensis	leaf	3400	4300	1.26
Hordeum vulgare	ears	1620	1850	1.14
*Pinus taeda**	bark	1560	1960	1.26
Polymers with type (d) curves				
Feijoa sellowiana	fruit (unripe)	4200	7800	1.86
Pinus longifolia	inner bark	3400	5900	1.74
Iris pseudacorus	fruit	3700	6200	1.66
Lotus tenuifolius	root	3100	10 000	3.22
Polymers with type (e) curves				
Cydonia oblonga	fruit (unripe)	4900	21 400	4.35
Cydonia oblonga	fruit (semi-ripe)	5500	22 600	4.09
Lotus pedunculatus	root	4500	34 000	7.39
Pinus radiata	needles	3000	10 700	3.57
Iris germanica	fruit	3200	9500	2.97
Musa sapientum	fruit skin (unripe)	3400	16 100	4.74
Freycinetia banksii	leaf	3900	23 000	5.90
Canna indica	leaf	3300	6700	2.03
Rhopalostylis sapida	leaf	2500	4200	1.68
Podocarpus totara	leaf	4200	9400	2.24
Polymers with type (f) curves				
Phormium cookianum	leaf	3500	154 000	44.0
Vaccinium corymbosum	fruit (unripe)	4400	63 000	14.3
Grevillea robusta	leaf	4500	79 000	17.6

*Type A polymer in the paper ref. [4].

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